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Assessment of Heavy Metals in Milk Produced by Black-and-White Holstein Cows from Moscow

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Abstract

Milk must comply with quality standards for proper human nutrition. The purpose of this study was to conduct a comparative microbiological and elemental analysis of milk quality from Black-and-White Holstein cows. The studies were conducted on the basis of livestock farming in January-October 2019, Moscow region, Russian Federation. The experiment included 60 cows, divided into 3 groups of 20 animals of full age. All cows were kept under identical conditions. The first group consisted of purebred Black-and-White cows; the second group included Holstein half breeds; and the third group included the third generation of Holstein crossbreeds. 500 samples of milk were taken from animals of each group. The second group exceeded in content of zinc by 0.5 times ($p \leq 0.05$). The third group was recorded with lower manganese by 2 times ($p \leq 0.001$) while the first group by 0.5 times ($p \leq 0.05$) compering to the second group. Milk in groups 3 and 2 had a significant excess of iron and copper concentrations by 1.5-2.0 times (at $p \leq 0.001$), comparing to group 1. The milk in group 1 (purebred Black-and-White cows) exceeded TLV (threshold limit value) for lead (by 2.2 times) and cobalt (by 2.5 times). The milk in this group also had a higher cadmium content compering to the other two groups (by 1.3–2.7 times). Holstein crossbreeding helps to increase the concentration of cobalt, zinc and manganese. Lead and cadmium accumulate in the milk of purebred Black-and-White cows.

Introduction

Milk and dairy products (cottage cheese, cheese, butter, etc.) – are one of the most important products in the human diet.[1] The role of milk in human life is difficult to overestimate; milk is especially critical for infants and young children.[2] The composition of milk includes all the nutritional components and vitamins necessary for humans. Milk is one of the

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products used in dietetics and therapy. Milk contains about 250 different nutrients, which include vital and easily digestible proteins, milk fats, carbohydrates, minerals, and vitamins.[3,4] The daily consumption of milk (1 L) satisfies a person's daily energy requirements per one-quarter, completely – in fats and phosphorus, 20% – in iron, two-thirds – in proteins, above normal (150%) – in calcium.[5,6] Moreover, human's needs for vitamins are also met – one third for vitamins A and C, and 5% for vitamin D.[7] Humans require about 300-400 kg of milk and dairy products, annually.[8]

Therefore, dairy products must comply with quality standards for proper nutrition. Milk obtained from infected animals (cows, goats, etc.) can often contain dangerous pathogens.[9] The violation of sanitary rules is another way for milk to become contaminated with bacteria during or after milking.[10] The consequence of infecting for human body can range widely, from mild to severe food poisoning.[11] Veterinary services are involved in monitoring the condition and quality of milk. The respective services should monitor the acidity, fat content and mechanical contamination of milk.

Another important area in the quality control of dairy products is researches related to the entry of heavy metals into the human body along with products. Recent studies have shown that about 70% of toxicants enter the human body with food intake.[14] About 10% of dairy products, in Russia and neighboring countries, contain salts of heavy metal.[15] Thus, by heavy metals are meant chemical elements with a molecular weight of over 40 units. Heavy metals are characterized by high toxicity and pronounced biological activity.[16] The increase of biological activity has severe consequences for the human body: metabolic disorders, as well as CNS disorders. Heavy metals also have carcinogenic and mutagenic effects, namely an increase in lipid peroxidation, disorders of calcium metabolism, a decrease in mitochondrial respiration, and the incorporation of metallic ions into the cell membrane. Led, mercury, cadmium, manganese, cobalt, zinc and copper ere the most commonly used heavy metals.[17]

There are no data that determine the effect of Holstein crossbreeding on the content of toxicants in dairy products. Thus, the considering topic is relevant.

The aim of this study is to compere a qualitative and quantitative indicators of milk samples from Black-and-White cows and their Holstein crossbreed, namely for the content of metals with an assessment of their possible correlations.

Materials and Methods
Study Design
The studies were conducted in January-October 2019 in the Moscow region (Russian Federation). The study base is a livestock farm specializing in cattle breeding, and the production of meat, dairy and grain products. These two areas determined the range of products sold by the farm – grain, sugar beets, meat and milk. High yields are associated primarily with the intensive way of farming that implies the great introduction of mineral and organic fertilizers into the soil, as well as pesticides. Thus, a high amount of heavy metals enters to the soil along with these compounds.

The sample included 60 cows, divided into 3 groups. Each animal corresponded to full age. 500 samples of milk were taken from animals of each group. The samples were taken at Lenin' crop production cooperative, Moscow region, Lukhovitsky district, Didinovo village. Groups formed on the basis of analogues. The first group consisted of purebred Black-and-White cows; the second group included Holstein half breeds; and the third group included the third generation Holstein crossbreeds. There were 20 animals in each group. Animals from all groups were kept under identical conditions, receiving the same food.

Analysis of Heavy Metals in Milk
Atomic absorption spectroscopy (AAS) was carried out on the same samples in order to determine the content of heavy metals in the Laboratory of Toxicology of the Russian Academy of Sciences.[3,5] The TLV of heavy metals for milk and dairy products was evaluated in accordance with "The maximum

acceptable limit of heavy metals and arsenic in raw materials and food products (approved by the Chief State sanitary doctor of the USSR March 31, 1986 N 4089-86) ",[21] as well as Singh articles.[3,5] To determine the concentration level of heavy metals, the following documents were used with the methods: for cobalt determine - GOST 30178-96, copper, zinc, lead and cadmium - GOST 30178-96, manganese - GOST 27997-88, iron - GOST 27997-88.

Statistical Analysis

Statistical data processing was performed with Past v. 3.0. The date, in the tables on toxicological tests, are given as the arithmetic mean and the error of the mean, with a significance level of p≤0.05.

Microbiological parameters are given as quantitative data in the corresponding tables. Student's t-test was used to evaluate differences between samples.

Results

The content of heavy metals in milk did not exceed the established standards, as presented in Table 1. Subsequently, milk is suitable for consumption.[3] None of the samples in all three groups contained mercury, which refers to heavy metals of the 1st hazard class.

There were found differences in the content of heavy metals between the three groups.

Table 1: Concentrations of heavy metals (mg/kg) in the milk of cows from three groups

Group	Cadmium	Lead	Cobalt	Zinc	Copper	Iron	Manganese
The TLV (threshold limit value) (mg/kg) of heavy metals in milk	0.02 – 0.03	0.05 – 0.1[a]	0.001 – 0.008	5.0	1.0	0.138 – 0.700	0.02 – 0.09
1 (purebred Black-and-White cows)	0.011± 0.002[b]	0.110± 0.017[a, b]	0.019 ± 0.002[a]	1.28± 0.18	0.13± 0.02[c]	0.55± 0.05[c]	0.051± 0.005[a]
2 (Holstein half breeds)	0.008± 0.001	0.075± 0.009	0.021± 0.002[a]	1.41± 0.16	0.11± 0.01	0.71± 0.07	0.062± 0.006[a]
3 (the third generation Holstein crossbreeds)	0.004± 0.001	0.080± 0.010	0.014± 0.001[a]	1.21± 0.11	0.21± 0.02[c]	0.82±0.08[c]	0.029± 0.003

Note: [a] - the content of heavy metals in the milk of the studied group is higher than the TLV (p ≤ 0.05), [b] - the differences are significant between the first and second, third groups (p ≤ 0.001), [c] - the differences are significant between the third and first groups (p ≤ 0.001).

An increase of heavy metals in milk was recorded in the following sequence: cadmium, cobalt – manganese – lead – copper – iron – zinc (min to max). The difference between zinc and cadmium can be two orders of magnitude (p≤0.001). The maximum concentration of cadmium and lead was recorded for the first group, namely 2 times higher comparing to the second and third groups (p≤0.001). The second group exceeded in content of zinc by 0.5 times (p≤0.05). The third group was recorded with lower manganese by 2 times (p≤0.001) while the first group by 0.5 times (p≤0.05) compering to

the second group. Milk in groups 3 and 2 had a significant excess of iron and copper by 1.5-2.0 times (at p≤0.001), comparing to group 1. Similar results were obtained by Singh *et al.,*[5]

Generally, a significant excess of cadmium, lead, and high cobalt concentrations were found in milk of the purebred cows from group 1. On the contrary, less toxic iron and zinc prevailed in milk of groups 2 and 3. High cobalt is also recorded in milk from group 2.

Discussion

The tendency to accumulate cadmium in the products of high-blooded Holstein cows (group 1) has been determined among three group. However, cadmium in the milk of the first group did not exceed the TLV level, therefore it's not toxic to humans. A higher concentration of cobalt and manganese was found in products of the second group compering to other groups. Meanwhile, the third group occupied an average position between the compared groups. Thus, the 'holsteinization' increases the milk productivity while maintaining its safety.

An increased content of lead was found in the milk of cows from the first group, exceeding the TLV standard (see Table 2). Thus, this product is harmful for human health. Exceeding the TLV for heavy metals in food is a threat to human health. Similarly, an unsafe concentration of cobalt was found in milk samples from the second group, and iron - in the milk of the second and third groups. The most toxic are lead and cobalt. An increased concentration of lead and cobalt in milk may be associated with the peculiarities of metabolic processes in the body of cows from the first and second groups, when some metals are excreted more slowly than others or get directly from the bloodstream into milk. This line of research needs further development in terms of biochemical metabolic processes and possible difference in parameters between various breeds of cows.

Heavy metals have a negative effect on human health. Some, for example, mercury and lead, can accumulate in the body and manifest only with time.[24] Lead primarily affects the nervous and cardiovascular systems, as well as the blood itself. Lead can block sulfhydryl groups in enzymes, *synthesizing porphyrins*. The effect of cobalt is also toxic. It can be manifested by dysfunction of the CNS, polycythemia, as well as disruption of the thyroid gland.[25]

Zinc is a strong mutagen since competitively interacts with other metals and enhances the negative effects of other pollutants.[26] Manganese effect has not been specifically studied yet, but its high concentrations can cause irreversible changes in gastrointestinal tract (due to shifts in the *cattle rumen microbiota*). It can also cause CNS disorders.[27] Highly toxic metals include copper, which effects *mitochondrial membrane permeability* by binding proteins and amino acids. Iron is able to influence the concentration of other metals in the body, the increase of cobalt, and the decrease (through excretion or binding) of copper, chromium, and calcium. Iron enhances inflammatory processes, its excess leads to dysfunction of the spleen, liver, and brain.[28]

All above mentioned elements were found in the milk of the cows examined, especially lead, iron, copper, cadmium. Based on the data obtained, full examination of milk after its production is required in order to avoid bacterial and heavy metals poisoning. Similar results were obtained by Singh *et al.,*3 that previously also found high cadmium in cow's milk in India. The same authors5 pointed out that the content of certain trace elements, mainly, manganese, calcium and phosphorus, may differ depending on the animal (buffalo, goats, and cows). According to our study results, the concentration of heavy metals in milk may also vary depending on the breed of the animal.

Mass mandatory veterinary inspection of animals and milking equipment must be involved in large farms in order for dairy products to meet the

Conclusions

The milk in group 1 (purebred Black-and-White cows) exceeded TLV for lead (by 2.2 times) and cobalt (by 2.5 times). The milk in this group had a higher cadmium content compering to the other two groups (1.3–2.7 times). 'Holsteinization' helps to increase the concentration of cobalt, zinc and manganese. Lead and cadmium accumulate in the milk of purebred Black-and-White cows.

Conflict of Interest
The authors declare no conflict of interest

References

1. Siivonen J., Taponen S., Hovinen M., Pastell M., Lensink B. J., Pyörälä S., Hänninen L. Impact of acute clinical mastitis on cow behaviour. *App Anim Behav Sci*. 2011; 132: 101-106.

2. Chouraqui J., Tavoularis G., Simeoni U., Ferry C., Turck D. Food, water, energy, and macronutrient intake of non-breastfed infants and young children (0–3 years). *Eur J Nutr.* 2020; 59: 67-80.

3. Singh M., Ranvir S., Sharma R., Gandhi K., Mann B. Assessment of contamination of milk and milk products with heavy metals. *Indian J Dairy Sci*. 2020; 72(6): 608-615.

4. Mainau E., Temple D., Manteca X. Welfare issues related to mastitis in dairy cows. *Farm Animal Welfare Education Center.* 2014; 10: 1-2.

5. Singh M., Sharma R., Ranvir S., Gandhi K., Mann B. Profiling and distribution of minerals content in cow, buffalo and goat milk. *Indian J Dairy Sci*. 2019; 72(5): 480-488.

6. Leitner G., Silanikove N., Jacobi S., Weisblit L., Bernstein S., Merin U. The influence of storage on the farm and in dairy silos on milk quality for cheese production. *Int Dairy J.* 2008; 18: 109-113.

7. Leitner G., Lavon Y., Matzrafi Z., Benun O., Bezman D., Merin U. Pricing of cow's milk in relation to bulk milk somatic cell count in the threshold range of 400×103 cells per milliliter. *Isr J Vet Med.* 2016; 71: 10-15.

8. Hogeveen H., Huijps K., Lam T. J. G. M. Economic aspects of mastitis: new developments. NZ Vet J. 2011; 59: 16-23.

9. Huijps K., Lam T. J., Hogeveen H. Costs of mastitis: facts and perception. *J Dairy Res.* 2008; 75: 113-120.

10. Losinger W. C. Economic impacts of reduced milk production associated with an increase in bulk-tank somatic cell count on US dairies. *J Am Vet Med Assoc.* 2005; 226: 1652-1658.

11. Leitner G., Krifucks O., Merin U., Lavi Y., Silanikove N. Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *Int Dairy J.* 2006; 16: 648-654.

12. Murphy S. C., Martin N. H., Barbano D. M., Wiedmann, M. Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *J Dairy Sci.* 2016; 99: 10128-10149.

13. Blum S. E., Heller E. D., Leitner G. Long term effects of Escherichia coli mastitis. *Vet J.* 2014; 201: 72-77.

14. Jesse E., Cropp B. Basic milk pricing concepts for dairy farmers. *Economic Res.* 2008; 180: 200.

15. Petrov N. I. Laboratory quality control of milk – to a new level. Zoo Industriya. 2001; 6: 16-18.

16. Katz G., Merin U., Bezman D., Lavie S., Lemberskiy-Kuzin L., Leitner G. Real-time evaluation of individual cow milk for higher cheese-milk quality with increased cheese yield. *J Dairy Sci.* 2016; 99: 4178-4187.

17. Merin U., Fleminger G., Komanovsky J., Silanikove N., Bernstein S., Leitner G. Subclinical udder infection with Streptococcus dysgalactiae impairs milk coagulation properties: the emerging role of proteose peptones. *Dairy Sci Technol.* 2008; 88: 407-419.

18. Gosstandart of Russia. GOST 10444.8-88. Food products. Method for determination of Bacillus cereus; 1996. http://docs.cntd.ru/document/1200021078. Accessed 15 June 2020.

19. Gosstandart of Russia. GOST P 52054-2003. Cow's milk raw. Specifications; 2004. http://docs.cntd.ru/document/gost-r-52054-2003. Accessed 15 June 2020.

20. Gosstandart of Russia. GOST 10444.2-94. Food products. Methods for detection and quantity determination of Staphylococcus aureus; 1996. http://docs.cntd.ru/document/1200021067. Accessed 15 June 2020.

21. Dokipedia. The maximum acceptable limit of heavy metals and arsenic in raw materials and food products (approved by the Chief State sanitary doctor of the USSR March 31, 1986 N 4089-86; 1986. https://dokipedia.ru/document/5182275. Accessed 15 June 2020.

22. Larsen L. B., Hinz K., Jørgensen A. L. W., Møller H. S., Wellnitz O., Bruckmaier R. M., Kelly A. L. Proteomic and peptidomic study of proteolysis in quarter milk after infusion with lipoteichoic acid from Staphylococcus aureus. *J Dairy Sci.* 2010; 93: 5613-5626.

23. Rovai M., Rusek N., Caja G., Saldo J., Leitner G. Effect of subclinical intrammamay infection on milk quality in dairy sheep: I. Fresh-soft cheese produced from milk of uninfected and infected glands and from their blends. *Small Rumin Res.* 2015; 125: 127-136.

24. Fleminger G., Heftsi R., Uzi M., Nissim S., Gabriel L. Chemical and structural characterization of bacterially-derived casein peptides that impair milk clotting. *Int Dairy J.* 2011; 21: 914-920.

25. Fleminger G., Ragones H., Merin U., Silanikove N., Leitner G. Low molecular mass peptides generated by hydrolysis of casein impair rennet coagulation of milk. *Int Dairy J.* 2013; 30: 74-78.

26. Abdelgawad A. R., Rovai M., Caja G., Leitner G., Castillo M. Evaluating coagulation properties of milk from dairy sheep with subclinical intramammary infection using near infrared light scatter. A preliminary study. *J Food Eng.* 2016; 168: 180-190.

27. Andreatta E., Fernandes A. M., dos Santos M. V., de Lima C. G., Mussarelli C., Marques M. C., de Oliveira C. A. F. Effects of milk somatic cell count on physical and chemical characteristics of mozzarella cheese. *Aus J Dairy Technol.* 2007; 62: 166-170.

28. Gemechu T., Beyene F., Eshetu M. Physical and chemical quality of raw cow's milk produced and marketed in Shashemene Town, Southern Ethiopia. *J Food Agric Sci.* 2012; 5(2): 7-13.

29. Kader A., Deb M., Aziz M. A., Sohag M. M. H., Rahman S. R. Evaluation of physico-chemical properties and microbiological quality of milk collected from different dairy farms in Sylhet, Bangladesh. *Food Sci Technol.* 2015; 3(3): 37-41.

30. Hossain M. B., Dev S. R. Physiochemical characteristics of various raw milk samples in a selected dairy plant of Bangladesh. *Int J Eng.* 2013; 1(3): 91-96.

31. Awan A., Naseer M., Iqbal A., Ali M., Iqbal R., Iqbal F. A study on chemical composition and detection of chemical adulteration in tetra pack milk samples commercially available in Multan. *Pak J Pharm Sci.* 2014; 27(1): 183-186.