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Method for Improving the Ecological and Consumer Properties of Cow Milk and Its Derivative Products by Detoxifying Various Toxicants

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Abstract.

The violation of the storage technology of feed crops results in the accumulation of nitrates, nitrites and mycotoxins.

The purpose of research is to develop a method for improving the ecological and consumer properties of the milk of lactation cows and its derivative products by the rational use of the antioxidant etoxin and Mold-Zap in the diets containing the subtoxic dose of nitrates and aflatoxin B_1.

Methods. During the experiment using the method of pairs-analogues, we formed 4 groups of cows. The experiment material was processed by means of the Excel software from the Microsoft Office package.

Results. Adding to the diet of the 3rd experimental group etoxin in a dose of 0.5 kg/ton and Mold-Zap in a dose of 0.5 kg/ton of compound animal feeding stuff showed a stimulating impact on the hematopoietic function of the cows of the 3rd experimental group due to which their blood contained more erythrocytes and hemoglobin in comparison with the control group with the simultaneous decrease in the concentration of methemoglobin. The ammonia content in the blood of the cows of the 3rd experimental group was 20.9% higher and the content of nitrates and nitrites, on the contrary, was 2.42 and 2.91 times lower. The enrichment of the cow diets with these preparations had a positive impact on the chemical composition of milk and that resulted in the decrease in the concentration of nitrates by 45.1%, nitrites by 55.3%, and aflatoxin M_1 by 48.9%. The analogues of the 3rd experimental group showed the increase in the share of a-casein and the diameter of the casein micelles and this means the improvement of the milk suitability for cheese. The cheese sample made from the milk of the animals of the 3rd experimental group showed the lowest concentration of nitrates – 68.5% and nitrites – 70.0%. The content of aflatoxin M_1 was 60.0% lower than in the control group. The level of nitrates, nitrites and aflatoxin in the cheese samples made from the milk of the cows of this group was lower than the maximum permissible concentrations.

Field of application. The environment protection and rational natural resource management.

Keywords: cows, nitrates, aflatoxin B_1, antioxidant, blood composition, detoxication of nitrates and aflatoxins, physical and chemical composition of milk, ecological and consumer properties of cheese.

INTRODUCTION

Urgency of research.

The intensive use of the industrial technologies in the agriculture that is accompanied by the wide use of nitrogen mineral fertilizers can provoke the risk of excessive concentration of nitrates and nitrites in the soil. Under the impact of enims of nitrate and nitrite reductases of plants, nitrates are reduced with the formation of the intermediate product – hydroxylamine that is involved into the protein metabolism [1].

Nitrates and nitrites, having absorbed into the blood stream, acidize the ferrous form of hemoglobin into the ferric form forming the methemoglobin. With an increase in the methemoglobin level in cows, the respiratory function of blood is impaired, the dispersion of milk lipids in milk is changed, the specific surface of the membrane of milk protein is increased and the size of fatty capsules is decreased [2].

During the storage, the feed stuff is damaged by mold fungus including Aspergillus flavus and Aspergillus

parasiticus. They actively produce mycotoxin aflatoxin B_1, possessing pronounced hepatotrophic action [3].

One of the most perspective directions of denitrification of milk products and prevention of mycotoxicosis is preventing the pollution of feed grain during the stage of storage. However, if the excessive concentration of the said xenobiotics (nitrates and aflatoxin B_1) in the feeds cannot be avoided, it is reasonable to use for feeding cows the preparations that are able to decrease the harmful influence of nitrates and mycotoxins on the productivity and metabolism. Such preparations include adsorbents, inhibitors of fungus and antioxidants [4].

The objective of research is to develop the method for improving the ecological and consumer properties of milk of lactation cows and its derivative products by using the antioxidant etoxin and the inhibitor of fungus Mold-Zap in the diets containing the subtoxic dose of nitrates and aflatoxin B_1.

MATERIALS AND METHODS OF RESEARCH.

To achieve the set goal, the scientific and economic experiment was conducted at the farm "Meat products", the Republic of North Ossetia-Alania, on the Schwyz breed cows. 4 groups of 10 cows each were formed from 40 cows selected according to the breed, age, live weight, productivity in the previous lactation, and fat content in the milk under the analogue principle.

During the research, the cows of all groups were put on the basic diet (BD). For the purity of the experiment taking into account the concentration of nitrates in the feeds, we strived for the availability of these toxicants in the diet of the animals of all groups at the level of a subtoxic dose – max. 0.03 g/kg of the live weight of cows [5] by means of adding the sodium nitrate preparation. The BD of the cows of the 1st, 2nd, and 3rd experimental groups included the tested preparations in the quantities stipulated by the scheme of the experiment (Table 1).

The milk productivity of cows was determined according to the results of the control milk yields performed once a month. On the same days, we determined the chemical composition of milk of the tested cows using the general methods.

The hematological research according to the general methods was performed to characterize the metabolism processes in the organism and the physiological condition of the cows. The concentration of hemoglobin in the blood of the lactation cows was determined by the spectrophotometric method [6].

The concentration of nitrates and nitrites in the blood samples, ruminal fluid and milk was studied colometrically in a PEC-M (photoelectrocolorimetric analyzer).

The concentration of aflatoxin B_1 in the feeds and aflatoxin M_1 in the samples of milk and cheese were determined by the method of enzyme immunoassay (EIA) using the test systems Ridoscreen® Aflatoxin Total.

The experimental material was processed statistically using the Excel software from the current Microsoft Office package.

RESULTS OF RESEARCH.

When performing the experiment, we studied the concentration of nitrate and nitrite ions and aflatoxin B_1 in the feed stuffs that were included into the diets of experimental cows (Table 2).

The feed stuffs showed no exceeding of the maximum permissible concentration (MPC) of nitrates and nitrites and also aflatoxin B_1. To estimate the denitrification properties of the tested preparations taking into account the nitrate ions in the feed stuffs, the diets of the experimental animals included sodium nitrate, on the base of the subtoxic level of nitrates in them – max. 0.03 g/kg of the live weight of cows [5]

Table 1.Scheme of the scientific and economic experiment

Group	Number of animals	Basic diet (BD)	Doses of added preparations		
			sodium nitrate, g/kg of live weight	etoxin, kg/ton compound animal feeding stuff	Mold-Zap, kg/ton compound animal feeding stuff
Control	10	BD	0.03	-	-
1 experiment	10	BD	0.03	0.5	-
2 experiment	10	BD	0.03	-	1.5
3 experiment	10	BD	0.03	0.5	1.5

Table 2. Concentration of nitrates, nitrites and aflatoxin B_1 in the feed stuffs, mg/kg

Feed stuffs	NO_3^-		NO_2^-		Aflatoxin B_1	
	MPC	actual value	MPC	actual value	MPC	actual value
Grass of oat – pea	500	14	10	0.31	-	-
Grass of rape	500	11	10	0.25	-	-
Grass of artificial pasture	500	14	10	0.11	-	-
Gramineous and various grasses hay	1000	44	10	0.25	0.05	traces
Corn silage	500	12	10	9.13	0.05	traces
Compound animal feeding stuff	300	3	10	0.03	0.05	0.03
Beet molasses	1500	27	10	0.34	-	-
Fodder beet	2000	36	10	0.27	-	-

During the intoxication of the organism with nitrates and nitrites, the morphological composition of blood is subjected to significant changes (Table 3).

Joint additives of antioxidant etoxin and the preparation Mold-Zap made a stimulating impact on the hematopoietic function of organisms of the cows of the 3rd experimental group due to which their blood contained (P<0.05) erythrocytes more by 1.51 × 10^{12}/l and hemoglobin – by 14.55 g/L with a simultaneous decrease in the level of methemoglobin – by 54.94% (P<0.05) in comparison with the cows of the control group.

Together with the morphological composition, we studied some biochemical blood values (Table 4).

During the process of detoxication of xenobiotics, the energetic metabolism of the cows of the 3rd experimental group was activated, which is confirmed by the statistically significant (P<0.05) increase in the glucose concentration in the blood serum by 34.3% in comparison with the control group.

In case of the joint supplement of the preparations of etoxin and Mold-Zap, the cows of the 3rd experimental group showed the optimization of the protein metabolism due to which in their blood statistically significant (P<0.05) crude protein was 7.22% higher in comparison with the control group and, on the contrary, acetone was 54.17% less (P<0.05).

The amount of ammonia had an inversely proportional ratio with the number of nitrates and nitrites of the experimental animals, that is, their concentration during the process of denitrification in the blood decreased. Therefore, the blood serum of the cows of the 3rd experimental group contained the statistically significant concentration of ammonia (P<0.05) that was 20.9% higher in comparison with the control group, and the concentration of nitrates and nitrites, on the contrary, was 2.42 (P<0.05) and 2.91(P<0.05) times less.

To study the efficiency of the use of the tested feed preparations, some physical and chemical properties of milk of the experimental cows were studied (Table 5).

Table 3. Morphological blood values of experimental cows

Value	Group			
	Control	1 experimental	2 experimental	3 experimental
Erythrocytes, 10^{12}/L	5.97±0.21	6.73±0.23	6.88±0.25	7.48±0.29
Leukocytes, 10^{9}/L	10.26±0.31	10.17±0.45	10.30±0.51	10.10±0.41
Hemoglobin, g/L	99.55±1.2	109.88±1.5	110.77±2.1	114.10±1.4
Methemoglobin, %	3.44±0.29	2.55±0.30	2.43±0.27	1.55±0.32

Note: n = 3

Table 4. Some biochemical blood values

Value	Group			
	control	1 experimental	2 experimental	3 experimental
Crude protein, g/L	72.23±0.51	74.83±0.58	75.28±0.45	77.45±0.32
Acetone, mmol/L	0.48±0.05	0.34±0.03	0.30±0.02	0.22±0.04
Sugar, mmol/L	2.48±0.07	2.88±0.04	2.94±0.09	3.33±0.12
Ammonia, mmol/L	4.25±0.12	4.88±0.19	4.94±0.14	5.14±0.18
Nitrates, mmol/L	10.24±0.19	6.21±0.20	6.12±0.22	4.22±0.25
Nitrites, mmol/L	0.332±0.004	0.208±0.005	0.200±0.006	0.114±0.003

Note: n = 3

Table 5. Chemical composition of milk of the experimental cows

Value	Group			
	control	1 experimental	2 experimental	3 experimental
Dry matter, %	12.30±0.14	12.63±0.12	12.68±0.11	12.78±0.15
Milk fat, %	3.62±0.05	3.75±0.06	3.77±0.05	3.83±0.07
Milk protein, %	3.34±0.04	3.43±0.05	3.45±0.06	3.51±0.05
Lactose, %	4.51±0.05	4.58±0.08	5.6±0.05	4.61±0.02
Ammonia, mg/L	2.08±0.05	2.63±0.07	2.73±0.07	3.14±0.09
Nitrates, mg/L	7.76±0.13	5.96±0.18	5.75±0.21	4.26±0.18
Nitrites, mg/L	0.152±0.004	0.102±0.003	0.097±0.005	0.068±0.006
Aflatoxin (M_1), mg/kg (MPC=0.005 mg/kg	0.0047±0.0003	0.0037±0.0002	0.0034±0.0004	0.0024±0.0007

Note: n = 10

Table 6. Milk suitability for cheese of the experimental cows

Value	Group			
	control	1 experimental	2 experimental	3 experimental
Average concentration of protein in milk, %	3.34±0.04	3.43±0.05	3.45±0.06	3.51±0.05
Share of casein, %	2.40±0.02	2.58±0.03	2.62+0.02	2.71±0.04
Composition of casein, %: α-casein	31.57±0.21	35.16±0.22	34.61±0.18	36.13±0.26
β-casein	53.41±0.24	53.71±0.30	53.55±0.28	53.62±0.33
γ-casein	15.02±0.19	11.13±0.16	11.84±0.19	10.25±0.13
Total	100.0	100.0	100.0	100.0
Diameter of casein micelles, °A	610±3.2	663±3.0	675±4.0	711±3.5
Output of cheese mass 45% fat content from 100 kg of milk, kg	9.77±0.24	10.42+0.30	10.54±0.29	10.89±0.33
Chemical composition of cheese, %: Dry matter	51.76±0.21	52.86±0.34	52.99±0.28	53.44±0.38
Fat in dry matter	44.86±0.20	44.96±0.37	45.02±0.39	45.09±0.46
Protein in dry matter	20.02±0.04	20.86±0.03	20.93±0.04	21.33±0.05
Nitrates, mg/kg	3.18±0.12	1.50±0.15	1.44±0.14	1.00±0.17
Nitrites, mg/kg	0.050±0.002	0.037±0.004	0.034±0.003	0.015±0.003
Aflatoxin M_1), mg/kg (MPC=0.0005 mg/kg	0.00030±0.0002	0.00023±0.0003	0.00021±0.0002	0.00012±0.0003

Note: n = 10

In the milk of the cows from the control group, the concentration of the dry matter was 12.30%, and its density was 27.72°A. Joint additives of the preparations etoxin and Mold-Zap made a positive impact on these values of milk of the animals of the 3rd experimental group and that allowed them to overcome the control group (P<0.05) in the density – by 0.61°A and the concentration of the dry matter in the products – by 0.48%.

The inverse proportion was established between the concentration of nitrates and nitrites in the milk, on one side, and ammonia, on the other side. Therefore, the highest concentration of ammonia was noticed in the milk of the cows of the 3rd experimental group – 3.14 mg/L, which was 50.9% (P<0.05) higher than in the control group.

Joint additives of the tested preparations provided the highest degree of the denitrification of the products of the cows of the 3rd experimental group, and because of it the concentration of nitrates in the milk was significantly lower (P<0.05) – by 45.1%, and the concentration of nitrites was 55.3% less than in the control group.

Besides, the milk of the animals from the 3rd experimental group contained a statistically significantly lower concentration of aflatoxin M_1 (metabolite of aflatoxin B_1) – 48.9% lower than in the control group. At this, the concentration of mycotoxin in the milk of cows of the compared groups was lower than MPC.

Taking into account the higher concentration of protein in the milk, the process parameters of the milk of animals were studied that were received by the processing of Ossetian cheese into samples (Table 6).

Additives of the mixture of the said preparations in the diets of cows of the 3rd experimental group contributed to a statistically significant (P<0.05) increase of protein in milk - by 0.21%, and the casein share in it – by 0.31% in comparison with the control group.

The use of these preparations had no effect upon the concentration of β-casein in the milk, but it provided a statistically significant increase in the share of *a*-casein in the products of the animals of the 3rd experimental group – by 4.56% with a simultaneous decrease in the concentration of γ-fraction of casein – by 4.77 % (P<0.05) in comparison with the control group. Taking it into account, the diameter of casein micelles in the milk casein of the animals from the 3rd experimental group was statistically significantly (P<0.05) higher than in the control analogues – by 16.6%.

The improvement of the protein metabolism under impact of the mixture of antioxidants allowed providing the highest output of the cheese mass with 45% fat content for the cows of the 3rd experimental group in comparison with the control group. In the cheese samples from the milk of the cows of the 3rd experimental group, there was a statistically significant (P<0.05) increase in the concentration of dry matter – by 1.68%, and protein in dry matter – by 1.31% in comparison with the control group.

The lowest concentration of nitrates and nitrites had the junket obtained from the milk of the cows of the 3rd experimental group, statistically significantly (P<0.05) exceeding the cheese mass of the control sample according to these parameters by 68.5 and 70.0%.

In the cheese samples made from the milk of the cows of the 3rd experimental group, the concentration of aflatoxin M_1 was 60.0% (P<0.05) less than in the control sample. At this, the concentration of this mycotoxin in the cheese samples made from the milk of the cows of the compared group was lower than MPC.

DISCUSSION OF RESEARCH RESULTS.

Joint additives of the antioxidant etoxin and preparation Mold-Zap made a stimulating impact on the hematopoietic function of the organism of cows of the 3rd experimental group due to which the concentration of the erythrocytes in their blood was higher with a simultaneous decrease in the level of methemoglobin. The terminal metabolite of the impact of nitrate-reducing enzymes is

ammonia, the level of which had the inversely proportional ratio with the concentration of nitrates and nitrites of the experimental animals, that is, their concentration in blood decreased during the process of denitrification. Therefore, the blood serum of the cows of the 3rd experimental group in comparison with the control group had a statistically significantly ($P<0.05$) higher concentration of ammonia (by 20.9%), while the concentration of nitrates and nitrites, on the contrary, was 2.42 times ($P<0.05$) and 2.91 ($P<0.05$) times less.

The additives of the mixture of the mentioned preparations into the diets of the cows of the 3rd experimental group contributed to a statistically significant ($P<0.05$) increase in the milk protein by 0.21% and the share of casein – by 0.31% in comparison with the control group. At the same time, the milk of the animals of the 3rd experimental group contained statistically less aflatoxin M_1 – by 48.9% in comparison with the control group. At this, the concentration of this mycotoxin in the milk of the cows of the compared groups was lower than MPC. Impact of the mixture of preparations of antioxidants allowed to provide the highest output of the cheese mass with 45% fat content – 10.89 kg from the cows of the 3rd experimental group, which was statistically significantly ($P<0.05$) higher than in the control group – 11.5%. The sample of cheese obtained from the milk of the cows of the 3rd experimental group had the lowest concentration of nitrates and nitrites, statistically significant ($P<0.05$) exceeding the cheese mass of the control sample according to these parameters – by 68.5 and 70%,respectively, that is the enrichment of the diets of cows with the subtoxic does of nitrates with the mixture of preparations made a positive impact on the process properties of milk and sanitary and hygienic qualities of its derivative products.

CONCLUSIONS:

1) The enrichment of the diets of the lactation cows with the increased concentration of nitrates by the mixture of the preparations of etoxin in a dose of 0.5 kg/ton and Mold-Zap in a dose of 1.5 kg/ton of the compound feeding stuff made a positive impact on the chemical composition and sanitary and hygienic qualities of milk and that resulted in the increase in the concentration of ammonia (by 50.9%) with a simultaneous decrease in the level of nitrates and nitrites, and in the statistically significantly ($P<0.05$) lower concentration of aflatoxin M_1 (by 48.9%), the level of which in the milk of cows was lower than MPC.
2) The joint use of the preparations of etoxin and Mold-Zap helped to increase the concentration of erythrocytes and hemoglobin in the blood of the cows of the 3rd experimental group with a simultaneous decrease in the concentration of methemoglobin by 54.94%, nitrates – by 2.42 and nitrites – by 2.91 times.
3) The increase in the level of *a*-casein in the products led to the increase in the diameter of the casein micelles. The improvement of the protein metabolism allowed providing the highest output of the cheese mass – 10.89 kg - from the cows of the 3rd experimental group, which is 11.5% higher than of the control group. The lowest concentration of aflatoxin M_1, nitrates and nitrites was shown by the sample of cheese obtained from the milk of the cows of the 3rd experimental group, which, as compared to the control sample, was lower by 60.0%, 68.5% and 70.0%, respectively.

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