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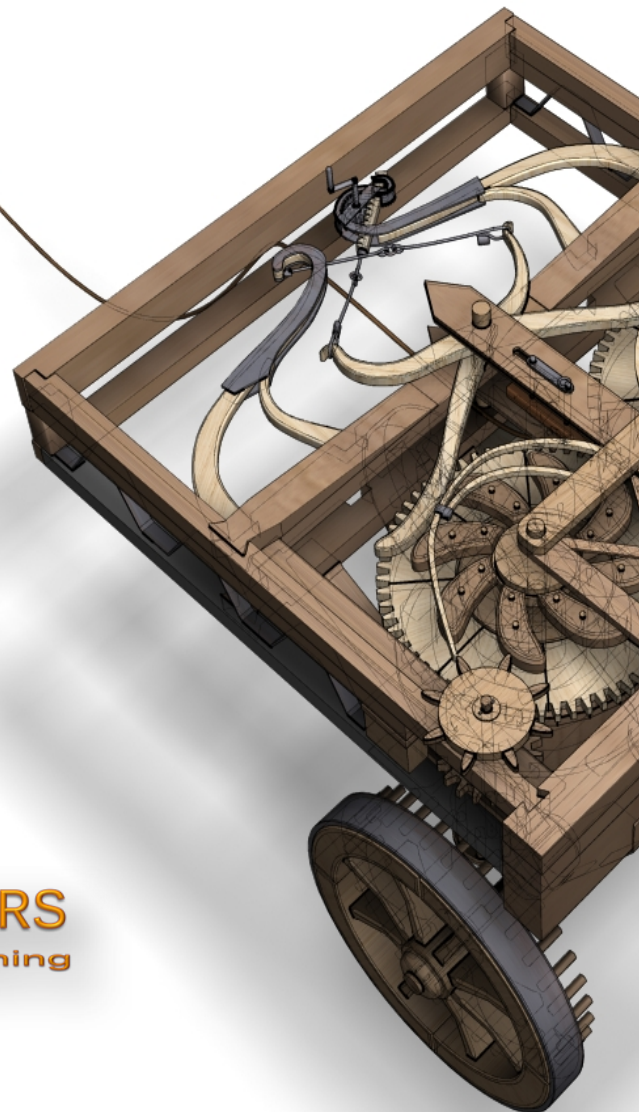
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Contents:

1	Comparison of Methodical Approaches to Environmental Risk Assessment Elena E. VOLKOVA, Evgeny A. KUZMIN	...497
2	Innovative Aspects of Development of the Waste Recycling Industry in the New Economic Context: Problems and Prospects Yuriy Vitaliyovich MOROZYUK, Antonina Vasilievna SHARKOVA, Irina Anatolievna Merkulina, Oksana Nikolaevna Vasilyeva	...507
3	The Formation of the Management System of Ecological, Social, and Economic Development of Rural Territories Using the Experience in European Union Oleg GORB, Ilona YASNOLOB, Dedukhno ALLA, Yulia KALIUZHNA	...516
4	The Effect of Chromium and Boron on the Lipid Peroxidation and Antioxidant Status (in Experiment) Marat IZTLEUOV, Zhanat UMIRZAKOVA, Erbolat IZTLEUOV, Sanimgul SAMBAEVA, Gulmira IZTLEUOVA, Dariga YESMUKHANOVA, Ainur AKHMETOVA, Raushan MEDEUOVA, Indira KOLISHBAEVA	...529
5	Environmentalization of Agriculture in Russia and the World Tatyana Mihailovna POLUSHKINA, Elena Georgievna KOVALENKO, Yulia Alekseevna AKIMOVA, Svetlana Andreevna KOCHETKOVA	...537
6	Investigating the Level of Environmental Awareness and Practices on Recycling of Solid Wastes at University's Campus in Malaysia Abdelnaser OMRAN, Muhammad BAH, Amir Hussin Baharuddin	...554
7	Method for Diminishing the Adverse Effect of Anthropogenic Heavy Metal Pollution on Poultry Meat Products Rustem B. TEMIRAEV, Mukhamed K. KOZHOKOV, Susanna K. CHERCHESOVA, Fatima F. KOKAEVA, Irina R. TLETSERUK	...567
8	Assessment for Quality of Life in Cities Taking into Account Ecological and Energy Factors Irina BELIK, Natalia STARODUBETS, Tamila ALIKBEROVA	...574
9	Empirical Study of Natural Adsorbents Efficiency in Animal Husbandry Under Anthropogenic Heavy Metal Pollution Marina G. KOKAEVA, Sergey I. KONONENKO, Eduard V. BESLANEEV, Gil'midin S. TUKFATULIN, Natalya V. LYASHENKO	...583
10	Factorial Structure of Community Intervention Strategies in Ecotourism A.VINODAN, James MANALEL, S. MEERA	...590

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11	Economic Research of Transfer of Technologies for Manufacturing High-Tech Production in Russia: Bio-Fuel Nikolai Ivanovich KUZNETSOV, Nadezhda Viktorovna UKOLOVA, Sergey Vladimirovich MONAKHOV, Juliya Anatolyevna SHIKHANOVA	...606
12	Role of Transnational Companies in Implementaion of the Import Phaseout Strategy in the Agroindustrial Sector Alexander M. ZOBOV, Ekaterina A. DEGTEREVA, Veronica Yu. CHERNOVA, Vasily S. STAROSTIN, Zhanna G. GOLODOVA	...612
13	Scenario Forecasting of the Reproduction Process in the Agriculture in View of Inflation Andrey N. BAYDAKOV, Lyudmila I. CHERNIKOVA, Anton V. NAZARENKO, Dmitry V. ZAPOROZHETS, Darya V. SIDOROVA	...620
14	Assessment of Willingness to Pay for Good Agricultural Practice Cabbage Kanokwan CHANCHAROENCHAI, Wuthiya SARAITHONG	...629
15	The Agricultural Production and Food Industry Development Trends in the Context of Food Security of Russia Inna BOLDYREVA, Olga ANDRYUSHCHENKO, Anastasia NIKITAEVA, Zoya UDALOVA, Janetta RUDASH	...642
16	Analysis of the Agro Risks of Import Substitution of the Food Production Alexander M. ZOBOV, Ekaterina A. DEGTEREVA, Veronica Yu. CHERNOVA, Vasily S. STAROSTIN, Zhanna G. GOLODOVA	...648
17	Factors Forming Transport-Communication Infrastructure of the Region: Russian Theory and Practice Viktor A. BLAGININ, Dmitriy A. KARKH, Elena V. KOLOTNINA	...657
18	Methodological Aspects of the Delimitation of Peripheral Areas: Approaches of Russian Scholars Elena B. DVORYADKINA, Catherine I. KAIBICHEVA, Julia S. BAUSOVA	...666
19	Technological and Economic Substantiation for Efficiency of Involvement in the Processing of Complex Reserves of Coal Deposits Liudmila I. SHULYATIEVA	...678
20	Distinctness and Endemicity of the Vegetative Cover of Altai Transboundary Mountain Region and its Conservation as a Part of the Strategy for Altai Sustainable Development Evgenia O. GARMS, Maria G. SUKHOVA, Olga V. ZHURAVLEVA, Andrey V. KARANIN, Alexander I. Minaev	...686

Call for Papers

Volume VIII - Fall Issues 2017

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Empirical Study of Natural Adsorbents Efficiency in Animal Husbandry under Anthropogenic Heavy Metal Pollution

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Abstract:

Increasing the quality of food products through the prudent use of natural adsorbents is becoming a topical problem in agriculture in conditions of anthropogenic heavy metal contamination. The purpose of the study is to work out a method for detoxifying heavy metals by using natural adsorbents such as local zeolite and vitamin C as an example of the implementation of standards and requirements for environmental food safety in animal husbandry in the territories with high anthropogenic pollution. Using the analogue method three groups of cows were formed with account of origin, age, body weight and lactation performance. To determine the heavy metal concentration, the spectrophotometric method was applied. It was experimentally established that in order to enhance the quality of dairy products in the territory with the man-made contamination, it is advisable to supplement the diets of lactating cows with zeolite and vitamin C (the recommended doses are 3% and 0.04% of the dry matter norm respectively). Due to the combined use of these natural adsorbents, zinc concentration in the cows' blood decreased 2.04 times, lead – 2.53 times and cadmium – 3.65 times. The cow milk increased in mass fraction of fat by 0.22% and protein – by 0.20%; at the same time zinc concentration lowered 3.47 times, lead – 2.76 times and cadmium – 3.09 times. At that, the content of these toxicants in dairy products was lower than the maximum allowable concentration (MAC).

Keywords: animal husbandry; anthropogenic pollution; heavy metals; natural adsorbents; detoxification

JEL Classification: O13; Q15; Q51

Introduction

Most large cities suffer from constant emissions of harmful substances into the atmosphere which exceed the maximum allowable concentration (MAC) in terms of many parameters. This factor causes the accumulation of heavy metals. In Russia, the Republic of North Ossetia – Alania (RNO – Alania) is one of such regions with high concentration of mining and processing enterprises. Large non-ferrous metal processing plants located in this territory are the main sources of anthropogenic environmental pollution with heavy metals. Over the last few years, the monitoring of heavy metals content in soil and fodder plants has indicated that lead content was 8–10 times higher than background levels (Kokaeva *et al.* 2015, Baeva and Tedtova 2009).

Given the high level of contamination of soils and fodder plants with various types of toxicants, including heavy metals, a number of technological methods are employed to reduce the toxicity of dairy products obtained from cows grown in the territory with the anthropogenic pollution. Environmental safety of milk in many aspects depends on feeding conditions for lactating cows. One of the ways of rational environmental management in the territory with the man-made pollution is to block migration of toxicants along the trophic chain: soil – plant – feed – animal – food products (Temiraev *et al.* 2009, Tedtova *et al.* 2009, Temiraev *et al.* 2011). The solution to this issue to a large extent determines food security in the region and commodity flows of food products (Kuzmin 2015, Kuzmin 2016).

Heavy metals are toxic due to the fact that they bind with proteins and form insoluble compounds altering properties and inactivating a number of vital enzymes. At the same time, these xenobiotics reduce the physicochemical and technological properties of dairy raw materials, making it difficult or impossible to prepare high-quality foodstuffs. Therefore, it seems urgent to raise the quality of dairy products and intensify metabolism in lactating cows through the rational use of natural adsorbents for the removal of heavy metals in industrialized territories in order to demonstrate the implementation of standards and requirements for environmental food safety in animal husbandry (Fyodorov and Kuzmin 2013, Temiraev *et al.* 2005).

1. Materials and methods

The experimental part of the work with cows of black-motley breed was carried out under the conditions of the agricultural-production cooperative “Vesna” in Digorskiy district of RNO - Alania. In the experiment, 30 cows were selected and divided into three groups of 10 cows each using the analogue-pair method with account of origin, age, body weight, previous lactation performance and milkfat percentage. The scheme of the scientific and production experiment is presented in Table 1.

Table 1. Scheme of the scientific and production experiment

Group	Number of cows in group	Basic diet (BD)	Dose of feed additives, % of the dry matter norm	
			Zeolite	Vitamin C
1-Control	10	BD	–	–
2-Experimental	10	BD	3.0	–
3-Experimental	10	BD	3.0	0.04

The cows in all three groups consumed diets balanced in accordance with the existing nutritional standards. However, the concentrations of zinc, lead and cadmium in these diets exceeded the maximum permissible level. The feeding regimen of the cows of the two experimental groups was supplemented with local zeolite (the Ursdon deposit, Digorskiy district of RNO – Alania) as a natural source of macro- and micronutrients and an adsorbent for the removal of heavy metal salts. Zeolite is also used as a medium for ion exchange in some biochemical transformations during the digestive process. Apart from zeolite, the diets of the 2-experimental group were supplemented with vitamin C, a natural adsorbent and antioxidant, in the form of the drug Ascorbic Acid.

Milk productivity of the cows used in the experiment was established by doing the control milking once a month. On the days of the control milking, the chemical composition of milk was determined according to conventional practices.

The content of heavy metals in the samples of feedstuffs, blood and milk was analysed using the atomic adsorption method with the help of the spectrophotometer AAZ-115-M1. The study of the digestive metabolism was implemented using conventional practices and based on the results of rumen contents analysis (Kurilov and Krotkova 1971) and morphological and biochemical blood parameters (Volgin *et al.* 2008) in the cows used in the experiment. All the figures collected during the experiment were subjected to Student's t-test analysis using Microsoft Excel.

2. Results and discussion

Special attention was paid to the content of heavy metals in animal feeds. At that, the concentration of toxicants in 1 kg of diet dry matter was an important indicator when analysing the cows' feeding regimens (Table 2).

Table 2. Concentration of toxicants in 1 kg of diet dry matter

Indicator	Unit	Diets	
		Summer	Winter
Zinc	mg	102.07	110.31
Lead	mg	28.99	32.50
Cadmium	mg	2.20	2.38

It was found that the total daily concentration of heavy metals in the dry matter of the winter diet was higher than that of the summer diet: zinc – by 8.07%, lead – by 12.11% and cadmium – by 8.18%. This is due to the locally produced coarse forage contained in the winter diet. At the same time, the concentration levels of these elements were elevated in both summer and winter diets of the experiment cows.

It is known that heavy metals can suppress the intensity of rumen metabolism, which is confirmed by the data of Table 3.

Table 3. Characteristics of rumen contents of the experiment cows

Indicator	Group n = 3		
	Control	1-experimental	2-experimental
pH	6.46±0.17	6.86±0.10	7.04±0.11
Ammonia, mmol/L	15.64±0.19	14.69±0.24	14.40±0.22
Ciliates, thousand/ml	358±5.3	428±4.5	442±4.8
Cellulolytic activity, %	15.18±0.44	18.48±0.41	18.56±0.37
Proteolytic activity, %	43.08±0.69	46.97±0.80	47.40±0.78
VFA, mmol/100 ml	11.11±0.15	12.47±0.11	12.65±0.12
of which, %:			
acetic acid	63.00±0.71	64.98±0.67	65.06±0.61
propionic acid	19.46±0.32	19.58±0.34	19.54±0.31
butyric acid	12.43±0.21	10.31±0.23	10.20±0.21
valeric acid	3.37±0.19	3.25±0.14	3.30±0.20
hexanoic acid	1.74±0.07	1.88±0.06	1.90±0.04

Due to the high concentration of macro- and microelements, zeolite when used in combination with vitamin C caused a shift in the pH of rumen contents towards neutral. At the same time, there was a significant ($P>0.95$) increase in the pH of rumen fluid by 8.24% in the 2-experimental group of cows if compared with the control analogues.

The combined use of zeolite and vitamin C led to a significant ($P>0.95$) increase in the number of ciliates of rumen contents by 84 thousand/ml in the 2-experimental group as compared to the control cows. Ciliates produce a large portion of cellulase, therefore, cellulase activity in rumen fluid of the cows of the 2-experimental group was significantly ($P>0.95$) higher by 3.38% than in the control animals.

The adsorption of heavy metals in the gastrointestinal tract intensified the synthesis of proteinases by forestomach microflora, so their activity in rumen fluid of the 2-experimental cows was significantly ($P>0.95$) higher by 4.32% than in the control group. Moreover, ammonia concentration in rumen fluid of the 2-experimental group was significantly ($P>0.95$) lower by 8.6% as compared to the control animals. This indicates that rumen microflora of the 2-experimental cows markedly better utilized ammonia for protein synthesis.

In contrast to the control analogues, the combined supplementation of zeolite and vitamin C to the diets of the 2-experimental cows resulted in significant ($P>0.95$) intensification of synthesis of volatile fatty acids (primarily acetic acid – by 2.06%) in rumen fluid and a simultaneous decrease in the level of butyric acid by 2.23% ($P>0.95$). In other words, this led to intensification of hydrolysis of animal feeds' cellulose and correction of metabolites' fermentation according to the desired direction, and this stimulated an increase in mass fraction of milk fat.

Hematologic parameters are the deciding factors in the assessment of level and direction of metabolism and physiological state of animals, therefore, we studied morphological blood composition of the experiment cows (Table 4).

Table 4. Morphological blood parameters

Group N = 3	Indicator		
	Erythrocyte count, 1012/L	Leukocyte count, 109 /L	Haemoglobin, g/L
Control	6.00±0.14	10.39±0.34	104.3±0.52
1-experimental	6.39±0.18	10.16±0.37	109.7±0.63
2-experimental	6.57±0.20	10.32±0.31	111.2±0.47

Zeolite and vitamin C admixed to the diets of the cows of the compared groups did not exert any considerable influence on leukocyte count in blood, since the cattle of the control group ($10.39 \times 10^9/L$) and the two experimental groups (10.16 - $10.32 \times 10^9/L$) did not demonstrate significant ($P<0.95$) differences.

The combined use of the tested drugs exerted a stimulating effect on the hematopoiesis in the cows of the 2-experimental group. They exhibited a significant ($P>0.95$) increase in red blood cell count by $0.47 \times 10^{12}/L$ and haemoglobin – by 6.9 g/L when compared to the control analogues.

Heavy metals can produce a depressing effect on redox reactions in the body of ruminant animals, and the rational use of natural adsorbents in the diets for excreting these toxicants allowed optimizing biochemical blood composition in the cows of the two experimental groups, as evidenced by the data presented in Table 5.

Table 5. Biochemical blood parameters

Indicator	Group n = 3		
	Control	1-experimental	2-experimental
Glucose, mmol/L	66.30±0.33	70.38±0.23	70.45±0.37
Total protein, g/L	74.20±0.30	80.38±0.35	81.47±0.21
Urea nitrogen, mmol/L	39.64±0.41	36.79±0.43	35.36±0.31
Ketone bodies, mmol/L	4.10±0.30	3.56±0.33	3.49±0.27
Calcium, mmol/L	10.82±0.28	11.66±0.21	11.72±0.31
Phosphorus, mmol/L	8.57±0.34	9.03±0.24	9.05±0.16
Zinc, mg/kg (MAC=22)	33.22±0.12	21.53±0.14	16.27±0.11
Lead, mg/kg (MAC=1.2)	1.85±0.02	0.96±0.01	0.73±0.01
Cadmium, mg/kg (MAC=0.05)	0.106±0.003	0.043±0.001	0.029±0.002

The combined intake of zeolite and vitamin C increased the amount of total protein and glucose in blood serum of the experiment animals, and this indicates that these natural adsorbents have a positive impact on metabolism of proteins and carbohydrates. Taking these drugs, the cows of the 2-experimental group experienced a significant ($P>0.95$) rise in the levels of total protein and glucose by 9.8% and 11.3% respectively.

Serum proteins serve as a source of building material for synthesis of proteins of various organs and tissues, as they are involved in nutrient transportation. Therefore, an increase in the concentration of total protein in blood of the 2-experimental group was accompanied by a significant ($P>0.95$) decrease in urea nitrogen and ketone

bodies in blood serum by 6.1% and 17.5% respectively. This signifies a deterioration in protein metabolism in the animals of the 2-experimental group fed diets with a high dose of distillers grains.

For mineral metabolism to be normal, lactating cows need high digestibility of calcium and phosphorus contained in feeds. It was found that due to the combined use of zeolite and vitamin C, the concentration of calcium and inorganic phosphorus in blood serum of the 2-experimental group increased significantly ($P>0.95$) by 8.3% and 5.6% respectively in comparison with the control animals.

Due to high detoxification properties of the natural adsorbents (zeolite and vitamin C), the concentration of zinc in blood serum of the 2-experimental group significantly ($P<0.05$) lowered 2.04 times, lead concentration – 2.53 times and cadmium – 3.65 times. At that, the levels of these elements in blood of the 2-experimental group did not exceed the maximum allowable concentration.

Detoxification of heavy metals through the prudent use of natural adsorbents in diets of lactating cows positively influenced their milk productivity (Table 6).

Table 6. Milk productivity of the experiment cows

Indicator	Group n = 10		
	Control	1-experimental	2-experimental
Average daily milk yield, kg	22.09±0.54	22.53±0.40	22.58±0.50
Milk containing, %:			
Fat	3.45±0.05	3.65±0.04	3.67±0.07
Protein	3.23±0.03	3.40±0.03	3.43±0.04
Yield of milk with 3.4%-standard fat content	22.41±0.30	24.18±0.21	24.37±0.26
Ratio to the control analogues, %	100.0	107.9	108.7

In terms of average daily yield of milk with natural fat content, the cows of the control group (22.09±0.54 kg) and the animals of the 1-experimental (22.53±0.40 kg) and the 2-experimental (22.58±0.50 kg) groups did not reveal any significant ($P<0.95$) differences. The combined intake of the natural adsorbents (zeolite and vitamin C) intensified cellulase production by rumen microflora, and this, in turn, accelerated cellulose hydrolysis by activating the fermentation of its metabolites with production of large amounts of acetic acid. This resulted in a significant ($P>0.95$) increase in milkfat by 0.22% in the cows from the 2-experimental group when compared to the control analogues.

Along with this, milk processing enterprises make purchases according to the yield of milk with standard fat content, which, in RNO – Alania, is equal to 3.4%. It was established that due to the combined use of the natural adsorbents (zeolite and vitamin C), the cows of the 2-experimental group showed a significant ($P>0.95$) increase in the yield of milk with standard fat content by 8.7% when compared to the animals of the control group. Disturbing the ecology of feeding brings about a marked negative effect on physical and chemical properties of lactating cows' milk (Table 7).

Table 7. Physical and chemical properties of milk

Indicator	Group n = 10		
	Control	1-experimental	2-experimental
Density, °A	28.17±0.11	28.77±0.13	28.84±0.14
Acidity, °T	17.84±0.22	17.77±0.22	17.89±0.19
Dry matter, %	12.28±0.08	12.78±0.05	12.82±0.10
Fat content, %	3.45±0.05	3.65±0.04	3.67±0.07
Protein content, %	3.23±0.03	3.40±0.03	3.43±0.04
Lactose, %	4.55±0.04	4.59±0.06	4.55±0.05
Ash, %	1.05±0.04	1.14±0.04	1.17±0.03
Vitamin C, mg/L	15.75±0.32	19.55±0.42	19.94±0.34
Vitamin A, mg/L	0.24±0.03	0.34±0.03	0.38±0.03

There were no significant ($P<0.95$) differences between the compared groups of cows with respect to milk acidity. At that, in terms of acidity, the samples of the experiment cows' milk were classed as 1st grade milk.

Due to the combined supplementation of zeolite and vitamin C to diets with a high content of heavy metals, the cows of the 2-experimental group demonstrated an improvement in a number of other physical and chemical parameters of milk. For example, in contrast to the control analogues, milk density of the 2-experimental animals increased significantly ($P>0.95$) by 0.67°A , dry matter content – by 0.54%, vitamin C – by 21.01% and vitamin A – by 36.84%.

Along with an increase in the percentage of milkfat, the joint use of the tested adsorbent drugs optimized proteinase secretion by rumen microflora. This contributed to a significant ($P>0.95$) improvement in milk protein content by 0.20% in the cows of the 2-experimental group in comparison with the control analogues.

The percentages of fat and protein in milk are of high importance when determining the purposes of milk production. Considering a significant ($P>0.95$) increase in mass fraction of fat by 0.22% and protein – by 0.20% in milk of the 2-experimental group against the control analogues, we carried out a comparative analysis of the diameter and number of fat globules, as well as composition and properties of milk protein in the cows selected for the experiment (Table 8).

Table 8. Diameter and number of fat globules, composition and properties of milk protein

Indicator	Group n = 10		
	Control	1-experimental	2-experimental
Diameter and number of fat globules			
Total fat, %	3.45 ± 0.05	3.65 ± 0.04	3.67 ± 0.07
Diameter of fat globules, μm	3.63 ± 0.03	3.58 ± 0.02	2.83 ± 0.02
Number of fat globules, billion/ cm^3	3.79 ± 0.09	3.86 ± 0.10	5.80 ± 0.09
Composition and properties of milk protein			
Total protein, %	3.23 ± 0.03	3.40 ± 0.03	3.43 ± 0.04
Casein, %	2.30 ± 0.02	2.49 ± 0.03	2.51 ± 0.02
Serum protein, %	0.93 ± 0.003	0.91 ± 0.004	0.92 ± 0.006
Casein composition, %: α -casein	32.51 ± 0.24	34.46 ± 0.20	34.63 ± 0.22
β -casein	52.15 ± 0.34	54.29 ± 0.41	54.51 ± 0.32
γ -casein	15.34 ± 0.14	11.25 ± 0.13	10.86 ± 0.17

It was established that the combined supplementation of zeolite and vitamin C to diets high in heavy metals exerted a negative impact on the diameter and number of fat globules. Milk of the 2-experimental group of cows, compared with the control animals, contained significantly ($P>0.95$) more fat globules by 53.0%, but their diameter reduced by 22.0% ($P>0.95$). It means that qualitative characteristics of milk fat of the 2-experimental cows indicate a deterioration in the properties of dairy raw materials for butter-making.

The adequacy of milk for cheese-making depends on composition and properties of its proteins. Milk of the 2-experimental group of cows was found to contain significantly ($P>0.95$) more casein by 0.21%. Only α - и β -casein coagulate under the influence of rennet enzyme, but γ -casein does not form part of casein micelles. Due to the combined use of the natural adsorbents (zeolite and vitamin C), casein in milk of the 2-experimental group of cows was significantly ($P>0.95$) richer in α - и β -fractions by 2.12% and 2.36% respectively compared with the control analogues, but at the same time it was lower in γ -casein – by 4.48% ($P>0.95$).

However, when studying consumer properties of dairy products, environmental safety of such products is as important as their traditional physical and chemical parameters. Environmental safety is based on the evaluation of the content of various toxicants in milk, including heavy metals (Table 9).

Table 9. Heavy metals content in milk of the experiment cows

Indicator	Group n = 10		
	Control	1-experimental	2-experimental
Zinc, mg/L (MAC=5.0 mg/L)	6.80 ± 0.14	2.61 ± 0.17	1.96 ± 0.12
Lead, mg/L (MAC=0.05 mg/L)	0.080 ± 0.003	0.045 ± 0.002	0.029 ± 0.004
Cadmium, mg/L (MAC=0.02 mg/L)	0.034 ± 0.001	0.019 ± 0.002	0.011 ± 0.002

The research results demonstrated that the natural adsorbents (zeolite and vitamin C), taken in combination, produced the most powerful detox effect in the body of the cows. The concentration of zinc in milk of the 2-experimental group significantly ($P < 0.05$) lowered 3.47 times, lead – 2.76 times and cadmium – 3.09 times when compared to the control animals. At that, the content of these heavy metals in dairy products of the 2-experimental group was also lower than the maximum allowable concentration.

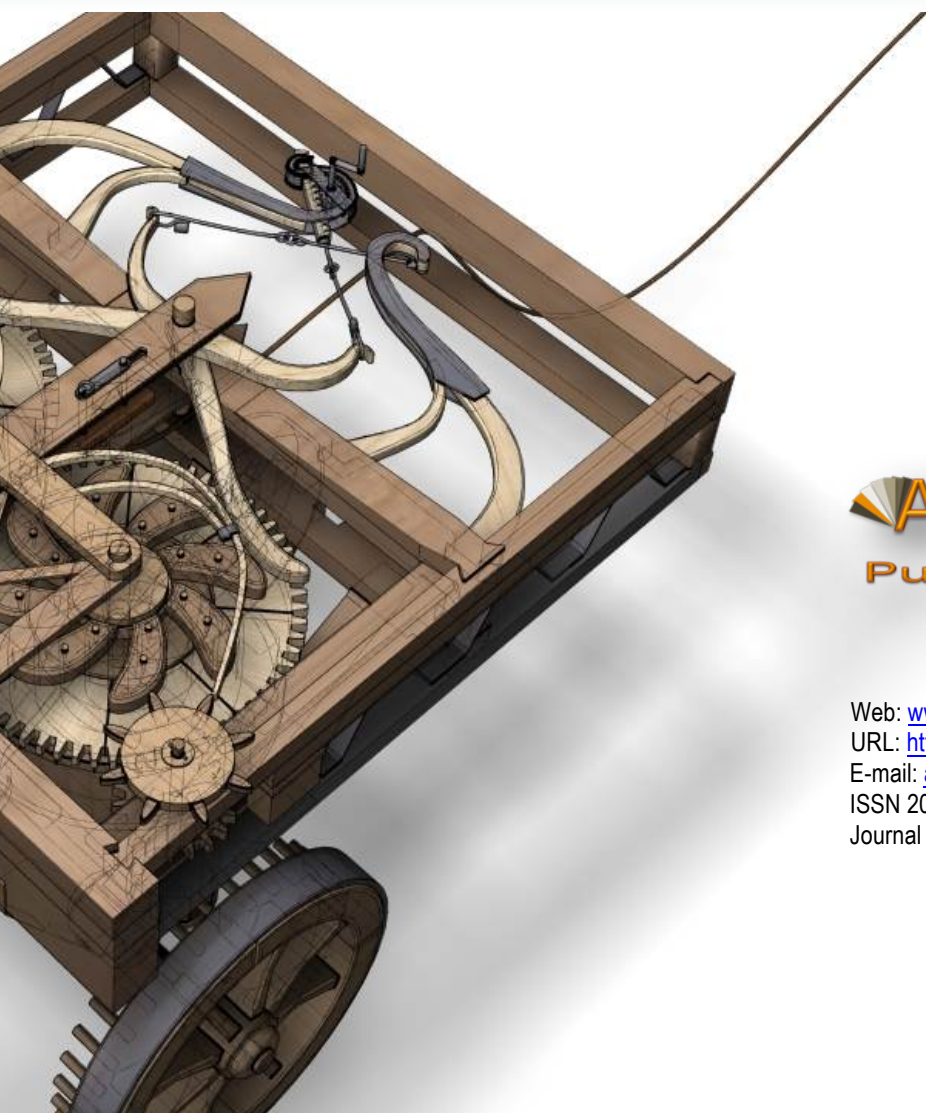
Conclusion

In the territories with high levels of anthropogenic contamination such as RNO – Alania, in order to enhance the quality of dairy products, improve sanitary-hygienic qualities of milk and intensify metabolism of lactating cows, it is advisable, during heavy metal detox, to supplement the diets with zeolite and vitamin C (the recommended doses are 3% and 0.04% of the dry matter norm respectively).

Zeolite and vitamin C, taken together, exerted the most pronounced detox effect in the body of the cows. The concentration of zinc in milk of the 2-experimental cows significantly ($P < 0.05$) lowered 3.47 times, lead – 2.76 times and cadmium – 3.09 times when compared to the control animals. At that, the content of these heavy metals in dairy products of the 2-experimental group of cows was also below the maximum permissible levels.

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