

## Trace elements in chelates form use in cows feeding in conditions of copper, zinc and manganese lack in vegetable feeds of Forest-Steppe zone of Ukraine

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### ABSTRACT

This article highlights the net research results on trace element composition of feed during 2012-2015, the effect of trace elements in chelates form feeding that are scarce in feed, on the concentration of copper (Cu), zinc (Zn) and manganese (Mn) in the blood and milk of the cows of the Ukrainian black-and-white milk breed. The general tendency to decrease the content of Cu, Zn, Mn in corn silage, alfalfa haylage, alfalfa hay, used in feeding cows of the Forest-steppe zone of Ukraine was revealed. The deficit of micronutrients in the diet of the cow ration was established at the level of 31, 175 and 39 mg/cow/day, and in the period of lactation, on average 32, 462 and 323 mg/cow/day, respectively, for Cu, Zn and Mn. In the control group of cows, the deficit of trace elements in the diet was offset by feeding the premix with sulfuric acid salts of Cu, Zn and Mn in a dose that offset their lack in feed for 100 %. Experimental animals of groups I, II and III fed premixes with complexes of chelates of these trace elements (in terms of pure element), which compensated their deficiency in feed of the diet by 100, 50 and 25 %, respectively. As a result of experimental studies was found that the concentration of Cu, Zn and Mn in the blood of all cows was within the limits of the physiological norm and in the dry period was somewhat higher than in lactation period. Relatively lower content of Cu, Zn and Mn in the blood of cows of the III experimental group in the dry period, compared with I, was determined by 14.5 %, 25.7 %, 13.2 % ( $p < 0.05$ ), respectively. During the lactation, a significant difference was found ( $p < 0,05$ ) relative to the third group in the concentration of trace elements in the blood of cows. Our experiments showed that the trace elements content in the milk of all cows didn't exceed the maximum permissible standards. A significantly higher concentration of Zn in the milk of cows of groups I and II was established relative to III by 22.0 % and 20.1 % ( $p < 0.05$ ), respectively. In relation to

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the control group, the use of trace elements chelates in feeding cows doesn't significantly affect the concentration of trace elements in the blood and milk of cows. To prevent microelement deficiency symptoms occurrence in cows, it's necessary to continuously monitor their content in the diet of the diet, and correct it by chelating complexes of trace elements, using a dose twice the rate of feeding of sulfuric acid salts.

**Keywords:** chelates, cows, milk, trace elements, blood

## INTRODUCTION

The balance of ration feeding cows with nutrients in accordance with physiological needs is the basis for the disclosure of genetic potential and for increases milk production without health harming of cows. The territory of Ukraine covers several geochemical provinces, which essentially differ in quantitative characteristics of mineral composition of soil and vegetation.

Trace elements are essential substances that play an important role in animals body and are part of proteins, hormones, directly involved in biochemical processes, also trace elements regulate metabolic processes, etc. (Spears, 2003; El Ashry et al., 2012). In certain geochemical zones of Ukraine can be a certain deficit or excess of trace elements. For example, Kharkiv region belongs to the Forest-steppe zone of Ukraine, which is characterized by a deficiency of copper, zinc and manganese in the feed of the main ration of cattle (Bogdanov et al., 2009; Rudenko et al., 2012).

During more than twenty years of research (1986-2009) at the Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, employees of the Laboratory evaluation of the quality of feed and animal products conducted monitoring of nutritional value assessment, chemical, vitamin and mineral composition of plant fodder in various regions of Ukraine. An information database for the forages chemical composition was created for the organization of a reasonable feeding of farm animals (Bogdanov et al., 2009). In investigated fodder cultures, trace elements deficiency and high coefficients of variation in the content of these substances were established by years of researches. A tendency towards further decrease in the concentration of a number of essential mineral elements in fodder for the period from 1991 till 2009 was noted (Bogdanov et al., 2009). Therefore, there is a need for constant monitoring of forages mineral value to prevent microelementosis occurrence in cows.

The advantage of using organic forms of trace elements in feeding animals has been highlighted in many scientific works (Kinal et al., 2004; Spears et al., 2004; El Ashry et al., 2012). The trace elements in the chelating compound are more bioavailable in the body than inorganic salts. Therefore, as we assume, it's possible to reduce the dose of trace elements

feeding several times. This will contribute to a significant reduction in the removal of trace elements into the environment with manure (Bogorodenko, 2016b).

Since copper and zinc are positioning both essential and heavy toxic metals, it's relevant to determine their concentration in blood and milk of cows for feeding easily accessible trace elements forms. Therefore, the purpose of our research was to: investigate the microelement composition of feeds and establish their deficit in the diet of cows; determine the effect of feeding chelates of trace elements on the concentration of copper, zinc and manganese in blood and milk of cows.

#### MATERIAL AND METHODS

To study the micronutrient composition of feed in the period 2012-2015, 20 samples of corn silage, 16 – alfalfa haylings and 18 – hay alfalfa from farms located on the territory of the forest-steppe zone, including in the Kharkiv region, were selected. Cu, Zn and Mn content in the feeds was determined by standardized atomic absorption method at the AAS-30 ("Carl Zeiss", Germany) spectrometer in the Laboratory evaluation of the quality of feed and animal products of Institute of Animal Science NAAS of Ukraine.

Experimental researches were an integral part of the scientific and economic experiment conducted in 2013 at the State Enterprise Experimental Farm "Gontarivka" of the Institute of Animal Science of NAAS, Kharkiv region (Ukraine). In accordance with the principle of analogues, taking into account age (2-3 lactation), live weight (538-560 kg), 40 cows of Ukrainian black-and-white milk breed were selected for three months prior to calving, according to the estimated date of calving and planned milk yield of 5000 kg. Cows were divided into four groups of 10 animals in each - one control group and three experimental groups. The preparatory period lasted 30 days, the trial – during the last two months before calving and the first four months of lactation. During the preparatory and experimental periods, the experimental animals fed the same background diet that is typical for the Ukrainian forest-steppe: corn silage, perennial grassland hay, alfalfa hay and concentrated fodder, and in the period of lactation to the diet, in addition to the main feed, molasses of beet feed were added.

Norms of feeding cows during the dry period and during lactation were calculated according to modern detailed rules taking into account the chemical composition and nutritional value of feed (Bogdanov and Kandyba, 2012). During lactation, the diet was counted on a cow with an average live weight of 550 kg, with a daily milk yield of 20 kg 4 % fat and balanced on the basis of the main nutrients in accordance with the norms.

The feeding and drinking regimen, the maintenance conditions, microclimate parameters in all groups were the same.

Daily requirement for essential microelements of cows during lactation, average live weight of 550 kg, with daily milk yield 20 kg 4 % fat is 142 mg/head for Cu, 948 mg/head for Zn and 948 mg/head for Mn (Bogdanov and Kandyba, 2012).

Feeding cows of different groups differed only in the type and concentration of trace elements. Control cows fed 1 % premix containing sulfuric acid salts of copper, zinc and manganese in a concentration that covered the deficit of the trace elements in feed by 100 % (per pure element) which fully provided a physiological need of cows in these elements. Experimental groups of cows, in contrast to the control group, received a different amount of trace elements. Experimental cows of the first group fed the same amount of trace elements, as in the control, but in the form of a chelating complex. In experimental groups II and III, cows received premix with investigated trace elements in the form of chelates whose concentration covered the deficiency of Cu, Zn and Mn in feeds by 50 and 25 %, respectively. For the experimental premixes preparation chelate of glycine hydrate of the trademark EcoTrace® (Germany) were used in the form of powder with the concentration of trace elements, based on a pure element: Zn – 25 %, Cu – 23 %, and Mn – 20 %. Oat bran was used to fill the premixes. Premixes were included in concentrated feeds in the amount of 1 %.

We conducted a complete chemical analysis of feeds of the cows' main diet according to generally accepted methods. In addition, in the feeds, blood plasma and milk of cows, the content of trace elements copper, zinc and manganese was determined by the standardized atomic absorption method on the spectrophotometer AAS-30, according to the method which was described by Price (1976).

Blood for research was taken from the jugular vein from 5 cows from each group in a month (in the dry period) and four months later (during lactation) from the beginning of the feeding of experimental premixes. The average sample of milk for the study was taken twice a day for 60 days after calving individually from 5 cows from each group.

The results of the researches were processed by the methods of variation statistics based on the probability criterion by Student-Fisher using the Microsoft Office Excel software.

## RESULTS

In the period from 2012 to 2015 inclusive, we studied the microelement composition of feeds of the forest-steppe zone of Ukraine, which are more often used in cattle feeding. Twenty samples of corn silage,

16 alfalfa hay and 18 alfalfa hay were analysed. Average values of the microelement composition of feed are given in Table 1.

Table 1. Trace elements composition of the main feeds of the cows of the Forest-steppe zone of Ukraine during 2012-2015, M  $\pm$  SE (standard error)

Feed	Samples number, n	Index, mg/kg of dry matter		
		copper	zinc	manganese
Corn silage	20	4.04 $\pm$ 0.280	15.41 $\pm$ 0,816	30.39 $\pm$ 1.352
Alfalfa silage	16	6.55 $\pm$ 0.339	18.57 $\pm$ 1,039	53.06 $\pm$ 2.835
Alfalfa hay	18	5.09 $\pm$ 0.267	17.68 $\pm$ 0,645	25.49 $\pm$ 1.172

Having analysed the data of the table 1, the deficiency of essential trace elements in the examined feed samples was confirmed. However, it has been found that, compared with the reference data (Bogdanov et al., 2009) for 2005-2009, the concentration of zinc in the dry matter of silage of corn and alfalfa grew by 9.3 % and 9.7 %, respectively. Copper content in corn silage increased by 49.6 %, and in hay it remained at the level of 2005-2009. The concentration of manganese in investigated feed decreased by 2.1 % in corn silage and by 7.3 % in alfalfa, comparatively with the previous period of research.

It was determined that in the dry matter of alfalfa haylage the content of copper and manganese decreased by 17.6 % and 8.8 %, comparing to the average reference data for the Kharkiv region (Forest-Steppe Zone of Ukraine) (Rudenko et al., 2012). Zinc content in the studied feed for the period 2012-2015 was at the reference data level: 18.20  $\pm$  1.56 – 18.57  $\pm$  1.039 mg/kg of dry matter (Rudenko et al., 2012).

During the scientific and economic experiment in 2013 to determine the effectiveness of substituting sulfuric acid salts of trace elements in their chelate complexes, the deficiency of copper, zinc and manganese in the diet was established. The conducted chemical analysis of feed showed that the lack of essential micronutrients in the main diet of cows during the dry period was approximately 31, 175 and 39 mg/cow/day, and in the lactation period-on average 32, 462 and 323 mg/cow/day, respectively, for copper, zinc and manganese, and was compensated according to the experimental scheme.

The effect of mineral feeding of cows with chelates of copper, zinc and manganese on the concentration of trace elements studied in the blood of animals is given in Table 2.

After analysing the results of the research of the microelement composition of the blood of cows, presented in table 2, it was established that the concentration of copper, zinc and manganese in the blood of the experimental cows was within the limits of physiological oscillations and in the dry period it was somewhat higher than during the lactation period. As

we assume this is due to the increased needs of the body of animals in trace elements and nutrients for the synthesis of milk and additional excretion of them with milk.

Table 2. Content of trace elements in the blood of cows

Group of cows	Index, $\mu\text{g}/100\text{ ml}$		
	copper	zinc	manganese
A month after feeding cows in the dry period			
Control	102.6 $\pm$ 4.91 <sup>ab</sup>	123.3 $\pm$ 6.09 <sup>ab</sup>	19.5 $\pm$ 0.75 <sup>ab</sup>
I	115.6 $\pm$ 4.78 <sup>b</sup>	136.0 $\pm$ 4.31 <sup>b</sup>	21.2 $\pm$ 0.90 <sup>b</sup>
II	112.9 $\pm$ 5.30 <sup>ab</sup>	128.5 $\pm$ 7.85 <sup>ab</sup>	20.3 $\pm$ 0.70 <sup>ab</sup>
III	98.8 $\pm$ 4.64 <sup>a</sup>	101.1 $\pm$ 9.72 <sup>a</sup>	18.4 $\pm$ 0.60 <sup>a</sup>
Four months after feeding cows supplements during lactation			
Control	88.8 $\pm$ 4.61 <sup>ab</sup>	111.6 $\pm$ 4.04 <sup>ab</sup>	18.0 $\pm$ 0.68 <sup>ab</sup>
I	89.5 $\pm$ 4.37 <sup>ab</sup>	125.9 $\pm$ 5.47 <sup>b</sup>	19.0 $\pm$ 0.60 <sup>b</sup>
II	95.1 $\pm$ 4.44 <sup>b</sup>	115.2 $\pm$ 5.78 <sup>ab</sup>	17.9 $\pm$ 0.48 <sup>b</sup>
III	79.9 $\pm$ 2.12 <sup>a</sup>	102.5 $\pm$ 4.87 <sup>a</sup>	16.1 $\pm$ 0.58 <sup>a</sup>

a-b Mean values with different letters are significantly different ( $p < 0.05$ )

Relative to the control group, no reliable intergroup difference was found for the studied parameters in the blood of cows during the dry period. However, there is a significantly lower content of copper, zinc and manganese in the blood of cows of the III experimental group, compared with I, by 14.5 %, 25.7 %, 13.2 % ( $p < 0.05$ ), respectively. During the lactation period, four months after the application of the trace element supplemented with feeding cows, it was established that, in comparison with the analogues of the third group, the concentration of copper was significantly higher in blood of animals of the II group by 19.0 % ( $p < 0.05$ ). It was found that during this research period, the concentration of zinc in the blood of animals of the first group significantly differed from this indicator in the third group by 22.8 % and at the level of the trend was higher than in the control group, by 12.8 %. The lowest content of manganese was detected in the blood of cows of the third experimental group, the absolute value approaching the lower limit of the physiological norm. The difference in this indicator in the second group was significant in favour of the first and second groups by 18.0 % ( $p < 0.01$ ) and 11.2 % ( $p < 0.05$ ), respectively. In blood counterparts in the control group the figure was higher at trends by 11.8 % compared to the third group.

After four months of experimental premix feeding, on the sixtieth day of lactation, the content of trace elements in the milk of all cows was within the limits of physiological oscillations and didn't exceed the maximum permissible standards (Table 3). A significantly higher concentration of zinc in the milk of cows of the first and second experimental groups was observed for the group III by 22.0 % and 20.1 %, respectively ( $p < 0.05$ ).

Table 3. Concentration of trace elements in milk of cows on the sixtieth day of lactation, M±SE, n = 5

Group of cows	Index, mg/kg of natural substance		
	copper	zinc	manganese
Control	0.050 ± 0.0053 <sup>a</sup>	2.86 ± 0.147 <sup>ab</sup>	0.046 ± 0.0047 <sup>a</sup>
I research	0.058 ± 0.0062 <sup>a</sup>	3.10 ± 0.162 <sup>b</sup>	0.052 ± 0.0036 <sup>a</sup>
II research	0.047 ± 0.0053 <sup>a</sup>	3.05 ± 0.099 <sup>b</sup>	0.054 ± 0.0042 <sup>a</sup>
III research	0.040 ± 0.0050 <sup>a</sup>	2.54 ± 0.145 <sup>a</sup>	0.041 ± 0.0047 <sup>a</sup>

Regarding the control group, feeding cows with chelate complexes of trace elements contributed to an increase in the content of zinc in the milk of cows in I and II experimental groups, but no statistically significant difference was found.

Regarding the content of copper and manganese in milk of cows, no reliable intergroup difference was found for these parameters. However, at the level of tendency, the higher concentration of copper in milk of cows and experimental group, compared with III, was noted at 40.0 %. The content of manganese in milk of cows of groups I and II exceeded this trend in the third group by 26.8 % and 31.7 % respectively.

#### DISCUSSION

The chemical composition of animal feed of the Forest-Steppe Zone of Ukraine, which was analysed for more than 20 years (1986-2009), has undergone significant changes in a number of important indicators of microelement nutrition of farm animals (Bogdanov et al., 2009). Researches results of microelement composition of animal feed during 2012-2015 confirmed the general tendency to decrease the content of copper, zinc, manganese in corn silage, alfalfa silage, alfalfa hay, which are used in feeding cows of the Forest-steppe zone of Ukraine. Therefore, during the rations preparation, it's necessary to take into account the place of forage cultures growth and carry out their complete zootechnical and mineral analysis for establishing the optimal ratio of the main components of nutrition in accordance with the norms of feeding cows.

Previous studies found that the use of chelating trace elements in feeding cows didn't have any negative impact on animal health. However, it was found that compensation of micronutrient deficiency only by 25 % due to chelates in the III experimental group was insufficient to improve the digestibility of nutrients in the body of cows, their physiological state, and to increase the milk productivity of cows compared with the control group (Bogorodenko, 2016a, 2016b; Kulibaba, 2016; Kulibaba et al., 2017).

In the case of this study, cows of the third group, which were fed with the smallest amount of trace elements due to chelates, had the lowest

values of the content of trace elements in blood and milk, as compared with those of other groups, and in some cases the difference was reliable. The best results for the studied parameters were found in cows of groups I and II, which fed the chelates of trace elements in an amount that replenished their deficiency in the diet by 100 % and 50 %. The concentration of investigated trace elements in the blood and milk of cows of group II was at the same level, or was higher than the similar indices in the control group, but not reliable.

Our data on the slight increase in the concentration of trace elements in the blood, which was not statistically confirmed, in cows who received the chelates of Zn, Cu and Mn instead of their inorganic salts, confirm the results presented by Pechova et al. (2006), Kinal et al. (2007), Cortinhas et al. (2012). However, in the studies of Iwańska et al. (1999) and Strusińska et al. (2003). indicated that the application of trace elements in organic forms increased their bioavailability to rumen microorganisms and at the same time the possibility of better transfer of trace elements to the tissues, including blood. Other scientists also pointed out this effect of the application of various forms of zinc and copper (Spears, 1996; Olson et al., 1999; Huert et al., 2002).

Our studies results are consistent with Kinal S. et al (2005, 2007), which observed a positive effect of feeding cows the bioplexes and the chelates of Zn, Cu and Mn on the mineral management of cows in the first period of lactation. These authors reported a higher content of zinc and copper in milk of these cows in comparison with milk of cows from the control group receiving trace elements in sulphate forms. Only in some cases authors established a significant difference in the studied parameters.

#### CONCLUSIONS

Compensation for the deficiency of copper, zinc and manganese in the diet of cows due to different doses of chelate complexes, compared with their sulfuric acid salts, doesn't significantly affect the concentration of trace elements in milk and blood of animals. However, while feeding cows with chelates of trace elements in a dose, which only compensates for their lack of diet in the diet of 25 %, there are significantly lower indicators of the content of copper, zinc and manganese in the blood and milk of cows. To prevent the occurrence of symptoms of trace elements deficiency in cows, it's necessary to constantly monitor their content in the ration, and adjust it to chelate complexes of trace elements, using a dose twice the rate of feeding of sulfuric acid salts (per pure element). This dose is sufficient to improve the trace elements composition of blood and milk of cows, increase their milk yield (Kulibaba S.V., 2016), and optimally from an environmental point of view by reducing the excretion of heavy metals with animal manure (Bogorodenko S.V., 2016b).



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