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CERTAIN BIOCHEMICAL PARAMETERS AND ACTIVITY OF ANTIOXIDANT ENZYMES IN BULL BLOOD UNDER THE INFLUENCE OF CHELATE CHROMIUM COMPOUNDS

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The aim of the study was to measure certain biochemical parameters in bull blood serum and activity of antioxidant protection enzymes under feeding of 15-month-aged bull calves with Chromium (III) in Chromium methionine chelate form. Four time periods with 15 days each were applied. At the 1st (preparatory) period, animals received the basic ration (BR), while at the 2nd, 3rd and 4th periods animals received Chromium (III) chelate compounds (0.8 mg of pure metal per day for each animal), as a solution added to the morning watering. Blood was obtained from the jugular vein 2 hours after the morning feeding, at the start of the 1st period and at the end of the 2nd, 3rd and 4th periods, and certain biochemical parameters were measured in blood serum. It is shown that the introduction of Chromium (III) in its methionine chelate form during morning feeding increased the level of urea (P<0.05) and decreased the level of ammonia in blood at the 45th and 60th days. It also decreased (P<0.05) glucose concentration at the 45th day from the beginning of the experiment. At the same time, Chromium supplement did not affect the content of total blood protein and the activity of superoxide dismutase. Catalase activity increased (P<0.05) at 45th and 60th days, while the activity of the glutathione peroxidase decreased (P<0.05) at the 60th day of treatment. As Chromium methionine chelate caused an increase in antioxidant enzymes activity and in certain biochemical parameters of blood, we may conclude that adding organic Chromium to the feeding of bull calves is advisable.

Keywords: CATTLE, MINERALS, CHROMIUM, CHELATE, COMPOUNDS, BLOOD, SYSTEM OF ANTIOXIDANT DEFENSE

ОКРЕМІ БІОХІМІЧНІ ПОКАЗНИКИ ТА АКТИВНІСТЬ АНТИОКСИДАНТНИХ ЕНЗИМІВ У КРОВІ БУГАЙЦІВ ЗА ДІЇ ХЕЛАТНОЇ СПОЛУКИ ХРОМУ

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Метою дослідження було визначити деякі біохімічні показники сироватки крові бугайців та активність ензимів антиоксидантного захисту при згодовуванні 15-місячним бугайцям Хрому (ІІІ) у формі Хром метіонінового хелату. Було застосовано чотири часових періоди по 15 днів кожен. У першому (підготовчому) періоді тварини одержували основний раціон (ОР), а в другому, третьому та четвертому періодах тварини одержували хелатні сполуки Хрому (ІІІ) (0,8 мг чистого металу в день для кожної тварини) у вигляді розчину під час ранкового випоювання. Кров отримували з яремної вени за 2 години після ранкової годівлі, на початку 1-го періоду та в кінці 2-го, 3-го і 4-го періодів для визначення у сироватці крові окремих біохімічних показників. Було показано, що додавання Хрому (ІІІ) у формі метіонінового хелату під час ранкової годівлі призвело до зростання рівня сечовини (P<0,05) та зниження рівня аміаку у крові на 45-ту і 60-ту доби. Також знизилася (P<0,05) концентрація глюкози на 45-ту добу від початку досліду. Водночас, додавання Хрому не вплинуло на вміст загального білка крові та активність супероксиддисмутази. Активність каталази зростала (P<0,05) на 45-ту і 60-ту доби, в той час як активність глутатіонпероксидази знижувалася (P<0,05) на 60-ту добу експерименту. Таким чином, додаткове введення у раціон

бугайців Хрому у дозі 0,8 мг чистого металу в день у вигляді хромметіонінового хелату оптимізує вивчені нами біохімічні показники та активність антиоксидантних ферментів у крові та покращує продуктивні якості тварин. Враховуючи те, що при згодовуванні хромметіонінового хелату реєструвалась вища активність антиоксидантних ензимів та окремих біохімічних показників крові, можна зробити висновок про доцільність додаткового введення в раціон бугайців органічного Хрому.

Ключові слова: ВЕЛИКА РОГАТА ХУДОБА, МІКРОЕЛЕМЕНТИ, ХРОМ, ХЕЛАТНІ СПОЛУКИ, КРОВ, ОБМІН ПРОТЕЇНІВ, ОБМІН ВУГЛЕВОДІВ, СИСТЕМА АНТИОКСИДАНТНОГО ЗАХИСТУ

НЕКОТОРЫЕ БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ И АКТИВНОСТЬ АНТИОКСИДАНТНЫХ ФЕРМЕНТОВ В КРОВИ БЫЧКОВ ПОД ДЕЙСТВИЕМ ХЕЛАТНОГО СОЕДИНЕНИЯ ХРОМА

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Целью исследования было изучить некоторые биохимические показатели сыроватки крови и активность ферментов системы антиоксидантной защиты при скармливании бычкам 15-месячного возраста Хрома (III) в форме Хромметионинового хелата. Было использовано четыре временных периода по 15 суток каждый. В первый (подготовительный)период животные получали основной рацион (ОР), во второй, третий и четвёртый периоды животные получали хелатное соединение хрома (III) (0,8 мг чистого металла в день на животное), в виде раствора во время утреннего выпаивания животных. Кровь получали из яремной вены через 2 часа после утреннего кормления в начале 1-го периода исследований и в конце 2-го, 3-го и 4-го периодов. В сыроватке крови изучали некоторые биохимические показатели. Показано, что при дополнительном введении Хрома (III) в форме Хромметионинового хелата приводило к возрастанию уровня мочевины ($P<0,05$) и снижению уровня аммиака в крови на 45-е и 60-е сутки. Также снижалась ($P<0,05$) концентрація глюкозы на 45-е сутки от начала опыта. При этом добавка хрома не влияла на содержание общего белка в крови и активность супероксиддисмутазы. Активность каталазы повышалась ($P<0,05$) на 45-е и 60-е сутки, в то время как активность глутатионпероксидазы снижалась ($P<0,05$) на 60-е сутки опыта. Учитывая то, что при скармливании Хромметионинового хелата, регистрировалось повышение активности антиоксидантных ферментов и некоторых биохимических показателей крови, можно сделать заключение о целесообразности дополнительного введения в рацион бычков органического Хрома.

Ключевые слава: КРУПНЫЙ РОГАТЫЙ СКОТ, МИКРОЭЛЕМЕНТЫ, ХРОМ, ХЕЛАТНЫЕ ВЕЩЕСТВА, КРОВЬ, СИСТЕМА АНТИОКСИДАНТНОЙ ЗАЩИТЫ

To organize the livestock farming effectively means to provide the animals with appropriate conditions that will suite their biological features and help them to realize their genetic potential effectively [1]. One of the basic factors of rational and complete feeding of cattle are feed minerals in optimal amounts and ratios.

Microelements are involved in the regulation of basic physiological processes. They are present in hormones, enzymes, vitamins, and participate actively in the metabolic functions of animal organism [2].

Chrome plays an important role in animal metabolism. Cr^{3+} is known to be an essential element for normal carbohydrate, protein, and lipid metabolism in human and animals [3]. The presence of chrome in nuclear acids and its effects on replication and translation processes indicate its great physiological importance. Animals on chrome-deficient diet show decreased ability to

incorporate the amino acids, especially glycine, serine, methionine, into proteins. Chrome is also essential for maintaining the structural stability of the protein molecules and nucleic acids, as well as for normal growth and development of the fetus. There are many reports that chrome increases insulin activity and lowers the blood sugar [4].

It is important to notice that this element is often under-estimated while developing norms of farm animals feeding. There is no data on its content in diets, soil, water, vegetable feed and in animals of different biogeochemical provinces. Moreover, understudied remains the effect of different doses and compounds of chrome on some levels of animal metabolism, for causing maximum positive effect on the vital functions of animals and their productive qualities.

The need for Chromium cannot be satisfied with vegetables [5], as most of chrome is retained in the roots and only little of it is transported to the aboveground parts. Studies [4, 5] of chrome addition to the animal rations showed changes of insulin, sugar and lipid blood rates in dependence of the amount and form of added chrome, and the duration of its use. For chrome deficit compensation, different compounds are used, but their different biological availability affects the effectiveness of its use.

Among the new ingredients of feed additives, increasing attention is drawn to microelement chelates . The combination of inorganic constituents (metals) with amino acids creates entirely new chemical compounds, which mechanism of action, physical and chemical characteristics significantly differ from those traditionally used in feeding (chlorides, sulfates, oxides). From a purely chemical point of view, chelates consist of a metal atom and the corresponding ligands, amino acids. The spatial configuration of the chelate contains a metal ion (complexing agent) in the center of the molecule, connected with amino acids (ligands). Amino acid chelate molecule has a symmetrical structure, and thus amino acids are tightly and stably bound to the metal atom. Therefore, unlike the inorganic microelement forms, chelates are stable both in acidic and in alkaline environments and do not lose their solubility [6]. It is known that inorganic metal salts are able to form insoluble complexes in the rumen, reducing the digestion efficiency. Thereafter, concentration of these microelements is decreasing, because their soluble form turns into insoluble.

According to this, the aim of our work was to study the effects of chromium (III) chelate compounds, added to the diet of calves, on certain parameters of protein metabolism and antioxidant defense. The chelate, in chromium methionine form, was synthesized at the Technology of biologically active compounds, Pharmacy and Biotechnology Department, National University «Lviv Polytechnic».

Materials and methods

The study was conducted at the experimental farm "CHISHKY", Institute of animal biology NAAS. We studied three black-motley breed bull calves aged 15 months, with fistulas imposed on rumen. The periods method was used. All manipulations with animals were corresponding to the European Convention «On protection of vertebrate animals used for experimental and scientific purposes» from 03.18.1986, the EU Directive № 609 from 24.11.1986, and «General ethical principles of animal experimentation», adopted by the first National Congress on Bioethics in Kiev, 2001 (protocol № 40 dated 15.07.13 by the Commission of bioethical expertise of the Institute of animal biology NAAS). Experiment lasted for 60 days and consisted of four periods of 15 days each. In the first period (preparatory), animals received the basic diet (BD). In the second, third and fourth periods, animals received a solution of chromium (III) chelate compounds in dose 0.8 mg of pure metal per animal daily, during the morning watering. Blood was get from the jugular vein and studied biochemically. Blood sampling was performed 2 hours after the morning feeding at the start of the 1st experiment period and at the end of the 2nd, 3rd, and 4th periods.

In the blood samples, the following parameters were studied: protein concentration (by Lowry) [7], concentration of ammonia (by the micro-diffusion method using the ammonium displacement from its salts with concentrated alkali solution, followed by its absorption with acid titrant) [8], glucose concentration (by o-toluidine method: heating glucose with ortho-toluidine in acetic acid solution forms a compound in amount proportional to the glucose concentration) [8], urea concentration (by a color reaction with diacethimooxyme) [9], glutathion peroxidase (GPO) activity (EC 1.11.1.9) (by the speed of GSH oxidation before and after incubation with tertiary butyl hydroperoxide) [10], superoxide dismutase (SOD) activity (EC 1.15.1.1) (by the level of enzyme inhibition of nitroblue tetrazolium recovery process in the presence of NADP and fenazyne metasulfate) [11], catalase activity (EC 1.11.1.6) (by hydrogen peroxide decay rate [12]. The obtained data were processed statistically. To determine significant differences between mean values, Student's t test was used.

Results and discussion

Studying bull calves watered with chromium methionine solution (fig. 1), we revealed that their blood sugar at research stages 2–4 was somewhat lower as compared to one in the period 1, and significant difference ($P<0.05$) was noted only in the 3rd research period.

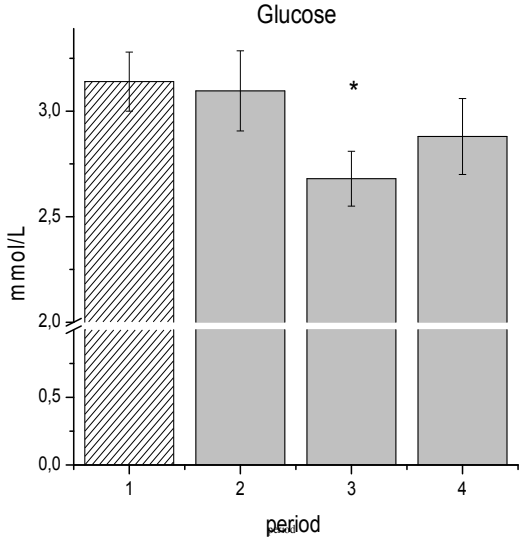

Fig. 1. The glucose blood rate in bull calves before and after chromium methionine administration

This can be caused by the inclusion of chromium methionine compounds into the diet. Chromium is known to affect glucose tolerance and carbohydrate metabolism. In particular, it enhances the activity of insulin that decreases glucose synthesis from propionic acid and other blood precursors and increases its metabolism in peripheral tissues by facilitated diffusion mechanism, i.e. the concentration gradient, with participation of protein carrier, thereby increasing its quantity [13, 14]. Protein synthesis plays the key role in the biochemical processes that underlie animal life. [15]. According to protein metabolism parameters (fig. 2), protein blood rate in periods 2-4 (when bull calves were exposed to chromium compound) had no significant differences ($P<0,05$) comparing with 1st period. This may indicate that chromium chelate has low effect on protein synthesis in liver.

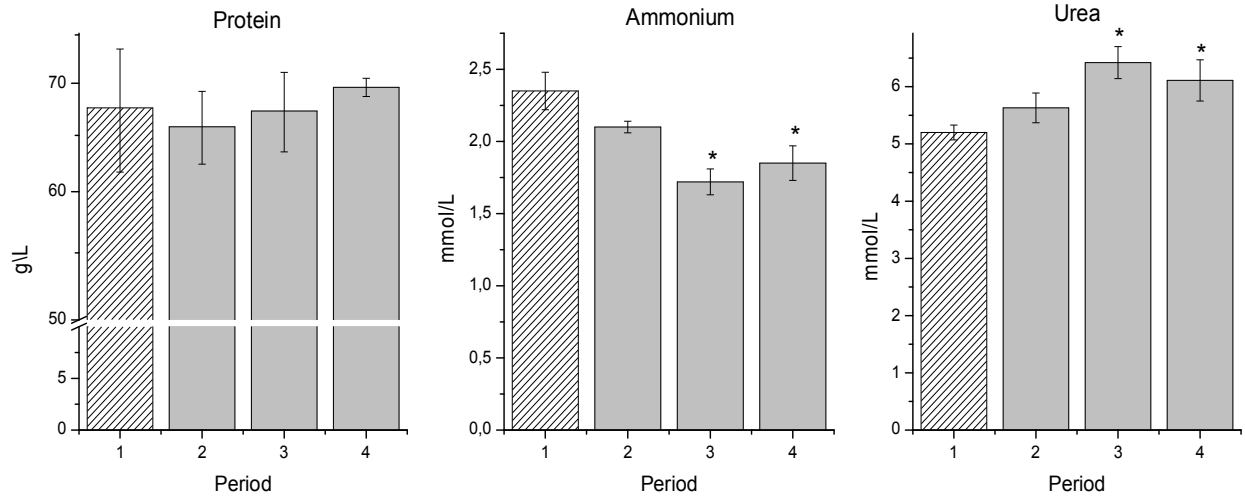

Fig. 2. Proteine, ammonium and urea content in bull blood before and after chromium methionine administration

As for ammonium, its presence in blood is known to be caused by amino acids deamination in liver and splitting of rumen content by bacteria. Analysis of the results showed a significantly lower ($P<0,05$) concentration of ammonia in the blood of calves, watered with chromium methionine, 26.8 % and 21.3 % in the 3rd and 4th research period respectively, comparing with 1st period. Urea concentration in the blood serum under chromium methionine action at all stages of the study was higher, as compared to one in the 1st period, and significant difference ($P<0,05$) was noted on days 45 and 60 from the start of the compound administration. These results suggest that, under the influence of chromium methionine, urea synthesis is enhanced by deactivation of ammonia, which is formed by the amino acids catabolism in tissues. Therefore, greater amount of nitrogen gets into rumen and is consumed by microorganisms.

Free radical oxidation processes (FRO) are underlying all cells metabolism and determine the organism's adaptive capacity to non-specific or damaging factors. A damage of physiological antioxidant protection system (APS) leads to free radicals and reactive metabolites formation, one of the main mechanisms that cause cell damage or death. We studied the action of chelated chromium form on some antioxidant protection enzymes performance level (fig. 3), in particular, we examined superoxide dismutase, catalase, and glutathione peroxidase activity. Thus, the activity of superoxide dismutase, as one of the key enzymes regulating peroxidation processes at the initiation stage, in the bull blood was slightly higher in 2nd period, indicating APS activation at the start of chromium compounds administration. In 3rd and 4th periods, superoxide dismutase activity decreased to the 1st period level, i.e., period prior to the introduction of chromium supplements. This may indicate a lack of excessive $O_2^{\bullet-}$ toxic radicals, and the organism eventually gets adaptated to the chromium methionine diet.

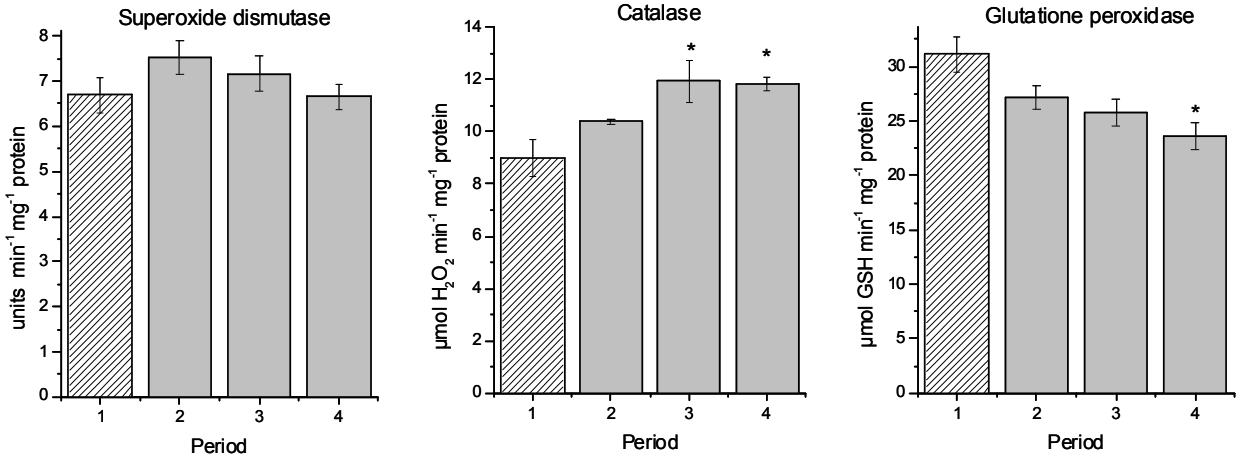

Fig. 3. Antioxidant protection system enzymes activity in bull blood before and after chromium methionine administration

More significant changes were observed in the activity of catalase — one of the most active APS enzymes. It prevents cell hydroxide and hydrogen excessive accumulation and is among the most long-active enzymes: one catalase molecule is capable to split 44,000 hydrogen peroxide molecules per second [16]. During the whole period of the experiment, 15.5–32.8 % increase in catalase activity was observed, although significant difference ($P<0,05$) was noted only at 45th day from the start of chromium methionine introduction.

Glutathione peroxidase is an important part of the enzymatic antioxidant defense system, its function is eliminating toxic hydroperoxide radicals via their reduction by glutathione. We observed a tendency of glutathione peroxidase activity decrease during the whole period of study. However, significant ($P <0.05$) decrease in the activity of this enzyme has been observed only on the 60th day from the beginning of the experiment. We can assume that the prooxidant action of chromium is done via hydroxyl radical production. Being an active form of oxygen, this radical enhances free radical oxidation, thereby causing a damaging effect and activating APS [17].

Conclusions

Adding Chromium (III) in chromium methionine form to the diet of bull calves causes blood urea increase and blood ammonium decrease at days 45 and 60, and blood sugar decrease at day 45 (from the start of the study). The chromium methionine effects on APS are shown by catalase activity increase at days 45 and 60, and glutathione peroxidase activity decrease at day 60.

Prospects for further research. To clarify the role of chromium in metabolic processes in cattle and appropriate requirements of it, it is advisable to study effects of other doses and forms of chromium on blood biochemical and metabolism parameters of the rumen contents of ruminants.

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