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IMMUNOBIOLOGICAL REACTIVITY OF CALF BODY DURING THE COMPATIBLE APPLICATION OF NANOTECHNOLOGICAL CITRATES OF TRACE ELEMENTS IN THE RATION

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Background. Nanotechnology in animal husbandry is an important area of research and development, which includes environmental sustainability, human health and food security. Nanotechnology has the potential to solve many questions related to animal health, production, reproduction, good hygienic practices during rearing and maintaining of animals. Therefore, the research aimed to find out the biological effect of separate and combined feeding of nanotechnological citrates that show high physiological activity in doses much lower than their mineral salts.

Materials and Methods. The research was conducted on 15 heifers and bulls divided into three groups five days after birth: control (I) and experimental (II, III) ones, five animals in each. Calves of group I received 240 kg of natural milk with the addition of a mineral-concentrate supplement (MCS) and grass hay for 60 days. Calves of group II were fed similar feed to that of group I with the replacement of MCS with a nutritionally equivalent quantity of cereal grains and soybeans and the introduction of nanotechnological citrates of microelements (NTC ME) I, Se, Cr, Cu, Co, Mn, Zn, Fe into milk in the proportion of 10 %. Calves of group III were fed with a complex of feeds of group I, adding 10 % of NTC ME. Physiological and biochemical indicators of blood and wool of calves were studied on the 10th, 40th and 70th days of growing period.

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Results. Statistically significant changes in the immunobiological indicators of the blood of calves of group II were found with an increase in the concentration of circulating immune complexes, haptoglobin, and sialic acids in the blood on the 40th day and ceruloplasmin, sialic acids, hexoses bound to proteins and chromium on the 70th day. An increase in the detoxification function of the calves' liver was recorded, with an increase in the blood level of phenol sulfates by 11.2 % and phenol glucuronides by 18.8% during this period. The combined use of 10 % NTC microelements and MCS in feeding calves of group III led to a statistically significant increase in the blood content of circulating immune complexes, phenol sulfates, phenol glucuronides, and zinc on the 40th and 70th days compared to their level in animals of the control group. Body weight gain in calves of group II was higher by 7.7 %, and in group III – by 4.3 % compared to the control.

Conclusion. The addition of NTC ME in the amount of 10 % of the rationed food for these elements to the milk for calves of group II, with the exclusion of the mineral-concentrate supplement from the feeding scheme, led to the activation of the immunobiological reactivity of the organism. The combined use of 10 % of NTC ME and mineral-concentrate supplement in the diet of calves of group III resulted in a lesser immunobiological reactivity of the organism, and strengthened the detoxification ability of the body.

Keywords: blood, biochemical parameters, calves, nanotechnological citrates of trace elements

INTRODUCTION

The regulation of animal micronutrient nutrition is based on using mineral salts of biotic elements synthesized by chemical methods (Ibatullin & Zhukorskyi, 2016). However, the low level of assimilation, safety, and biological effectiveness do not meet the physiological needs of the body and environmental requirements (Antonyak & Vlizlo, 2013; Koleshuk *et al.*, 2020; Lesyk *et al.*, 2022). Therefore, active scientific research is conducted for new safe and ecological sources of obtaining and ways of using biologically active additives in animal husbandry based on biotechnological and nanotechnological synthesis (Borisevich *et al.*, 2010; Iskra, 2012).

Nanotechnology holds promise for animal health, veterinary medicine, and other areas of animal production (Kuzma, 2010). Nanotechnology is no longer a concept or theory of the new world, but has become a new enabling technology over the years, with enormous potential to revolutionise agriculture and livestock development over the world (Patil *et al.*, 2009). Nano-sized minerals, vitamins or supplements developed for food application in human beings can also be used for animal feed. Nano-sized additives have also been specifically developed for animal feed. Nano-sized liquid vitamin mixes are available for use in poultry and livestock feed (Manuja *et al.*, 2012). The Cu (II)-exchanged montmorillonite nanoparticles (MMT-Cu) have been used to investigate the effect on growth performance, digestive function and mucosal disaccharidase activities of weaned pigs (Manuja *et al.*, 2012). The addition of MMT-Cu to the pigs diet increased the average daily weight gain, feed efficiency, and digestibility as compared with those of the control and copper sulphate groups (Manuja *et al.*, 2012).

In Ukraine, an innovative method of obtaining organic compounds of microelements in nanotechnological citrates (NTC ME) has been developed (Kosinov & Kaplunenko, 2009; Kaplunenko & Kosinov, 2017; Stoika, 2017), which shows high biological activity in doses much lower than their mineral salts and is relevant in animal husbandry (Manuja *et al.*, 2012; Vlizlo *et al.*, 2018; Iskra *et al.*, 2017; Fedoruk *et al.*, 2021). However, the study of the physiological activity of a mixture of biotic elements in nanotechnological citrates on calves during the milk feeding period was performed for the first time. The research aimed to determine the physiological activity of separate and combined feeding of nanotechnological citrates and mineral salts of trace elements, which are standardized in the nutrition of calves during the milk feeding period.

MATERIALS AND METHODS

The research was conducted on bulls and heifers that were divided into three groups five days after birth; each group containing five animals. Calves of the first (control, I) group received 240 kg of natural milk in 60 days according to the drinking scheme and were fed with a mineral-concentrate supplement (MCS) and grass hay (Ibatullin & Zhukorskyi, 2016). Animals of group II received concentrates in the form of crushed grains of cereals and soybeans with milk containing NTC ME in the 10 % proportion (Kosinov & Kaplunenko, 2009; Kaplunenko & Kosinov, 2017) of their content in the MCS, mg/animal/day, instead of MCS during the growing periods: I – 0.25 -0.5; Se – 0.075–0.15; Cr – 0.1–0.2; Cu – 3.0–6.0; So – 0.30–0.45; Mn – 18.0–30.0; Fe – 23.0–40.0; Zn – 20.0–33.0. Calves of group III were fed fodder included in the diet of group I combined with administration of NTC ME with milk according to the scheme of group II. Equal amounts of NTC ME were added to one-time portions of milk in individual bowls. For the study, blood was taken from the jugular vein, and wool was cut from the dorsal area at the end of adaptation on the 10th, 40th, and 70th days of the experiment. The intensity of growth of calves was controlled by body weight indicators twice a month and average daily growth during the period of research, morbidity, and survival of animals. The content of circulating immune complexes, CIC (according to the description Hrynevych, 1981); ceruloplasmin, sialic acids, hexoses bound to proteins (according to the description Anasashvili, 1968); haptoglobin (according to the description Hariachkovskyi, 2005); free phenols, phenol sulfates, and phenol glucuronides (according to the description Palfii *et al.*, 1974) was determined in the blood. Chromium, copper, zinc, and manganese content in blood and wool was determined by dry ashing with photometry of ash dissolved in HCl on an atomic absorption spectrophotometer SF 115–PK Selmi, 1995 (according to the description Vlizlo *et al.*, 2012).

Experimental data from different groups were expressed as mean values (M) ± standard error (S.E.M.). Statistical comparison between groups (the control and experimental (I, II; I, III) and experimental (II, III) was calculated through ANOVA (followed by Scheffe post hoc test) using Microsoft Excel statistical package. Confidence level of P values ≤0.05 was statistically significant in all cases.

A permission to conduct the research was obtained from the Committee on Bioethics of the Institute of Animal Biology NAAS of Lviv (Protocol No. 131; of March 20, 2023). All international, national and institutional guidelines for the care and use of animals were followed (European convention, 1986).

RESULTS AND DISCUSSION

The biological influence includes the growth and development of the organism, the state of the detoxification capacity of the liver and the body (free and conjugated phenols), homeostasis, energy (hemoglobin, haptoglobin), mineral (ceruloplasmin, the content of manganese, copper, zinc in the blood and chromium in the blood and wool), carbohydrate-protein metabolism (hexoses bound to proteins, sialic acids), immunobiological reactivity (circulating immune complexes). The studied indicators are informative regarding the possible toxic effect of the applied nanotechnological compound of microelements, their influence on immunobiological reactivity, growth and development of the organism.

Studies of the immunobiological reactivity of the body based on the content of glycoproteins and CIC revealed an increase in ceruloplasmin in the blood of the calves of the experimental groups during the first month by 8.0 and 5.0 %, and in the second month by 10.2 (P<0.05) and 2.5 % (**Table 1**).

A similar trend towards a higher level of haptoglobin – 11.1 % (P <0.05), sialic acids (6.1 and 17.1 % (P <0.05), hexoses, bound with proteins (during the second month of life (P <0.05)), in the blood of calves of the experimental groups was noted. The level of CIC in the blood increased during the first month of administering NTC ME in calves of groups II and III (P <0.01) and during the second month – for group III (P <0.05).

Table 1. **Content of glycoproteins and circulating immune complexes in the blood of calves (M±m, n = 4)**

Indicators	Groups	Periods of the research		
		Preparatory	The experimental month of feeding	
			1	2
Ceruloplasmin, s.u.	I II III	163.3±5.04 162.3±6.77 159.3±6.49	297.8±9.24 321.5±11.44 313.0±7.23	302.8±9.42 334.0±8.88* 310.5±8.05
Haptoglobin, g/L	I II III	1.38±0.090 1.37±0.035 1.34±0.020	1.26±0.045 1.40±0.033* 1.32±0.029	1.41±0.033 1.56±0.059 1.50±0.089
Sialic acids, s.u.	I II III	340.0±11.24 340.3±9.06 332.4±6.33	150.5±7.24 172.3±5.23* 159.7±6.17	177.4±10.89 207.8±6.75* 191.5±3.66
Hexoses bound to proteins, g/L	I II III	3.31±0.08 3.20±0.14 3.30±0.09	2.24±0.041 2.40±0.057 2.30±0.052	2.07±0.046 2.43±0.151* 2.29±0.113
Circulating immune complexes 3.5 %, s.u.	I II III	126.0±0.02 128.0±0.88 122.0±0.88	120.0±0.88 129.7±0.35** 126.7±0.40**	135.0±0.88 135.0±0.96 139.0±0.62*

Note: statistically significant difference in groups II, III compared to group I (control): * – (P ≤0.05), **– (P≤0.01)

An increase in concentration of ceruloplasmin, haptoglobin, and sialic acids and hexoses bound to proteins in the blood of the calves of the experimental groups compared to the control indicates the activation of immunobiological reactivity of their body both during the period of separate feeding of NTC ME in group II and its combination with MCS in group III. Data from the literature indicate that the differences in the action of NTC ME compared to inorganic salts are due to their increased physiological activity and intensity of absorption in the alimentary canal (Borisevich *et al.*, 2010; Vlizlo *et al.*, 2018; Iskra & Antonyak, 2018). In particular, adding Cr, Se, Co, and Zn NTCs to cows' diet stimulated liver protection, the exchange of Ca, P, vitamin E, and the secretory function of the mammary gland (Khomyn *et al.*, 2015). Under the conditions of the complex application of Fe, Zn, Mn, Cu, and Co NTC in piglet feeding, the resistance of their organism and adaptive capacity increased (Borisevich *et al.*, 2010). Strengthening the detoxification function of the body with an increase in the conjugation of free phenols in the liver was also noted in other studies under the effects of NTCs of some trace elements in agricultural and laboratory animals (Vlizlo *et al.*, 2018; Iskra & Antonyak, 2018). This may be related to the catalytic activation of the enzyme link of hepatocytes (Gulich *et al.*, 2018; Fedoruk *et al.*, 2021; Lesyk *et al.*, 2022). An increase in the level of Cr in the blood combined with its decrease in the wool of calves in the second month of administering NTC ME may indicate the regulatory influence of the limiting mechanisms of the absorption of this element, which is 1–3 % of the entry into the alimentary canal (Iskra, 2012; Khomyn *et al.*, 2015; Vlizlo *et al.*, 2018). Thus, the combined use of 10 % of nanotechnological citrates of I, Se, Cr, Cu, Co, Mn, Zn, and Fe in the diet of calves of groups II and III of their normalized need and mineral-concentrate supplement fed to the control group led to the activation of the immunobiological reactivity of the organism, an increase in the intensity of their growth, average daily weight gains, and total body weight.

The concentration of Zn (P <0.05) increased in the blood of calves of experimental group III during the first experimental period. Concentrations of Cr and Cu increased in experimental group II (P <0.05) during the second experimental period (**Table 2**). However, the level of chromium in the wool of the calves of the experimental groups was lower during the second month (P <0.05).

Table 2. **The content of trace elements in the blood and wool of calves, mg/L (M±m, n = 4)**

Trace elements	Groups	Research periods					
		Preparatory		The experimental month of feeding			
				1		2	
		blood	wool	blood	wool	blood	wool
Cr	I	0.80±0.07	1.76±0.10	1.90±0.04	1.66±0.24	1.16±0.01	1.89±0.06
	II	0.80±0.05	1.69±0.14	1.95±0.03	2.51±0.31	1.67±0.01*	1.61±0.09*
	III	1.01±0.04	1.67±0.14	1.78±0.03**##	2.75±0.42	1.60±0.02	1.70±0.09*
Cu	I	0.56±0.04	0.43±0.02	0.50±0.05	0.39±0.06	0.43±0.06	0.27±0.06
	II	0.68±0.05	0.37±0.04	0.50±0.07	0.31±0.03	0.52±0.05*	0.24±0.04
	III	0.55±0.02	0.34±0.05	0.51±0.09	0.37±0.03	0.69±0.07	0.30±0.07
Zn	I	3.84±0.57	15.5±0.36	3.19±0.56	11.0±2.93	4.30±0.92	16.2±0.57
	II	3.82±0.36	16.0±0.32	3.68±0.25	13.6±2.61	4.42±0.70	17.2±1.08
	III	4.00±0.33	14.6±0.37	4.23±0.25*	12.7±2.38	4.78±0.92	17.5±0.63
Mn	I	0.65±0.02	0.31±0.08	0.44±0.08	0.37±0.02	0.47±0.06	0.52±0.07
	II	0.55±0.05	0.29±0.03	0.36±0.09	0.32±0.02	0.38±0.02	0.54±0.04
	III	0.66±0.01	0.36±0.04	0.43±0.04	0.42±0.02#	0.49±0.06	0.62±0.05

Note: statistically significant difference in groups II, III compared to group I (control): * – (P ≤0.05); statistically significant difference in group III compared to group II: # − (P ≤0.05), ## – (P ≤0.01)

In the first month, the detoxification ability of the liver was pronounced only in calves of group III, while in the second month it was noted in both experimental groups (II and III), which was confirmed statistically. The concentration of phenol sulfates and phenol glucuronides increased in the blood of calves of the II and III groups, which were given 10 % of NTC ME of their normative consumption in the form of mineral salts (**Table 3**). The concentration of free phenols in the blood of the calves of the experimental groups was not significantly higher – 5.2–4.5 % (1st month) and 6.9–9.5 % (2nd month) compared to the control.

Table 3. **Fractional composition of phenols in the blood of calves (M±m, n = 4)**

Indicator	Groups	Research periods		
		Preparatory	The experimental month of feeding	
			1	2
Free phenols, μmol/L	I II III	15.9 ± 1.31 17.0 ± 0.43 17.5 ± 0.87	15.5 ± 0.42 16.2 ± 1.75 16.3 ± 0.74	18.9 ± 1.02 20.2 ± 0.74 20.6 ± 1.04
Phenol sulfates, μmol/L	I II III	19.2 ± 1.03 20.9 ± 1.13 21.6 ± 1.30	22.1 ± 1.03 20.1 ± 0.85 26.9 ± 0.72**##	24.2 ± 0.62 26.9 ± 0.60* 28.5 ± 1.04*
Phenol glucuronides, μmol/L	I II III	41.4 ± 2.38 44.1 ± 1.96 44.1 ± 4.10	45.6 ± 2.30 51.3 ± 2.33 58.1 ± 2.86*	50.0 ± 0.55 59.4 ± 2.13** 56.1 ± 2.83

Note: statistically significant difference in groups II, III compared to group I (control): * – (P ≤0.05), **– (P ≤0.01); statistically significant difference in group III compared to group II: ## – (P ≤0.01)

Analysis of changes in body weight of calves shows insignificant differences between the control and experimental groups. The body weight of calves of groups II and III at the end of the experiment was higher by 3.7 and 2.3 %, respectively (**Table 4**).

Table 4. **The intensity of growth of calves aged 10–70 days (M±m, n = 5)**

Indicator	Groups		
	I	II	III
Body weight of animals in the preparatory period, kg	35.6±1.61	35.5±2.40	35.8±3.10
Body weight of animals at the end of the experiment, kg	68.2±2.20	70.7±1.45	69.8±3.47
Body weight gain during the period of the experiment, kg	32.6±1.43	35.1±2.02	34.0±2.29
Average daily weight gain, g	543.3	585.0	566.7

The total body weight gain in groups II and III during the research period exceeded that of the animals of the control group by 7.7 and 4.3 %, respectively. The average daily weight gain during 60 days of the experimental period was 543.3 g (group I), 585.0 g (group II), and 566.7 g (group III). Thus, feeding calves with milk containing 10 % NTC

ME with the addition of dry grain concentrate to the diet (group II) and under the conditions of combined use of NTC ME and inclusion of MCS (group III) showed a significantly pronounced biological effect and an increase in the intensity of growth of calves compared with control.

CONCLUSIONS

1. The addition of NTC of I, Se, Cr, Cu, Co, Mn, Zn, and Fe in the proportion of 10 % of the normalized need for these elements to the milk of calves of group II, with the exclusion of the mineral-concentrate supplement from the feeding scheme, led to the activation of the immunobiological reactivity and resistance of the organism.
2. The combined use of NTC ME and a mineral-concentrate supplement in the diet of calves of group III resulted in a less pronounced activation of the immunobiological reactivity of the organism and strengthened the detoxification ability of the body.
3. The activation of the immunobiological reactivity of the calf organism led to an increase in the intensity of their growth, average daily weight gains, and total body weight, which indicates a higher physiological activity of the separate use of nanotechnological citrates of trace elements in the diet in a dose of 10 % of the recommended norms.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors received no specific funding for this work and declare no conflict of interest.

Human Rights: The article does not contain any experiments with humans.

Animal Rights: All international, national and institutional guidelines for the care and use of animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [V.V.; F.R.]; methodology, [F.R.; K.I.; Kh.M.; Ts.M.; Kh.M.]; investigation, [F.R.; V.V.]; resources, [O.D.]; data curation, [F.R.; Ts.M.]; writing – original draft preparation; [F.R.; Ts.M.]; writing – review and editing, [F.R.; Ts.M.]; visualization, [Ts.M.] super-vision, [V.V.; F.R.]; project administration, [F.R.; K.I.; Ts.M.]; funding acquisition, [–].

All authors have read and agreed to the published version of the manuscript.

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ІМУНОБІОЛОГІЧНА РЕАКТИВНІСТЬ ОРГАНІЗМУ ТЕЛЯТ У РАЗІ КОМПЛЕКСНОГО ЗАСТОСУВАННЯ НАНОТЕХНОЛОГІЧНИХ ЦИТРАТІВ МІКРОЕЛЕМЕНТІВ У РАЦІОНІ

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Вступ. Нанотехнології у тваринництві є важливими сферами досліджень і розробок, включаючи екологічну стійкість, здоров’я людини та продовольчу безпеку. Нанотехнології мають потенціал вирішити багато таємниць, пов’язаних зі здоров’ям тварин, виробництвом, відтворенням, належною гігієнічною практикою під час вирощування й утримання тварин. Тому метою досліджень було з’ясувати біологічний ефект роздільного та комбінованого згодовування нанотехнологічних цитратів, які виявляють високу біологічну активність у дозах, значно менших за кількість їхніх мінеральних солей.

Матеріали та методи. Дослідження проводили на 15 телицях і бугайцях, сформованих через 3–5 діб після народження в контрольну (I) та дослідні (II, III) групи, по 5 тварин у кожній. Телята I групи протягом 60 діб отримували 240 кг натурального молока з додаванням мінерально-концентратної добавки (МКД) і трав'яного сіна. Телятам II групи згодовували комбікорм, подібний до I із заміною МКД рівноцінною за поживністю кількістю зерна злаків і сої з введенням у молоко нанотехнологічних цитратів мікроелементів (НТЦ МЕ) I, Se, Cr, Cu, Co, Mn, Zn, Fe у кількості 10 %. Телят III групи утримували за набором кормів I групи з додаванням 10 % НТЦ МЕ. Досліджували фізіолого-біохімічні показники крові та шерсті телят на 10-ту добу, на 40-ву і 70-ту доби вирощування.

Результати. Встановлено вірогідні зміни імунобіологічних показників організму телят Д-II групи за підвищення на 40-ву добу в крові концентрації циркулюючих імунних комплексів, гаптоглобіну, сіалових кислот, а на 70-ту добу – церулоплазміну, сіалових кислот, зв'язаних з білками гексоз і Хрому. Відмічено підвищення детоксикаційної функції організму телят, водночас у крові за цей період зріс вміст фенолсульфатів на 11,2 % і фенолглюкуронідів на 18,8 %. Комбіноване застосування 10 % НТЦ мікроелементів і МКД у годівлі телят III групи призводило до вірогідного підвищення вмісту в крові циркулюючих імунних комплексів, фенолсульфатів, фенолглюкуронідів і цинку на 40-ву і 70-ту добу, порівняно з їхнім рівнем у тварин контрольної групи. Приріст живої маси телят II групи був вищим на 7,7 %, а III групи – на 4,3 %, порівняно з контролем.

Висновки. Додавання до молока телят II групи НТЦ МЕ у кількості 10 % від нормованої потреби в цих елементах, за виключення зі схеми годівлі мінерально-концентратної добавки, призводило до активації імунобіологічної реактивності організму. Сумісне застосування 10 % НТЦ МЕ та мінерально-концентратної добавки в раціоні телят III групи характеризувалося послабленням імунобіологічної реактивності організму, однак зростанням детоксикаційної здатності.

Ключові слова: кров, біохімічні показники, телята, нанотехнологічні цитрати мікроелементів

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