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THE INFLUENCE OF POLYPHENOL EXTRACT FROM NETTLE (*Urtica dioica*) ON THE ZINC CONCENTRATION IN THE BLOOD SERUM OF ROOSTERS

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Abstract

Zinc, being a trace element necessary for vital activity, is indispensable for the survival of living organisms. This element is of major importance in animal nutrition, but it also contributes to the metabolic activity of over 200 enzymes of living organisms, cell division and synthesis of DNA, proteins, tissue growth and development, immune system functioning, bone mineralization, blood coagulation, carrying out spermatogenesis, etc. Through its biological properties, it stimulates digestion, assimilation and has an important role in the activity of the reproductive organs and the metabolism of the digestive tract. It is found that zinc can exert a protective effect against testicular damage and plays an essential role in maintaining reproductive functions. Feed is the main source of zinc for animals, only a small part can be obtained from drinking water. Foods differ in their content of zinc. The daily ration is dependent on gender, age and the general state of the body`s health. Based on these considerations, this study was dedicated to the research of the quantitative changes of this element under the influence of polyphenols from nettle.

Key words: *food ration, microelements, metabolism, zinc.*

INTRODUCTION

Zinc is involved in numerous physiological and biochemical processes of living organisms. This element participates in the synthesis of protein and nucleic acids, being also involved in the process of transcription and transmission of the genetic message (Juravliova et al., 2007). By its involvement in various biochemical reactions and being part of various enzymes with antioxidant action, it participates with protective functions against reactions with the formation of free radicals and in maintaining the antioxidant status of the body (Panasenko et al., 2018). The optimal concentrations of zinc in reproductive material, organs and systems of the body can beneficially influence reproductive biotechnologies, such as artificial insemination, embryo transplantation, stimulation and synchronization, superovulation, transgenesis, sperm selection, cloning, obtaining chimera animals, etc., open up enormous possibilities in the field of animal breeding both from the point of view of increasing the effectiveness of breeding selection, as well as and increasing the body's reproductive capacities (Balan et al., 2022), possesses significant influence on apoptosis processes (Pang et al., 2013), apoptosis being as a fundamental biological phenomenon that is a special, genetically programmed form of physiological cell death and is a prerequisite for the normal development and reproduction of living biodiversity, including the functionality of the reproductive system, and implicitly also of the spermatogenesis process (Balan et al., 2021).

Zinc cations activated at the cell level are involved in catalytic and regulatory functions, being also part of the cell structure. The biological need for zinc is due to the fact that it contributes to the normal functioning of major systems and processes, which primarily manifest themselves by balancing the evolution of homeostasis in the processes of cellular absorption and its distribution according to its

involvement in cellular metabolism, implicitly also its excretion (Gammoh & Rink, 2017).
In connection with the lack of specialised zinc deposition and storage systems in living organisms, it is necessary that the body of animals and birds be supplied with a sufficient amount of zinc. Therefore, balancing the feed ration by including feed with an optimal zinc content in its composition will contribute to the physiological evolution and maintenance of zinc homeostasis (Mills, 2013). The major demands of the body on a daily basis are predetermined by a number of factors, including the age, sex and body weight of the animals and their maintenance and exploitation conditions (Jeong & Eide, 2013).
Zinc deficiency depends not only on the food ration, but also on a number of internal physiological and biochemical factors, which can condition its homeostasis due to the following causes: non-fermented vegetable ligands, such as phytate, some dietary fibers and helitic lignin that inhibit zinc absorption. Other factors influencing zinc assimilation are the ions of different metals with similar valence and the same oxidation state (Cu^{+2}, Cd^{+2}, Ni^{+2}, Co^{+2} and Fe^{+2}). The optimal concentration of zinc in the body is maintained through the sustained absorption of casein, including histidine and methionine. The highest intensity of absorption and metabolism is produced by zinc bound to polar amino acids (aspartate, cysteine and histidine), being important in the processes of enzymatic catalysis and metallothioneine (MT) (Reiber et al., 2017). In this vein, MTs are small proteins, rich in the amino acid cysteine, which play an essential role in the metabolism of zinc and in maintaining the homeostasis of metals in the body, in particular, they bind the ions of zinc and copper (Maret, 2000). Moreover, TMs have a high affinity for zinc, making it easier to capture and store in cells, which helps regulate the availability of zinc for various biological processes. One of the main functions of TM in the body is to transport metal ions by binding and releasing zinc in a controlled manner and maintaining an adequate balance of zinc in tissues and cells, preventing both deficiencies and poisoning, and contributing to the detoxification of the body by preventing the toxic accumulation of heavy metals essential and non-essential. For zinc the main carrier is considered albumuna binding around 80% of the total zinc content (Ruttkay-Nedecky et al., 2013). This detoxification occurs through mechanisms of bdisplacement of these metals from bioenergetic cell complexes, where their neutralization, destruction and evacuation from the body occurs (Pihteeva, 2009). At the same time, TMs have the ability to neutralize reactive oxygen species (ROS) and protect cells against oxidative stress, a process in which zinc plays an important role in enzymatic function and this process occurs as needed by the release of zinc by metallothioneins for its use in various enzymatic processes or for protein synthesis (Rostan et al., 2002). Zinc here has cofactor functions for enzymes such as superoxide dismutase (SOD), which neutralizes superoxide free radicals and helps reduce oxidative damage in cells, and by supporting the function of antioxidant enzymes, zinc helps prevent oxidation of lipids, proteins, and DNA (Abel & de Reuiter, 1989). Therefore, TMs are essential for the management and distribution of zinc in the body, ensuring the optimal functioning of biological systems dependent on this metal, as well as cellular protection against oxidative stress and metal toxins.
At the same time as protein compounds, a number of other chemical elements, vitamins, metals, acids, including folic acid, bioavailability and content correlate with the amount and intensity of zinc absorption in the body, also participate in the metabolism of zinc.
For optimal economic efficiency of the poultry breeding industry, it is necessary to maintain the physiological metabolism of the organism of the poultry breeding herd according to the variety of nutrition that provides for the elaboration of poultry feed rations, balanced also by zinc content (Petcu et al., 2023). This goal stems from the need to ensure an adequate intake of zinc in the daily diet of birds, which contributes to strengthening the body's natural antioxidant system, being an important element in the prevention and management of oxidative stress. In this context, the main goal of our research was to develop and test biologically active, inexpensive and ecological feed substances that regulate oxidative processes and enhance the reproductive properties of birds.

MATERIALS AND METHODS

The research was carried out on *birds Gallus gallus* by including 10 breeding males divided into two batches (control batch - LM and experimental batch - LE), 5 roosters, each kept in individual cages. The conditions of maintenance, feeding and watering of birds have been unified. Prior to the initiation of the experimental research, the pre-experimental period of preparation and adaptation of the animals to new maintenance conditions was carried out within 30 days. Later followed the experimental period, also within 30 days. Throughout the experiment, the animals were monitored by daily clinical examination, morning and evening, with evidence of the values of the physiological indices of the birds' body.

Roosters from LE were subjected to the influence of hydroalcoholic extract of polyphenols from nettle (*Urtica dioica*) with a total antioxidant activity of 33.2 mg gallic acid equivalent per 100 g, which was administered daily *per bone* at a dose of 1 ml/head. Blood samples were collected in compliance with veterinary sanitary requirements with a frequency of once every 15 days. The general blood analysis was performed in the Biochemistry Laboratory of the Institute of Physiology and Sanocreatology using established standard methods and techniques. In order to determine the influence of zinc on the antioxidant, enzymatic and protein status in the rooster blood serum, the investigations in the blood serum were carried out by standard methods within the Specialized Biochemical Laboratory of the University of Medicine and Pharmacy "Nicolae Testimițeanu". The data of the research results were statistically processed with the determination of the error threshold.

RESULTS AND DISCUSSIONS

Hematopoiesis is a complex process that underlies the daily formation of blood cells, which are the essential elements of the circulatory system. Zn^{2+} is an important micronutrient for animal health, playing a significant role in various biological and physiological functions and in this context, several studies mention its role in both immune system cells and hematopoietic cells, supporting enzymatic, structural and metabolic regulatory functions. A lack of zinc can lead to decreased activity of antioxidant enzymes, increasing the body's vulnerability to free radicals and cell damage.

The research included hematological investigations of breeding roosters on the influence of zinc on the functional status of blood and immunoreactive components. The results of the research are presented in Table 1.

Table 1. Influence of zinc on the functional status of immunoreactive components in rooster blood serum

Groups	Erythrocytes, 10^{12}/L	Hemoglobin, g/L	Hematocrit, %	Lymphocytes, 10^3/L	Monocyte, 10^3/L	Euzinophil, 10^3/L
Control	3.2±0.33	138.6±3.51	44.9±1.60	86.0±14.80	5.0±1.42	3.0±1.00
Experimental	3.6±0.32	151.0±2.07*	49.5±1.73	92.0±15.40	6.6±1.46	5.0±1.00

Note: * - the differences are statistically true between the experimental and control group ($P<0.05$).

The data presented in Table 1 established that there are changes in some blood components, which participate in different physiological and immunological processes, namely, erythrocytes play an important and decisive role in the health of birds, by transporting oxygen in the body and eliminating CO_2. These cells are represented with a value of 3.22±0.33 (10^{12}/L) in LM with a tendency to increase to 3.64±0.32 (10^{12}/L) in LE. This increase is produced, possibly by a favorable action of zinc on blood components, which in combination with the copper content influences the fermentation systems.

The results of the table established changes in the quantitative content of hemoglobin, where values of 138.6±3.51 g/L were obtained for the reference batch and 151.0±2.07 g/L for the experimental batch ($p<0.05$). The significant increase in hemoglobin denotes about a beneficial influence of zinc on this index, which is of particular importance in the physiological functionality of the cells and tissues of the body of roosters. In addition, this component contributes to the maintenance of gas metabolism and participates in the detoxification of the body by removing carbon dioxide and other substances resulting from gas metabolism.

The percentage content of hematocrit also undergoes changes. From the results obtained, a quantitative increase trend can be observed from 44.9±1.60% in the reference batch to 49.5±1.73% in the experimental batch. Changes in increasing hematocrit help maintain the balance between the formic elements of the blood and blood plasma, which can maintain an efficient metabolism in supplying the body with energy substances and a more effective detoxification of the body's cells and tissues.

Lymphocytes are a fundamental type of immune system cells that also possess immune status with an essential role in the body's defense against heterogeneous substances and for adaptive immune responses. In particular, in LM the limfocytes constituted 86.0±14.80 10^3/L, and in LE – 92.0±15.40 10^3/L. Some authors are of the opinion and claim that zinc at high doses can reduce the lymphocyte response, and in low doses it can stimulate their activity (Faber et al., 2004). Thus, zinc plays an important role in the functioning and regulation of lymphocytes and is essential for an effective immune response and for maintaining the health of the immune system, and its proper administration in the body can improve the immune response, especially in situations of deficiency or in the context of oxidative stress.

Monocytes, like lymphocytes, undergo insignificant growth changes from 5.0±1.42 10^3/L in LM to 6±1.46 10^3/L in LE and are part of the immune system with an important role in defending the body and maintaining tissue homeostasis. Here the presence of the sun can stimulate the activity of monocytes, increasing their capacity for phagocytosis and cytokine production, thus contributing to a more efficient immune reaction. Therefore, monocytes are essential cells in the immune response, and their reaction with zinc can influence how they perform their functions, being relevant for understanding immunological mechanisms.

Another indicator with a no less important role that undergoes insignificant changes are eosinophils, which are white blood cells, part of the immune system, involved in immunological responses and allergic reactions. As a result of the research, the following evolutionary trends were obtained, for LE the amount of 5.0±1.00 10^3/L was recorded against 3.0±1.00 10^3/L in LM. The tendency to increase the activity of eosinophils can be determined by the influence on the physiological functioning of the immune system, including the activity of eosinophils. It is important to note that, although zinc is essential for immune functioning, the specific reaction of eosinophils with zinc may vary depending on the immunological context, as their presence in the lumen of the gastrointestinal tract conditions various biological processes (Berek, 2018).

Next, the influence of zinc from the hydroalcoholic extract of polyphenols on the antioxidant status of the reproductive body was investigated under experimental conditions. The results of the research are presented in Table 2.

Table 2. Influence of zinc on enzyme and protein antioxidant status in rooster blood serum

Groups	Zinc, µM/L	SOD, u/c (min/L)	Catalase, µM/L	G-GTP, u/L	G-S-T, nM/sL	Total protein, g/L
Control	31.2±1.27	110.9±0.30	26.2±0.37	9.3±0.40	19.2±0.38	61.2±0.74
Experimental	34.4±0.80	127.5±0.40*	29.2±0.82*	10.8±0.66	22.0±0.51*	68.0±0.31*

Note: * - the differences are statistically true between the experimental and control group ($P<0.05$).

The zinc values shown in Table 2 showed a slight upward trend and constitute an amount of 34.42±0.80 µM/L for LE and 31.2±1.27 µM/L for LM. It is known that the major role of zinc for the body is the indispensability and contribution to the physiological functionality of vital systems and processes. As a result of the numerous physiological processes, zinc deficiency in the body can have various negative consequences, including the influence on the recruitment of granulocytes, the contribution to the evolution of oxidative stress with the production of ROS and the decrease in the fertility of reproductive cells, on the chemotaxis, of leukocytes and the phagocytic capacity of macrophages (Hasan et al., 2016; Lee et al., 2012; Gao et al., 2018).

Table data established significant changes in SOD content in LE, indicating a value of 127.5±0.4 u/c (min/L) compared to 110.9± 0.30 u/c (min/L) in LM ($P<0.05$) under the influence of the experimented extract compounds, in particular zinc. SOD has an essential role in neutralizing superoxide free radicals by catalyzing the superoxide radical conversion reaction and here it is important to mention that zinc itself does not have a direct role in the free radical neutralization reaction, but it is essential for the structure and function of SOD. The SOD reaction with zinc is rather indirect and is not a main reaction in the catalysis of free radical detoxification. Zinc participates in certain forms of SOD reactions by stabilizing the enzyme by coordinating and stabilizing the active center of the enzyme, but does not participate directly in the superoxide radical reduction reaction (Zelko et al., 2002). Thus, maintaining an adequate zinc balance is crucial for the effectiveness of the antioxidant response and for reducing the harmful effects of oxidative stress on the body.

Catalase research recorded a LE content of 29.2±0.82 μM/L compared to 26.2±0.37 μM/L in LM ($P<0.05$) depending on the zinc content. It is known that the biological role of catalase consists in preventing the accumulation of hydrogen peroxide, which is formed during the displacement of superoxide-anion. Catalase helps maintain redox balance in cells by reducing oxidative stress caused by free radicals, but it does not react directly with zinc in the usual way to perform its antioxidant function (Mahaseth & Kuzminov, 2017). Therefore, zinc can help support the antioxidant system, but it does not directly influence the reaction of catalase with hydrogen peroxide.

The content of gamma glutamyltranspeptidase (GTP) in the blood serum of roosters with a significant role in the detoxification process and in the antioxidant defense of cells in LE has a tendency to increase to 10.8±0.66 u/L, compared to 9.3±0.40 u/L in LM, which demonstrates an insignificant influence of the zinc content due to quantitative changes in this index. GTP being a zinc-dependent enzyme, which means that zinc is essential for its catalytic activity through the transfer of gamma-glutamyl groups with subsequent participation in antioxidant metabolism. GTP contributes to the neutralization of ROS, combating oxidative stress thus reducing oxidative damage while maintaining intracellular redox balance. In this sense, its action is considered an antioxidant mechanism, although under certain conditions, increased levels of GTP may indicate increased oxidative stress (Ndrepepa & Kastrati, 2016).

The study of the total protein content established a significant increase in the value from 61.2±0.74 g/l to 68.0±0.31 g/l, corresponding for LE and LM ($P<0.05$). Given that total protein plays an important role in chemical reactions involving zinc, especially due to its content of functional groups, in the context of antioxidants, this increase in its content contributes to the neutralization of ROS and the reduction of oxidative stress. Moreover, zinc contributes to the maintenance of the three-dimensional structure of proteins, protecting them from denaturation and the formation of reactive species with direct influences on antioxidant protection (Gammoh & Rink, 2017). Therefore, total protein exerts an antioxidant role in the reaction with zinc by forming stable complexes with zinc, thus reducing unwanted catalytic reactions and contributing to the stability and functioning of natural antioxidant systems. It helps maintain redox balance and protects cells from oxidative stress.

CONCLUSIONS

The zinc content of the hydroalcoholic extract of polyphenols from nettle (*Urtica dioica*) has a positive effect on the haematological components and the immune system of roosters, contributing to the improvement of immunoreactive parameters in blood serum, which indicates that zinc plays an essential role in supporting immunological function and maintaining the cellular functionality of the haematopoietic system, including in the context of oxidative stress.

Supplementing the feed ration of breeding roosters with zinc-rich components has a beneficial effect by contributing to the improvement of metabolic function and stimulation of the antioxidant system, thus reducing cell damage caused by oxidative stress, and research results suggest that this supplementation is an opportunity to prevent zinc deficiency and It can be an effective strategy for maintaining functionality, reproductive performance and for extending the life of breeding roosters under stress.

The zinc in plant polyphenolic components plays an essential role in modulating the redox balance, contributing to the reduction of ROS, protecting cells from oxidative stress and supporting the health and efficient functioning of the birds' body.

Zinc supports the body's detoxification mechanisms, participating in the activation of antioxidant enzymes by influencing fermentative reactions and other biochemical processes dependent on it, being essential for the optimal functioning of enzymes and for maintaining homeostasis in cells.

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