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Research on Physico-Chemical Properties of Milk and Milk Derived Products Made in Technogenic Area

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Abstract

The research purpose is to study an effect of additives (chelaton adsorbent and santochinum antioxidant) in lactating cows's diets with increased contents of heavy metals and nitrates on physico-chemical and working properties of milk. The basic approach to the research of this problem was in making experimental groups of cows. Efficiency of the co-fed adsorbent and antioxidant to detox from heavy metals and nitrates was estimated with spectrophotometry by measuring their concentration in milk and its derived products. The findings were statistically processed with SNEDECOR software. The article presents experimental data that substantiate efficiency of chelaton adsorbent and santochinum antioxidant co-added in cow's diets with increased levels of heavy metals and nitrates to improve physico-chemical and working properties of milk. Thanks to their synergetic affect, in animals of Experimental Group III, the piloted preparations provided significantly ($P<0.05$) increased levels of milk fat (by 0.22%) and protein (by 0.22%). In the same group, these preparations contributed to the significant ($p<0.05$) decrease in levels of zinc (by 4.75 times), lead (by 2.65 times) and cadmium (by 5.65 times), nitrates (by 2.63 times), and nitrites (by 3.3 times). Levels of these elements were not beyond maximum allowable concentrations (MAC) in milk. The article's content is of practical value for agricultural enterprises to detox cow's milk from heavy metals and nitrates with co-fed chelaton and santochinum preparations.

Keywords: milk, heavy metals, nitrates, adsorbent, antioxidant, cheese, dairy products.

INTRODUCTION

Physico-chemical and working properties of milk depend on animals' genetic potential, season, compositions and nutritional values of diets, and other factors. Moreover, a diet composition for lactating cows substantially adjusts levels of all its components in milk [1].

Among other Russian regions, the Republic of North Ossetia-Alania (the RNO-Alania) is considered one of the most disadvantaged in terms of its environmental safety of the soil and forage crops. Local vegetable feed-stuff polluted with heavy metals due to large-scale enterprises of mining and non-ferrous metals industries located in the region [2] are a serious danger. In the Mozdok District of the RNO-Alania, we should take into account high levels of heavy metals, especially zinc, lead, and cadmium accumulated by forage crops cultivated at local farms. Toxicity of these elements depends on the fact that with proteins they form insoluble complexes that can significantly change properties of some essential enzymes often inactivating biochemical reactions, in which they participate [3].

Agricultural enterprises in the Mozdok District of the RNO-Alania extensively apply nitrogen fertilizers, which are fraught with a risk of excessively accumulated nitrates and nitrites in forage crops. They penetrate in cow's milk with feed-stuff. Moreover, in an animal's body, toxicity manifests itself and it sharply strengthens when nitrates turn into nitrites as in animals' blood haemoglobin turns into methaemoglobin and causes hypoxia. Simultaneously available in milk, heavy metals, nitrates, and nitrites do not only adversely influence milk physico-chemical properties, but also cause worsened raw milk working parameters [4].

There are evidences of a positive effect of additives (adsorbent preparations) in diets with increased levels of heavy metals and nitrates on yield and quality of milk among lactating cows, Such adsorbent preparations bind these xenobiotics and remove them from the gastrointestinal tract. However, due to high adsorption capacity, along with toxicants, many adsorbents can take out other useful biologically active substances from the digestive tract of ruminants [5]. Therefore, to achieve a better detoxification effect, other biologically active additives are used along with adsorbents to inhibit excretion of biogenic compounds.

The research aim is to review the effect of additives (chelaton adsorbent and santochinum antioxidant) on physico-chemical and working properties of milk in lactating cows' diets with increased contents of heavy metals and nitrates.

MATERIAL AND METHODS

The experimental part of the research was performed at the agricultural production cooperative (APC) "Polyakov" in the Mozdok District of the RNO-Alania. The research object in the course of research and manufacturing experiment included Black-and-White cows of the second lactation. They were divided into three groups with 10 animals each, using the analogue method and considered breed, age, body weight, milk yield, and milk fat content from previous lactation.

The schedule for the research and manufacturing experiment is given in Table 1.

Table 1: Schedule for the research and manufacturing experiment with cows

Group	Number of animals, animal units	Feeding specifics
I Control Group	10	Basic diet (BD)
II Experimental Group	10	BD+chelaton in amount of 1.0 kg/ton of feed-stuff
III Experimental Group	10	BD+chelaton in amount of 1.0 kg/t+santochinum at dose of 0.5 kg/t of feed-stuff

Piloted preparations (chelaton adsorbent and santochinum antioxidant) were given to animals as added into a composition of the standard feed-stuff at doses given in Table 1.

Chelaton is one of the most effective chelating compounds designed to bind metal ions and other xenobiotics. Chelating in the lactating cows' rumen minimizes formation of free radicals, which is very important in enzymatic processes in proventriculus microflora. Some microorganisms living in the rumen become more active in their synthesis of nitrates involved in denitrification processes. To detox from heavy metals and nitrates, chelaton preparations should be added in feed-stuff of lactating cows at dose of 1.0 kg/t [6].

Santochinum is an antioxidant preparation, which, depending on its purification degree, has different names. Russia produces 72%-santohinum called quinol with the content of its main active substance (2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline) up to 98%. As an antioxidant, this preparation is included in composition of feed-stuff for cows at dose of 0.5 kg/ton of feed [7].

Cows’ lactation performance in the compared groups was estimated with monthly monitoring of milk yields. On the same days of milk yields, we estimated levels of fat, protein, lactose, ash, density, and acidity following conventional guidelines.

To study effects of piloted preparations on cheese-making properties of milk from experimental cows we made samples of Ossetian brined cheese. At the same time, we took into account a duration of milk caseating with chymosin (rennet).

Research of working properties of milk from experimental animals was made by producing butter samples. The product was manufactured from an output of cows’ daily milk yields. At the same time, butter-making properties of raw milk were explored by cream whipping time.

Heavy metals available in samples of feed, milk, and cream were detected using atomic absorption with spectrophotometer ASR-115-M1. Nitrates and nitrites in milk and diary products were detected with the spectrophotometer to measure absorbency of the wavelength of 538 nm with quartz cells with 10 and 20 mm-optical path length.

Digital data, obtained in the research and production experiment, were statistically processed according to Student's methods using a personal computer with SNEDECOR software.

Research was completed in the framework of the basic part of the governmental terms of reference on the subject “Development of Resource-saving Technology to Produce Environmentally Friendly Foods in Technogenic Area of the RNO-Alania”.

RESULTS

With the findings from the chemical analysis of feed used in diets of experimental cows at APC “Polyakov,” we identified that their energy and nutritional value were in compliance with average regional values for the RNO-Alania.

In the compared groups, summer diets of cows among own-produced feedstuff included green fodder: winter rape grass and artificial pasture grass based on alfalfa and timothy.

Winter diets of experimental animals included own-produced feedstuff with rough and succulent food (oat and vetch hay), corn silage, and fodder beet.

However, own-produced feedstuff had excessive contents of heavy metals and nitrates. Besides, summer and winter diets included a standard feedstuff for dairy cattle. Therefore, we have estimated levels of heavy metals and nitrates in the composition of summer and winter diets.

The research has revealed that summer and winter diets for experimental animals show excessed maximum allowable concentrations (MAC): for zinc by 46.2 and 50.3%, for lead by 33.8 and 37.6%, for cadmium by 39.3 and 43.1%, for nitrates by 16.2 and 19.7%. As for nitrite levels, figures did not go beyond maximum allowable concentrations. Higher levels of these toxins in the winter diet are due to own-produced hay and silage available in the diet composition.

By energy and nutrients, cows' physiological needs in the compared groups were met within valid standards of feeding with the exception of zinc, lead, cadmium, and nitrates.

In the course of the experiment based on monthly control milk yields, we studied an influence of piloted feed preparations on experimental cows' lactation performance (Table 2).

Table 2: Milk yield of experimental cows

n = 10

Index	Group		
	Control Group 1	Experimental Group II	Experimental Group III
Milk yield, kg	5,651 ± 36.7	5,669 ± 37.0	5,765 ± 39.0
Fat content, %	3.56 ± 0.04	3.74 ± 0.05	3.78 ± 0.04
Protein, %	3.25 ± 0.05	3.41 ± 0.03	3.47 ± 0.04
Basic fat milk yield (3.4%), kg	5,917 ± 21.4	6,236 ± 23.5	6,409 ± 21.7
As % of Control	100.0	105.4	108.3
Absolute yield, kg: Milk fat	201.77 ± 0.26	212.02 ± 0.24	217.92 ± 0.30
As % of Control	100.0	105.1	108.0
Milk protein	183.66 ± 0.23	193.31 ± 0.22	200.04 ± 0.28
As % of Control	100.0	105.2	108.9

The comparative evaluation of indicators for milk yield of natural fat content during lactation shows that by this indicator between Control Group I (5,651 ± 36.7 kg) and Experimental Groups II (5,669 ± 37.0 kg) and III (5,765 ± 39.0 kg) there are no statistically significant (P>0.05) differences.

Many toxicants, including heavy metals and nitrates, can have a depressing effect on a synthesis of milk lipids and protein, as they are able to inhibit an activity of enzymes involved in generation of these milk components in the animal's lacteous gland. Our findings have supported this assumption. Cows in Control Group I during all their lactation time showed the lowest milk fat (3.56%) and protein (3.2 5%) contents. The most favourable effect on the synthesis of milk fat and protein in the lacteous gland was made by co-added chelaton adsorbent and santochinum antioxidant into diets. Owing to this, animals in Experimental Group III compared to the Control Group significantly had in their milk 0.22% more ($P<0.05$) milk fat and 0.22% more protein. This points out to the synergetic effect of the piloted preparations on the synthesis of lipids and protein in the lacteous gland by means of more active enzymes of proteolytic and lipid spectrum.

In terms of a stimulating effect of the antioxidant and adsorbent on lactation processes, we estimated the absolute yield for milk fat and protein. It has been found that in the lactation period in Control Group I, the absolute yield of milk fat was 201.77 ± 0.26 kg and for protein, it was 183.66 ± 0.23 kg. As for lactation performance, animals in Experimental Group III were significantly ($P<0.05$) ahead of their control counterparts by 8.0 and 8.9% respectively.

In our country, to estimate economic efficiency of milk production, calculations go from the yielded milk output of basic fat. For the Republic of North Ossetia-Alania, this figure is 3.4%. We have identified that with co-fed chelaton and santochinum preparations in diets with increased levels of heavy metals and nitrates, Experimental Groups III by this parameter outwent the controls by 8.3%. At the same time, the difference was statistically significant ($P<0.05$).

We have reviewed an influence of piloted preparations on physico-chemical parameters of animal milk between the compared groups (Table 3).

Table 3: Physico-chemical properties of cow's milk

n = 10

Index	Group		
	Control Group 1	Experimental Group II	Experimental Group III
Density, ^{0}A	27.69 ± 0.13	28.04 ± 0.12	28.29 ± 0.16
Acidity, ^{0}T	17.93 ± 0.14	17.87 ± 0.010	17.97 ± 0.20
Dry substance, %	12.21 ± 0.11	12.55 ± 0.13	12.66 ± 0.14
Milk fat, %	3.56 ± 0.04	3.74 ± 0.05	3.78 ± 0.04
Milk protein, %	3.25 ± 0.05	3.41 ± 0.03	3.47 ± 0.04
Lactose, %	4.63 ± 0.06	4.61 ± 0.07	4.61 ± 0.08
Ash,%	0.77 ± 0.004	0.79 ± 0.005	0.80 ± 0.004
Calcium,%	0.19 ± 0.003	0.19 ± 0.002	0.21 ± 0.003
Phosphorus,%	0.12 ± 0.001	0.13 ± 0.001	0.14 ± 0.001
Zinc, mg/l (MAC 5.0 mg/l)	6.84 ± 0.17	2.56 ± 0.14	1.44 ± 0.15
Lead, mg/l (MAC 0.05 mg/l)	0.069 ± 0.003	0.035 ± 0.004	0.026 ± 0.002
Cadmium, µg/l (MAC 0.02 mg/l)	0.028 ± 0.002	0.0177 ± 0.003	0.005 ± 0.001
Nitrates, mg/l	6.44 ± 0.28	3.63 ± 0.24	2.45 ± 0.20
Nitrites, mg/l	0.10 ± 0.002	0.05 ± 0.001	0.03 ± 0.003

The comparative evaluation of milk acidity in lactation has showed that by this index between the Control (17.93^{0}T) and Experimental Groups II (17.87^{0}T) and III (17.97T$^{0)}$ there are no any statistically significant ($P>0.05$) differences.

Milk density value directly depends on dry substances available in it. In milk of animals in the controls, the dry substance level was 12.21%, and its density was 27.69 A^0. With co-fed chelaton and santochinum preparations, in milk of animals in Experimental Group III we observed a significant ($P<0.05$) increase in density by 0.60 A^0 and in dry substances - by 0.45% compared to the controls.

In turn, concentrations of other milk components are less subject to changes under an influence of the food factor, so the milk chemical composition is relatively steady with no observed significant differences in milk between the compared groups ($P>0.05$) by lactose and ash levels.

Along with these indicators of milk chemical composition. in experimental cows, we have also reviewed contents of various pollutants of chemical nature that significantly impair bionomic and nutritional qualities of milk products and milk by-products. With this in mind and in comparison with the controls, we have found that the antioxidant and adsorbent co-added in diets in Experimental Group III, provided a significantly ($P<0.05$) reduced level of zinc (by 4.75 times), lead (by 2.65 times), and cadmium (by 5.65 times). At the same time, levels of these elements in no case were beyond MAC in milk of animals in Experimental Group III.

The co-fed adsorbent and antioxidant also had a positive impact on denitrification in experimental animals. Therefore, in milk of cows in Experimental Group III compared to the controls, there were significantly ($P<0.05$) reduced nitrate and nitrite levels, by 2.63 times and 3.33 times accordingly. This assumes a high detox effect of these preparations in bodies of lactating cows.

It is known that levels of milk fat and protein mostly define which dairy products will be produced from raw milk, i.e. butter or cheese making. Cheese suitability of milk mainly depends on an amount and a quality of milk protein, specifics of which is given in Table 4.

With significant ($p<0.05$) superiority of Experimental Group III over the controls by milk protein level by 0.22%, there were no significant differences in casein contents in dairy raw materials of animals between the compared groups ($P <0.05$). Besides, in levels of whey proteins in milk, cows in Experimental Group III were significantly ($P<0.05$) inferior to the controls by 0.18%. Milk of cows in Experimental Group III compared to the controls by percentage of whey proteins of milk protein mass fraction was more saturated (by 3.6%).

Table 4: Composition and properties of milk protein

n = 10

Index	Group		
	Control Group 1	Experimental Group II	Experimental Group III
Total protein, %	3.25 ± 0.05	3.41 ± 0.03	3.47 ± 0.04
Casein, %	2.49 ± 0.004	2.51 ± 0.004	2.53 ± 0.005
Casein % of total protein	76.5	73.5	72.9
Whey proteins, %	0.76 ± 0.001	0.90 ± 0.003	0.94 ± 0.004
% of whey proteins of total protein	23.5	26.5	27.1
Casein composition, % α-casein	34.47 ± 0.22	33.56 ± 0.26	33.12 ± 0.24
β- casein	54.21 ± 0.16	53.88 ± 0.14	53.61 ± 0.13
γ-casein	11.32 ± 0.15	12.56 ± 0.17	13.27 ± 0.12
Rennet coagulation, min.	26	31	36
Milk consumption, kg/kg of cheese	10.37	10.78	11.08

According to the findings, milk of animals in Experimental Group III vs the controls in composition of casein had significantly ($P<0.05$) less α- and β-fractions (by 1.35 and 0.60%) with the simultaneously increased concentration of γ-casein by (1.95%) ($P<0.05$). Among all the casein fractions, α- and β-casein influenced by chymosin may only coagulate (γ-casein is not a part of casein micellae), so cheese-making properties of cow's milk in experimental groups were getting worse.

In the light of higher protein content in cow's milk in Experimental Group III, for this group raw material consumption to produce 1 kg of cheese was 6.85% lower than in the controls.

Going from these differences in the structure of casein fractions, we observed worse rennet coagulation of milk among cows in experimental groups. As for speed of rennet coagulation, milk of animals in all the groups belonged to type 2 (preferable) but compared to the controls products from milk in Experimental Group III exposed to chymosin produced by Meito (Japan) coagulated 10 minutes later. Furthermore, the rennet clot of milk in Experimental Group III was flabbier and not elastic. This evidences that milk from Experimental Group III is not reasonable to be used for cheese-making.

Based on cheese-making results, it seemed appropriate to process milk from experimental animals into butter as butter-making properties of raw materials depend on fat dispersion (Table 5).

Table 5: The diameter and number of milk fat globules

Index	Group		
	Control Group 1	Experimental Group II	Experimental Group III
Total fat, %	3.56 ± 0.04	3.74 ± 0.05	3.78 ± 0.04
Diameter of fat globules, μm	2.81 ± 0.04	3.29 ± 0.04	3.59 ± 0.03
Number of fat gobules, bln./cc	5.70 ± 0.06	4.33 ± 0.07	3.92 ± 0.05

With the adsorbent and antioxidant, co-added into diets, we observed more preferable properties of fat globules' diameter and number. Thus, compared to the controls, in Experimental Group III, there was a significantly ($P<0.05$) increased diameter of fat globules, by 27.76%, with simultaneous reduction in their quantity, by 31.23% ($P<0.05$). This says of better butter-making properties of raw milk in Experimental Group III.

Further whipping of cream got from milk produced by the compared groups was at temperature of 7°C. At the same time, we have studied the effect of the piloted preparations on physico-chemical properties of butter (Table 6).

Table 6: Physico-chemical properties of cream and butter

Index	Group		
	Control Group 1	Experimental Group II	Experimental Group III
Fat weight content in cream, %	32.58 ± 0.16	33.88 ± 0.12	34.27 ± 0.14
Creaming duration, min.	62	52	46
Cream acidity, °T	14.60	14.58	14.56
Peroxide value	0.13	0.14	0.14
Iodine number	33.24	32.87	32.85
Cream sort	2	1	1
Fat rate, %	96.56 ± 0.13	97.58 ± 0.15	97.86 ± 0.11
Butter acidity, °K	0.81	0.80	0.78
Zinc, mg/kg (MAC 5.0 mg/kg)	7.04 ± 0.05	2.44 ± 0.07	1.36 ± 0.06
Lead, mg/kg (MAC 0.05 mg/kg)	0.071 ± 0.002	0.036 ± 0.004	0.028 ± 0.003
Cadmium, mg/kg (MAC 0.02 mg/kg)	0.031 ± 0.04	0.016 ± 0.04	0.007 ± 0.05
Nitrates, mg/kg	5.33 ± 0.22	2.55 ± 0.21	1.75 ± 0.27
Nitrites, mg/kg	0.07 ± 0.003	0.04 ± 0.002	0.01 ± 0.001

Cream made by whipping milk of animals from Experimental Group III had more favourable physico-chemical properties. Compared to the control samples, this was evident in the significantly increased ($p<0.05$) fat mass fraction (1.69%) and whipping shortened by 16 minutes. At the same time, cream from raw milk of cows in Experimental Groups II and III was classified as grade No. 1, while in Control Group I as grade No. 2. Along with this, in a transformation of cream into butter, milk fat was better used for products given by cows from Experimental Group III, 97.86 ± 0.11%, that is significantly higher ($P <0.05$), 1.30%, compared to the controls.

In research of butter consumer-oriented characteristics, its ecological and biological value is significant. It has been found that with the co-fed chelaton and santochinum products, animals in Experimental Group III compared to the controls showed significantly ($P<0.05$) decreased concentrations of zinc by 5.18 times, lead by 2.54, cadmium by 4.43, nitrates by 3.05, and nitrites by 7.00 times. At the same time, the fact comes under notice that in butter samples derived from raw milk of cows in Experimental Group III, concentrations of zinc, lead, and cadmium were sub-tolerant.

DISCUSSION

Findings shown that the more favourable effect on physico-chemical and working characteristics of milk was made by chelaton adsorbent and santochinum antioxidant co-added in diets of lactating cows with increased contents of heavy metals and nitrates. These preparations had not any significant impact on natural fat milk yield. Among all the studied physico-chemical parameters of milk, co-fed preparations of chelaton and santochinum had the most effect on animals from Experimental Group III, providing a significant ($P<0.05$) increase in milk fat content by 0.22% and protein by 0,22%. The antioxidant and adsorbent co-added in diets for cows of Experimental Group III compared to the controls provided significantly ($P<0.05$) reduced concentrations of zinc, lead, cadmium, nitrates, and nitrites. At the same time, the level of these elements was in no case over MAC in milk of animals in Experimental Group III.

Among the all casein fractions, influenced by chymosin, α- and β-casein may only coagulate (γ-casein is not a part of casein micellae), so cheese-making properties of milk from cows in Experimental Groups were getting worse. Furthermore, the rennet clot of milk from Experimental Group III was flabbier and less elastic. This suggests that milk from cows in experimental groups was not effectively used for cheese-making.

Reviewing characteristics of the adsorbent and antioxidant, co-added in diets, we observed more preferable properties of fat globules' diameter and quantity. Thus, compared to the controls, in Experimental Group III, there was a significantly ($P<0.05$) increased diameter of fat globules (by 27.76%) with a simultaneous reduction in their quantity (by 31.23%) ($P<0, 05$). This says about better butter-making properties of raw milk in Experimental Group III. In Experimental Group III, in butter sample, we observed significantly ($P<0.05$) decreased levels of zinc, lead, cadmium, nitrates, and nitrites. Butter obtained from milk in Experimental Group III, had sub-tolerant concentrations of zinc, lead, and cadmium.

CONCLUSION

To improve physico-chemical and working properties of milk under the conditions in the technogenic area with increased concentrations of heavy metals and nitrates, in diets of lactating cows it is reasonable to add chelaton adsorbent in an amount of 1.0 kg/t and santochinum antioxidant at dose of 0.5 kg/ton of feed-stuff.

With precedence of cows from Experimental Group III over their control counterparts by content of milk protein by 0.22%, their raw milk is not reasonable to be used in cheese-making, as in their composition they have significantly less casein ($P <0.05$) than in α- and β-fractions. Furthermore, the rennet clot got of milk from Experimental Group III was flabbier and less elastic.

It makes sense to process milk from cows of the Experimental Groups III to get butter because of higher butterfat content (by 0.22%), increasing the diameter of fat globules with simultaneous reduction in their number that says of better butter-making properties in their raw milk. Along with this, in Experimental Group III, in the butter sample, we observed significantly decreased concentrations of zinc ($P <0.05$), lead, cadmium, nitrates and nitrites. In butter, produced from milk of cows from Experimental Group III, concentrations of zinc, lead, and cadmium were sub-tolerant.

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