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The effect of drugs "Enteronormin" and "Zeleris" on the antioxidant potential of young calves

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The article deals with study of the effect of the synbiotic drug "Enteronormin" in a complex with the trace elements iodine and selenium on the indicators of lipid peroxidation and the activity of antioxidant protection system in the body of calves and to the comparison of its action with the antibiotic "Zeleris". In the last decade, the study of the role of various essential trace elements in various aspects of the regulation of metabolic homeostasis and the state of the immune potential of young cattle remains relevant. In this concern, it is important to emphasize the role of iodine and selenium, as well, as biologically active compounds of new products, among which synbiotic drugs are becoming widespread. In view of this, the development of new effective immunotropic drugs, their comparative study with existing drugs and traditional antimicrobial drugs, such as antibiotics, is actual. Therefore, the purpose of the research, the results of which are presented in this article, was to conduct a comparative study of the effect of the antibiotic "Zeleris" and the complex use of the synbiotic drug "Enteronormin" together with iodine and selenium on the indicators characterizing the activity of lipid peroxidation processes and the level of antioxidant protection system in the body of young calves. As a result of the research, it was found that feeding calves the drug "Enteronormin" in combination with selenium and iodine leads to an increase in the activity of the enzyme glutathione peroxidase and the level of reduced glutathione in the erythrocytes of calves, which is noted at 50- and 60-day age. Such changes, in turn, logically lead to a decrease in the number of lipid peroxidation products in their body. In contrast, we did not detect similar effects from the use of the antibiotic "Zeleris" during the study. Thus, it is concluded that feeding calves with the drug "Enteronormin" together with iodine and selenium leads to an increase in the activity of the key enzyme of the antioxidant defense system — glutathione peroxidase and the level of reduced glutathione in the erythrocytes of the blood of calves. These changes, in turn, lead to a decrease in the number of lopid peroxidation products in their body. However, similar effects from the use of the antibiotic "Zeleris" were not detected. The data obtained may indicate a positive effect of the drug "Enteronormin" in combination with iodine and selenium on the activity of the antioxidant defense system in the body of calves.

Key words: calves, glutathione peroxidase, reduced glutathione, TBA-active products, hydroperoxides, iodine, selenium

Introduction

An important problem in the Western region of Ukraine remains the insufficient intake of dietary elements such as iodine and selenium into the human and animal body. Deficiency of trace elements in the animal body leads to the development of microelement diseases, which are endemic diseases, which, in turn, is associated with the insufficient content of active forms of trace elements in soils, water sources and plants [1, 2, 8, 9]. In order to ensure the proper level of iodine intake into the human and animal body, a large number of methods have been developed for enriching human food and animal feed with this trace element. However, the vast majority of these methods are based on the use of inorganic iodine compounds, which is not effective in terms of their absorption by the body, and the compounds themselves are unstable and have time to decompose to a large extent before they enter the body. Another problem is the balance of such drugs and supplements with the another important element, such as selenium, which significantly affects the absorption of Iodine in the body [13].

In this aspect, it is relevant to conduct research to determine the role of iodine and selenium and other trace elements and biologically active compounds in the form of new drugs and feed additives in the regulation of metabolism and the state of the immune potential of the body of farm animals, in particular young cattle. The experiments that we conducted in previous years confirmed the powerful modulating effect of selenium compounds on the functional potential of immunocompetent cells, as well as the associated activity of the antioxidant defense system of the body as a whole. In addition, it should be noted that the effect of selenium on immune function is primarily associated with its antioxidant properties [6, 9, 10]. Iodine is no less important in this regard, which prevents metabolic disorders in tissues and supports the body's protective reactions, accelerates the formation of new immunocompetent cells [3]. The class of so-called synbiotic drugs deserves special attention [7]. In our case, the basis of such a synbiotic preparation is a complex, which includes probiotics, in particular, lactic acid bacteria *Lactobacillus* spp. and *Enterococcus* spp., spore-forming bacteria *Bacillus subtilis* and prebiotics — water-soluble chitosan and microbiological chitons. It must be noted that such preparations are used in a wide range of domestic animals, including pond fish and bees. At the same time, it is extremely important to clarify the role of the components of the preparation in the regulation of immune function in animals. In view of this, the development of new effective immunotropic agents, their comparative study with existing methods for restoring the body's immune potential and traditional antimicrobial drugs, such as antibiotics, and a comprehensive study of their effect on the animal body are relevant. The above undoubtedly justifies the importance and relevance of studying another aspect of such an impact, namely, the study of the effects of a synbiotic preparation containing trace elements on the activity of the antioxidant defense system in the body of young cattle.

Thus we conducted a comparative study of the dynamics of the effect of the antibiotic "Zeleris" and the complex use of the synbiotic drug "Enteronormin" together with the trace elements iodine and selenium on indicators characterizing the activity of lipid peroxidation processes and the level of antioxidant protection in the body of young calves.

Materials and Methods

The study was conducted in a private farm (TF Dmytriv LLC "Barkom") on calves of the black-and-white dairy breed of 10-day age. It was formed three groups of calves-analogues weighing 40–50 kg: a control and two experimental groups of 15 animals each. Feeding and keeping of animals of the control and experimental groups met the existing requirements. Animals of the control group at the age of 10 days were injected intramuscularly with a 0.9% sodium chloride solution at a dose of 5 ml/animal. Calves of the first experimental group during the specified period were injected once with the antibacterial drug "Zeleris" at a dose of 1 ml/10 kg of body weight. The calves of the second experimental group were given the drug "Enteronormin" in the amount of 1 g/10 kg of body weight per day according to the following scheme: the first time the studied remedy was given with water at 10-day age, for six consecutive days, the next time the drug was given at 24-day age for two days. Before use, to activate the synbiotic drug, the required amount of "Enteronormin" was dissolved in the solution "Iodis concentrate + Se" (water containing biologically active iodine ions and selenium citrate) in a ratio of 1 to 5 and was exposed for 16 hours at room temperature. At the same time, calves of this group, starting from 10- to 65-day age, were given an aqueous solution of Iodine and Selenium, using the drug "Iodis-concentrate + Se" as a source of trace elements, at a dose of 25 mg I/1 t of water. At 10, 25, 50, and 60 days of age, blood was taken from the jugular vein from each group of calves before morning feeding for biochemical studies.

In blood plasma, the content of lipid hydroperoxides was determined in the reaction with ammonium thiocyanate after precipitation of proteins with a solution of trichloroacetic acid and extraction of lipids with ethanol. The content of TBA-active products in blood plasma was determined in the reaction of malondialdehyde with thiobarbituric acid. In blood erythrocytes, the activity of glutathione peroxidase was determined by the rate of glutathione oxidation in the presence of tert-butyl hydroperoxide. The level of reduced glutathione (GSH) was determined by the level of formation of thionitrophenyl anion as a result of the interaction

of SH-groups of glutathione with 5,5'-Dithiobis-2-nitrobenzoic acid. All of the above mentioned methods are described in the proper sections of the "Handbook of Laboratory Research Methods" [12]. The obtained digital results were processed by the method of variational statistics using the Student's criterion with the *Microsoft Excel* program.

Results and Discussion

As can be seen from the data presented in tables 1 and 2, we found significant changes in glutathione peroxidase activity, reduced glutathione content, and lipid peroxidation products under the action of the studied drugs.

In particular, as can be seen from the data presented in table 1, the decrease in the content of TBA-active products and lipid hydroperoxides in the blood of 50-day age calves of the second experimental group, which received the synbiotic preparation "Enteronomin" and the trace element complex of iodine and selenium, was observed. Thus, the content of TBA-active products in the blood of calves of the experimental group was 1.35 times lower, the content of lipid hydroperoxides, respectively, was 1.5 times lower, compared with calves of the control group (P≤0.001). At the same time, it was found that the use of the antibiotic "Zeleris" in 50-day-old calves led to a certain increase in the level of peroxidation products in their blood. In particular, the content of TBA-active products in the blood of 50-day-old calves of the first experimental group was 1.26 times higher, the content of lipid hydroperoxides was 1.42 times higher than in calves of the control group (P≤0.001). It is known that antibiotics have a certain toxic effect on the body of animals in which they are used, which is also accompanied by a pronounced prooxidant effect [4]. During the next 10 days of the experiment, this negative effect of the antibiotic was leveled. The explanation of such changes requires further research, but it can be assumed that this may be associated with the intensive postnatal development of the organism, which is accompanied by the active formation of the protective adaptive systems of the organism during this period.

A similar picture of a decrease in the content of lipid peroxidation products under the influence of a synbiotic preparation in a complex with trace elements was observed in calves of 60-day age, however, these changes were expressed to a somewhat lesser extent (P≤0.05). These data indicate that the use of a synbiotic preparation in a complex with microelements leads to a decrease in lipid peroxidation products in the blood of calves during the studied period. The following results allow us to reveal the reason for such a decrease in the intensity of the formation of peroxidation products. Unlike animals that were treated with the drug "Enteronormin", the use of the antibiotic "Zeleris" led to a certain increase in lipid peroxidation processes, in particular in 50-day-old calves.

Table 1. Content of TBA-active products and lipid hydroperoxides in the blood plasma of calves (M±m, n=5)

Animal groups	Indicators	
	TBA-active products, nmol/ml	Lipid hydroperoxides, U/ml
10-day-old calves		
Control	3.79±0.25	0.32±0.013
Experiment 1	3.80±0.21	0.30±0,017
Experiment 2	3.60±0.14	0.31±0.014
25-day-old calves		
Control	3.87±0.20	0.32±0.023
Experiment 1	3.83±0.15	0.31±0.012
Experiment 2	3.72±0.13	0.33±0.015
50-day-old calves		
Control	4.2±0.05	0.33±0.013
Experiment 1	5.3±0.10***	0.47±0.012***
Experiment 2	3.01±0.07***	0.21±0.007***
60-day-old calves		
Control	4.0±0.05	0.30±0.010
Experiment 1	3,9±0.06	0.33±0.015
Experiment 2	3.1±0.08***	0.27±0.005*

Table 2. Glutathione peroxidase activity and the level of reduced glutathione in erythrocytes of calves (M±m, n=5)

Animal groups	Indicators	
	Glutathione peroxidase, mmol GSH/min. mg protein	Reduced glutathione, μmol/g
10-day-old calves		
Control	30.45±0.22	0.46±0.02
Experiment 1	29.00±0.54	0.50±0.03
Experiment 2	32.00±0.20	0,53±0.02
25-day-old calves		
Control	30.61±0,20	0.49±0.005
Experiment 1	28.53±0,92	0.54±0.3
Experiment 2	30.23±0,29	0.58±0.03*
50-day-old calves		
Control	30.72±0.34	0.47±0.01
Experiment 1	29.41±0.61	0.42±0.02
Experiment 2	44.24±2.22***	0.79±0.02***
60-day-old calves		
Control	31.65±0.38	0.60±0.08
Experiment 1	30.66±0.38	0.52±0.01
Experiment 2	37.26±0.88***	0.73±0.02

Note. * – P≤0.05 the statistically significant differences in animals of this experimental group compared to animals of the control group; ** – P≤0.01 the same; *** – P≤0.001 the same

From the data presented in table 2, it can be seen that the activity of the enzyme glutathione peroxidase in the blood erythrocytes of calves of the second experimental group, which received "Enteronormin" with trace elements at 50-day age, was 1.44 times higher, and the level of reduced glutathione in blood plasma was 1.68 times higher (P≤0.001) than these indicators in the blood of calves of the control group.

At the next stage of the experiment, namely in calves of 60-day age, only a slight increase (P≤0.001) in the activity of glutathione peroxidase was observed in the second experimental group, which received the synbiotic preparation together with trace elements. It is noteworthy that the use of the antibiotic did not affect the activity of glutathione peroxidase at all stages of the study. We also noted a slight increase in the content of reduced glutathione in calves of 25-day age, which received the synbiotic preparation together with trace elements (P≤0.05). These data may to some extent indicate the positive effect of the drug "Enteronormin" together with iodine and selenium on the content of glutathione, which plays a certain role in the antioxidant defense system. In our opinion, the main antioxidant effect of the studied drug is primarily associated with the content of the trace element selenium in its composition, which is part of the active center of glutathione peroxidase, the key enzyme of the glutathione link of the antioxidant system [5]. Our study demonstrates a more pronounced positive effect on the balance of pro- and antioxidant processes when using the synbiotic drug in a complex with the trace elements iodine and selenium, compared to the effect of the antibiotic "Zeleris".

Summing up the obtained results, we can conclude that feeding calves the drug "Enteronormin" in combination with selenium and iodine leads to an increase in the activity of the key enzyme of the antioxidant defense system — glutathione peroxidase and the content of reduced glutathione in the erythrocytes of calves. Such changes, in turn, logically lead to a decrease in the number of LPO products in their body. In contrast, similar effects from the use of the antibiotic "Zeleris" were not detected during our study.

In connection with the results obtained, it would be advisable to establish the influence of individual components of the drug on the activity of antioxidant defense processes in the body of calves in further studies, namely to determine the role of individual microelements and the synbiotic component of the feed additive. A comprehensive study of the state of the antioxidant and immune systems of the body under these conditions is also considered promising.

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Вплив препаратів «Ентеронормін» та «Зелеріс» на антиоксидантний потенціал організму телят раннього віку

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Стаття присвячена вивченню впливу синбіотичного препарату «Ентеронормін» у комплексі з мікроелементами Йодом і Селеном на показники перекисного окиснення ліпідів та активність антиоксидантного захисту в організмі телят та порівнянню його дії з антибіотиком «Зелеріс». В останнє десятиріччя залишається актуальним дослідження ролі різних ессенціальних мікроелементів, зокрема Йоду і Селену, а також біологічно-активних сполук у складі нових засобів, широкого розповсюдження серед яких набувають синбіотичні препарати, у різних аспектах регуляції метаболічного гомеостазу та стану імунного потенціалу молодняка великої рогатої худоби. З огляду на це, актуальними є розробка нових ефективних імунотропних препаратів, порівняльне їх дослідження із вже наявними засобами та традиційними антимікробними препаратами — такими, як антибіотики. Тому метою досліджень, результати яких представлені у цій статті, було проведення порівняльного дослідження впливу антибіотика «Зелеріс» та комплексного застосування синбіотичного препарату «Ентеронормін» разом із Йодом та Селеном на показники, які характеризують активність процесів пероксидації ліпідів, та рівень антиоксидантного захисту в організмі телят раннього віку. В результаті проведених досліджень встановлено, що випоювання телятам препарату «Ентеронормін» у комплексі з Селеном та Йодом призводить до зростання активності ензиму глутатіонпероксидази та рівня відновленого глутатіону у еритроцитах телят, що спостерігали у 50- та 60-добовому віці. Такі зміни логічно призводять до зменшення кількості продуктів ПОЛ в їхньому організмі. На противагу цьому, подібних ефектів від застосування антибіотику «Зелеріс» у дослідженні нами не виявлено. Тож робимо висновок, що випоювання телятам препарату «Ентеронормін» разом з Йодом та Селеном призводить до зростання активності ключового ензиму системи антиоксидантного захисту — глутатіонпероксидази та рівня відновленого глутатіону в еритроцитах крові телят. Ці зміни, у свою чергу, ведуть до зменшення кількості продуктів пероксидації в їх організмі. Водночас аналогічних ефектів від застосування антибіотику «Зелеріс» не виявлено. Отримані дані можуть свідчити про позитивний ефект препарату «Ентеронормін» у комплексі з Йодом та Селеном на активність антиоксидантної системи захисту в організмі телят.

Ключові слова: телята, глутатіонпероксидаза, відновлений глутатіон, ТБК-активні продукти, гідропероксиди, Йод, Селен

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