Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF POLYPHENOLS OF GREEN WALNUT EXTRACT ON ZINC HOMEOSTASIS AND ITS ROLE IN THE ORGANISM OF BREEDING ROOSTERS

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Abstract

In this paper were analyzed the scientific bibliographic sources regarding the physiological and biochemical role of zinc on the tissues and organs of the organism of breeding roosters. In addition, in laboratory conditions, our researchers studied hematological, biochemical indices as well as glycine, glutamate and cysteine content in blood serum, seminal plasma and reproductive cells of breeding roosters. It is established that this element has a beneficial action on the organism of breeding roosters, maintaining and stabilizing the indices listed above at the level of physiological norms, compared to the roosters from the control group, and at the same time participating in the antioxidant protection of cells and maintaining cell homeostasis by balancing transmembrane metabolism.

***Key words**: blood serum, cells, cell membranes, metabolism, zinc.*

INTRODUCTION

Being a necessary element for living organisms, zinc participates in all metabolic processes of the body, being a component of more than 7200 enzymes (Kimura & Kambe, 2016). One of the main roles of this element is the synthesis of protein and nucleic acids, at the same time stabilizing the structure of DNA, RNA and ribosomes, playing an important role in the translation process (Juravliova et al., 2007). It also has an important role in cell growth and division, participates in the stabilization and permeability of cellular and intracellular membranes, in membrane transport processes (Williams, 2012), the formation of antioxidant status as a protector of free radical reactions (Panasenko et al., 2018), possesses significant influence on the immune system (Daaboul et al., 2012) and apoptotic processes (Pang et al., 2013), osteogenesis, hemopoiesis, tissue respiration, growth, brain formation and its neurotransmitter function advocating as a neuromodulator and neuromediator (Li et al., 2001), reproduction and development of the fetus (Sheibak, 2015). In the cell zinc cations have a catalytic, structural and regulatory function. They catalyze the hydrolysis of peptides, proteins of some etheric substances and aldehydes (Salinikova, 2012). Zinc enhances the activity of osteoblasts and inhibits the activity of osteoclasts. In osteoblasts zinc can enhance cell proliferation, alkaline phosphatase activity and osteogenic effect (Hie & Tsukamoto, 2011).

Zinc plays an important role in the normal development of the testicles, as well as in spermatogenesis and possibly in the hormonal regulation of male reproductive function (Favier, 1992).

Zinc is a cofactor for more than 80 enzymes and is of great importance for the stability of such macromolecules as ribonucleic acid and DNA, as well as for protein synthesis, cell division and the stability of cell membranes (Favier, 1992). In addition, zinc is a component of superoxide dismutase, one of the key antioxidants.

The concentration of zinc in the male reproductive system significantly exceeds the same in other organs and tissues. Zinc is mainly eliminated by the prostate, but is also found in significant amounts in maturing spermatozoa, where its concentration is correlated with the level of oxygen consumption and the stability of nuclear chromatin (Kruczynski et al., 1985).

A series of scientific works prove to us that the use of antioxidants in the correction of male infertility is completely justified (Favier, 1992; Kruczynski et al., 1985). The fact that the effectiveness of many antioxidants has not been confirmed in all studies may indicate that their effects were not strong enough to be identified in these studies. There are regimes for monitoring the stable functioning of the reproductive system and the spermatogenesis process, which use various combinations of antioxidant substances and which, of course, in turn will allow to obtain a maximum effect of blocking reactive oxygen species.

With the discovery of the zinc-finger structural motif in proteins, the presence of the structural function of zinc was proven. Zn^{2+} cations regulate both the fermentative activity and the stability of proteins, acting at the same time as an activator ion or an inhibitory ion. The regulation of zinc accessibility in eukaryotes is primarily achieved by the compartmentalization of zinc and the functioning of the metallothionein/thionein system, which allows to control the zinc content in cells. The biological necessity of zinc is confirmed by the existence of homeostatic mechanisms which regulate its absorption, distribution, cellular needs and excretion (Gammoh & Rink, 2017).

In order to maintain an adequate homeostasis of zinc, a sufficient daily consumption is necessary, because compared to iron, the body lacks a specialized system for its deposition. The highest concentrations of zinc are found in muscles, bones, skin and liver (Mills, 2013). The required amount of zinc depends on age, sex, weight, direction of animal exploitation and food ration (Jeong & Eide, 2013).

Relatively, the richest nutrients in the zinc content of the food ration are bran, grists and food yeasts. Therefore, most food rations need to be evaluated in the content of the amount of polyphenols in the food ration of animals in order to obtain a more effective metabolism of micro- and macroelements.

Zinc deficiency can be caused by the following causes: unfermented plant ligands and some dietary fibers, which inhibit zinc absorption. Other factors, which influence the assimilation of zinc, are the ions of different metals. In particular, the absorption of zinc is inhibited by bivalent ions, such as Co^{+2}, Ni^{+2}, Cu^{+2}, Fe^{+2} and Cd^{+2}, while at the same time the ions of Mg^{+2} do not influence this process. A positive influence on the absorption of zinc are casein, histidine and methionine. Biological additives with zinc possess different bioavailability. Zinc bound with amino acids such as aspartate, cysteine and histidine have the highest absorption concentration, followed by chloride, sulfate and zinc acetate, while zinc oxide has the lowest bioavailability (Reiber et al., 2017).

Metallothioneins (MT) are cysteine-rich proteins that bind metal ions, particularly zinc and copper (Maret, 2000). There are four different classes of MT: MT-1 and MT-2 are spread throughout the organism; their basic function is to maintain cellular homeostasis of zinc and chelation of heavy metals. The expression of MT-3 and MT-4 is limited by the paternal cell type, where MT-3 the priority is detected in the brain, but MT-4 in the stratified epithelium (Kimura & Kambe, 2016; King, 2011).

The basic functions of metallothioneins in the body are related to the transport of metal ions, the maintenance of redox reactions and protective functions. Binding of heavy metals in various bioenergetic complexes takes place momentarily at the introduction of metals into the body by any method and regardless of concentration (Shafran et al., 2003). This bond has a dynamic character, that is, the metal "migrates" from complexes with a weaker resistance to complexes with a stronger resistance (Pihteeva, 2009). MT are reasonably considered to be proteins involved in the detoxification of essential and non-essential metals (Ruttkay-Nedecky et al., 2013).

A study by Formigari A. et al. pointed out the protective effects of zinc and the zinc-MT complex in oxidative stress induced by copper and iron and the apoptosis observed during it (Formigari et al., 2007).

In addition to metallothioneins there are other proteins that bind zinc and act as a system, ensuring the storage and control of zinc release. Albumin binds around 80% of the zinc in the plasma and is considered the basic transporter of zinc. The interaction between albumin and Zn^{+2} have a special importance for the supply of tissues and organs with this microelement. Albumin carries out the transporter of zinc absorbed in the liver, facilitates the consumption of Zn^{+2} by endothelial cells and erythrocytes. Complexes of zinc with albumin have increased kinetics of metabolism and facilitate the modulation of free zinc in plasma.

In any case, the increase in the content of fatty acids, observed in various metabolic disorders or pathological states of the organism, which can cause a hypoalbuminemia, causes a significant decrease in the binding and the stoichiometric ratio Zn^{+2}/albumin, but at the same time the zinc cations not influencing the stoichiometric binding of fatty acids (Sheibak, 2015; Lu et al., 2008). Also, among the zinc-binding proteins are alpha-2-macroglobulin, histidine-rich glycoprotein and protein family S100 (Gilston et al., 2016). Besides these proteins in the metabolism of zinc also participate some chemical elements and some vitamins, such as: copper, iron and folic acid.

It is scientifically proven that the ingestion of large amounts of zinc over a period of several weeks can interfere with the metabolic processes of copper bioavailability. Massive ingestions of Zn cause the intestinal synthesis of metallothionein, which in turn captures copper in the intestinal cells and prevents its systematic absorption. Balancing the food ration in the relative content of zinc does not affect the absorption of copper, as well as any deviations to increase the concentration of copper do not influence the absorption of zinc.

Excess iron can decrease the absorption of zinc, this can happen in pregnant sows or newborn piglets during the treatment with iron preparations of iron deficiency anemia, which is very common in these animals during the given period.

Large amounts of dietary calcium in the ration of animals decrease zinc absorption not only in mature animals, but also in youth.

The bioavailability of folic acid from food is increased by the action of a zinc-dependent enzyme, suggesting a possible interaction between zinc and folic acid. Older studies claimed that small doses of zinc decrease the absorption of folic acid and conversely large amounts of folic acid affect the amount of zinc taken up by the body.

Apart from this, numerous scientific researches have shown that zinc also has antioxidant properties (Rostan et al., 2002). So, it has been demonstrated that zinc diminishes the damage of cells and their genetic apparatus, as a result of the influence of ultraviolet irradiation and increases the resistance of skin fibroblasts to oxidative stress damages (Richard et al., 1993). To a certain extent, this effect is conditioned by the influence of zinc-containing enzymes and proteins that participate in the elimination of ROS (active oxygen radicals), in particular these are superoxide dismutases (SOD) and MT (Abel & de Reuiter, 1989), but the antioxidant potential of zinc is not limited to them. It is assumed that zinc can replace the metals themselves, which actively participate in the reactions of free radical formation (iron, copper), but alone does not participate in the redox reactions (Rostan et al., 2002). The given hypothesis is confirmed by observations, which prove to us, that the activity of SOD in those systems, in respect of which it is proved, that the protective influence of the action of zinc is insufficient to achieve such a level of protection against free radicals.

MATERIALS AND METHODS

To carry out the research in the study were included 10 breeding roosters, which were divided into two groups of five birds each (control group and experimental group). Roosters in the control group were administered *per os* hydro-alcoholic polyphenol extract from green walnuts in a dose of 1 ml with a total antioxidant activity of 1.7 g gallic acid equivalent per 100 gr. The extract was administered during two cycles of spermatogenesis. Hematological, biochemical indices and amino acid content in blood, seminal plasma and reproductive cells were studied.

RESULTS AND DISCUSSIONS

In Table 1 are presented the hematological indices of reproductive roosters included in the experiments to study the influence of zinc on the functional state of the immunoreactive blood components.

Table 1. Hematological indices of reproductive roosters included in experiments to study the influence of zinc on the functional status of immunoreactive blood components

Groups	Leukocyte, 10^9/L	Erythrocytes, 10^12/L	Hemoglobin, g/L	Hematocrit, %	Lymphocytes, 10^3/L	Monocytes, 10^3/L	Eosinophils, 10^3/L
Control	37.6±4.1	3.22±0.33	138.6±3.51	44.9±1.6	92±15.4	6.6±4.46	5.0±1.0
Experimental	41.9±3.62	3.64±0.32	151±2.0	49.5±1.73	86±14.0	5.0±1.73	3.0±1.0
Reference values	20-40	3.4	138	36.8	70.06	6.7	6.0

The supply of the organism with the necessary amounts of zinc is of great importance for the blood components of the immune system. Zinc influences the response of lymphocytes, mitogens and cytokines, serves as a cofactor of the thymus hormone - thymulin, participates in the transduction of leukocyte signals. In cell culture high concentrations of zinc in serum-free medium stimulate monocytes to secrete anti-inflammatory cytokines (Wellinghausen et al., 1996).

In the result of the research, it is observed that the leukocytes, also called white blood cells, under the influence of zinc concentration from the hydroalcoholic polyphenol extract from green walnuts have a higher mathematical and biological value compared to the control group, indicating the figures of 41.9±3.62, while in the control group indicating a value of 37.6±4.1.

Erythrocytes also play a very important and decisive role in the health of birds and animals, in that they carry fresh oxygen throughout the organism. These cells show a value of 3.22±0.33 (10^12/L) compared to 3.64±0.32 (10^12/L) in the experimental group, which testifies about a beneficial influence on blood components of zinc in combination with copper and by activating some fermentation systems.

Hemoglobin is the protein inside erythrocytes, and it is also the one that ensures the transport of oxygen throughout the body. In addition to transporting oxygen, erythrocytes also have the role of removing carbon dioxide from the organism, transporting it to the lungs, to be eliminated with the exhaled air.

The lymphocytes present in most vertebrates, being also a component of the immune system, responsible for the body's defense reactions and the production of antibodies against substances they consider foreign and are presented with the following values in the experimental group 86±14.0, compared to the control group with 92±15.4 10^3/L. An insignificant effect of zinc on the proliferation of lymphocytes is demonstrated in the presence of mitogens - phytohemagglutinin or concanavalin A. It has been shown that zinc has an immunoregulatory effect, that is, it reduced the response of lymphocytes at high doses and increased it at low doses (Faber et al., 2004).

Monocytes being involved in the defense against bacterial infections, maintain their values within the limit of 5.0±1.73 in the experimental group, compared to the control group with a value of 6.6±4.46 10^3/L.

Eosinophils are involved in several pathological processes, such as allergic, parasitic and neoplastic ones (cancer), they are considered destructive effector cells in the final stage that have a role in parasitic infections and allergic reactions by releasing acid hydrolases of the lysosomal type and histones. However, eosinophils are also multifunctional leukocytes involved in inflammatory and physiological immunity. Under homeostatic conditions, eosinophils are particularly abundant in their own lamina of the gastrointestinal tract, where they are involved in various biological processes within the gastrointestinal tract.

At the same time, the influence of zinc on the antioxidant and protein status was evaluated and researched. The results are presented in Table 2.

So as is known from research results and bibliographic data, superoxide dismutase (SOD) is the key enzyme of the antioxidant system, which manifests important protective roles against cellular and histological damage produced by ROS (reactive oxygen species).

Table 2. Influence of zinc on antioxidant and protein status

Groups	SOD, u/c (min/L)	G-GTP, u/L	Catalase, μM/L	G-S-T, nM/sL	Prot. tot., g/L
Experimental	127.5±0.4	10.8±0.66	29.2±0.82	22.0±0.51	68.0±0.31
Control	110.93±0.30	9.33±0.40	26.23±0.37	19.21±0.38	61.21±0.74

Superoxide dismutase is the antioxidant factor, which catalyzes the dismutation of superoxide anion into hydrogen peroxide and molecular oxygen, and prevents the formation of peroxynitrite and hydroxyl radical, which can damage tissue components. Taking into account the fact that, depending on the metal ion in the active center of the ferment, several SOD isoenzymes are highlighted, of which the greatest activity is possessed by Cu -, Zn-SOD. From the data of the table, we observe changes between the SOD content in the experimental group indicating a value of 127.5±0.4 u/c (min/L)compared to the control group with a value of 110.93±0.30 u/c (min/L), which testifies to the changes, which take place under the influence of zinc from extract of green walnuts.

Catalase is one of the key enzymes of the antioxidant system, which performs the function of antiperoxide protection in conditions of oxidative stress and the intensified formation of active forms of oxygen. This enzyme of major importance has the property of being produced when the organism needs it. From the obtained results we observe a difference between the values of the control group 26.23±0.37, compared to the experimental one which has a value of 29.2±0.82 μM/L.

Glutathione-S-transferases (GSTs) are a group of enzymes that are important in blocking many xenobiotics in living organisms. Enzymes protect cells against toxic substances by conjugating the thiol group of glutathione with electrophilic xenobiotics and therefore, defend cells against the mutagenic, carcinogenic and toxic effects of the compounds. GST activity has been shown to be present in plants, insects, yeast, bacteria and in most animal and human tissues, especially in the liver, which plays a key role in detoxification. From the obtained results we notice that this enzyme changes in the experimental group and has a value of 22.0±0.51 nM/sL compared to the control group with a value of 19.21±0.38 nM/sL. Total protein, represents the total sum of all serum proteins, which circulate in the vascular bed and part of the basic component of the blood, the main proteins being free albumin bound with zinc and globulin, which has firm bonds with zinc, in turn being an active element in more than 300 metallo-fermentative reactions, it has a decisive role in the synthesis and decomposition of nucleic acids and protein metabolism, nitrogen metabolism, as well as participates in antioxidant protection (Fredricks et al., 1960). From the research results, it can be observed that experimental data were obtained, which indicate a value for the control group of 61.21±0.74 g/L, and for the experimental group – 68.0±0.31 g/L, which demonstrates an influence of zinc on the amount of protein in the blood serum.

Glutathione is a tripeptide consisting of three amino acids: glycine, cysteine and glutamine, which participates in the synthesis of leukotrienes and is a cofactor of the enzyme glutathione peroxidase.

In these studies, amino acids, which are closely related to glutathione, were determined in the blood serum, seminal plasma and reproductive cells. The research results are presented in Table 3.

As follows, from the obtained results, it is observed, that glutathione is a valuable antioxidant and is produced by almost all cells of the organism. However, a higher concentration of amino acids is observed in the seminal plasma, which are dependent on glutathione, which in turn depends on the level of zinc in the body.

Table 3. The content of glycine, glutamate and cysteine in blood serum, seminal plasma and reproductive cells of roosters

Amino acids	Blood serum, (mcm/ 100 ml)	Seminal plasma, (mcm/ 100 ml)	Reproductive cells, (mcm/ 100 ml)
Cysteic acid	1.41±0.79	2.04±0.34	0.66±0.04
Glutamic acid	8.10±2.05	207.54±17.36	7.57±0.22
Glutamine	32.23±9.18	757.26±42.11	21.75±0.18
Glycine	46.47±2.27	37.56±2.01	8.88±0.08
Cysteine	1.84±0.34	9.76±0.97	1.56±0.07

CONCLUSIONS

Zinc is an indispensable microelement for the functioning of every cell in the human body. Zinc deficiency affects the processes of growth and development. The best way to prevent zinc deficiency is a well-balanced ration and supplemented with vegetable polyphenolic components to prevent zinc deficiency in cells and tissues of the body, also once obtaining a more efficient metabolism and detoxification of the body by stimulating fermentative reactions and other zinc-dependent processes in living organisms.

Studying and analyzing the research results, we notice that zinc is an element that participates in the antioxidant protection of cells and the maintenance of cell homeostasis by balancing transmembrane metabolism.

From the research carried out by researchers, it appears that it is necessary to monitor the level of zinc content in living organisms by balancing the food ration and maintaining an adequate metabolism, thus ensuring the organism a good functioning of all organs and systems.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 “Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability”.

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