Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 1, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CHANGES OF THE GLUTATHIONE CONTENT IN THE BLOOD SERUM OF ROOSTERS UNDER THE INFLUENCE OF POLYPHENOLS EXTRACTED FROM NETTLE *(Urtica dioica)*

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Abstract

Oxidative stress has long been implicated in the development and progress of various disorders of living organisms. Glutathione is a natural antioxidant that possesses a major regenerative and detoxifying potential. Glutathione synthesis occurs continuously in almost all cells to maintain redox balance. Ensuring an adequate level of glutathione is vitally important, therefore the role of the glutathione system in maintaining the antioxidant status of the organism is essential. Normally, the formation of free radicals and underoxidized metabolic products occurs continuously during the body`s biochemical reactions. The balance is maintained by antioxidant enzymes that can neutralize molecules with a high oxidative potential. Glutathione is a unique peptide found in the cells of all eukaryotes. This compound plays a leading role in cellular metabolism, actively maintains the redox potential, regulates the detoxification processes of xenobiotics of endo- and exogenous origin, both directly and as a substrate for a number of enzymes. This paper is an analysis of the results obtained from the administration of polyphenols from nettle and their influence on zinc metabolism.

Key words*: glutathione, nutrition, oxidation, polyphenols.*

INTRODUCTION

Intensification of the growth and improvement of farm animals and poultry requires monitoring the state of the body's antioxidant system, otherwise oxidative stress can develop. In order to maintain the functionality of the body's organs and organ systems, it is necessary to stop the development of oxidative stress, through the inclusion of various remedies with antioxidant activity, including those of plant origin. The biochemical structure of herbal remedies is conditioned by their adaptation in enzyme metabolism through evolution, possessing a more pronounced degree of bioavailability compared to antioxidant remedies of synthetic origin (Balan et al., 2024).

The biochemical structure of herbal remedies is close to the structure of metabolites of living organisms, which is conditioned by adaptation by evolution, and correspondingly, these remedies are more easily subject to the influence of fermentative systems, compared to synthetic analogues. In this way, the issue of research and study of new opportunities and phytoprotective sources of natural antioxidants is currently pursued.

Glutathione is the most quantitative intracellular antioxidant, having a role in maintaining redox homeostasis and cellular protection against oxidative stress by neutralizing reactive oxygen species (Forman et al., 2009). It plays an essential role in maintaining the redox state of cells due to its ability to act as a reducing agent.

Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) with multiple functions in living organisms (Diaz Vivancos et al., 2010; Sies, 1999). Reduced glutathione (GSH) constitutes an active thiol group in the form of cysteine compounds, which acts as an antioxidant directly by interacting with reactive oxygen species (ROS) and electrophiles, in

particular, acts as a cofactor for various enzymes (Cooper et al., 2011). At the same time, the glutathione content in the intracellular environment has a moderate stability ensured by the cleavage of the peptide bonds formed by the α-carboxyl groups of amino acids by means of intracellular peptidases, but not by the γ-carboxyl groups by carboxylation or decarboxylation reactions, which do not directly involve the peptidases. Furthermore, for the regulation and maintenance of redox balance in cells, reduced glutathione (GSH) and oxidized forms of glutathione (GSSG) act together with other redox compounds, such as nicotinamide adenine dinucleotide phosphate (NADP+) generated by glucose metabolism pathways and protection of cells from oxidative stress (Jones et al., 2011).

GSH is often referred to as the main antioxidant (Bishayee et al., 2010) and being a tripeptide consisting of three amino acids: glutamate, cysteine and glycine plays an essential role in protecting cells against oxidative stress and oxidative damage by maintaining redox homeostasis. GSH is considered as a key element of the antioxidant defense system by neutralizing ROS species and reactive nitrogen species (RNS) thus preventing cell damage and is essential for metabolic processes (Pizzorno, 2014).

Oxidative stress is triggered by the imbalance between the production of ROS and the inability of antioxidant biological systems to eliminate them. In this situation, GSH is the basic redox agent in most aerobic organisms and a fundamental element in fine-tuning oxidative stress. GSH is involved in numerous vital functions, such as free radical scavenging, cysteine delivery, protein thiol status maintenance through redox exchange reactions, modulation of DNA synthesis, processes related to molecular microtubulation, as well as cell immune functions (Lu, 2009). Therefore, GSH plays an important role in cell metabolism, cell differentiation, proliferation, and apoptosis, and reducing ROS and other oxidizing molecules.

The function of ROS and RNS elimination is of great importance for metabolic functioning, and in this vein, GSH can directly bind some ROS species or serve as an essential source of reducing power for certain antioxidant systems (Bray & Taylor, 1993). At the same time, GSH by direct reaction considerably reduces ROS and RNS, but also other radicals, especially HO•, HOCl, RO•, $RO^{2\bullet}$,1 O^2 and $ONOO^-$ and is involved in the detoxification of xenobiotics and products derived from the oxidation of lipidaldehydes (4-hydroxy-2-nonenal as a product of the oxidation of unsaturated fatty acids), facilitating their elimination from the body (Lang et al., 2002). In addition, GSH serves as an electron donor for various antioxidant enzymes, including glutaredoxins, glutathione peroxidases, and glutathione S-transferases, and may be involved in the synthesis of ergothioneine (EGT), another enzyme-catalyzed antioxidant similar to glutathione with a significant role in cellular protection against oxidative stress. In turn, EGT is involved in the chelation of divalent metals (Co, Cu, Hg, Ce, Pt) and in radiation reactions by physically deactivating high-energy molecules through energy transfer (Borodina et al., 2020; Yadan, 2022). At the same time, ergothioneine helps to regenerate glutathione, thus helping to maintain its optimal levels in cells, and the presence of both antioxidants has a synergistic effect, improving the body's ability to cope with oxidative stress.

In the context of the above, the purpose of the present research was to study the possibilities of influencing the glutathione content in the blood serum of roosters under the influence of polyphenolic compounds of natural origin.

MATERIALS AND METHODS

The research was carried out on 10 breeding roosters, which were divided into two batches of five birds each (control and experimental batch). The birds were fed and maintained under identical, analogue investigation conditions, with the same microclimate, technological elements, watering, feeding, veterinary medical assistance, etc. in order to prevent the variation of the sample. The roosters in the experimental batch were administered *per bone* hydroalcoholic extract of polyphenols from nettle (*Urtica dioica*) in a dose of 1 ml with a total antioxidant activity of 33.2 mg gallic acid equivalent per 100 g, and the roosters in the reference batch were administered by the same method with five ml

of saline solution. The well-known methodology of investigation of hematological and biochemical indices in the blood plasma of birds was applied, as well as the determination of the content of free amino acids in seminal plasma and reproductive cells was applied. The sperm from the males was collected by the accepted method of abdominal massage. The semen underwent centrifugation (1000 rpm/min, 10 min) to separate the seminal plasma from the reproductive cells. The reproductive cells subsequently followed the technological process until the amino acid content was determined. The seminal plasma underwent double centrifugation under the conditions mentioned for complete removal of sperm debris from the plasma. The seminal plasma was transferred and stored in the freezer at a temperature of -45°C with subsequent transmission to the profile laboratory for the determination of amino acid content.

The biometric processing of the experimental results was carried out in accordance with the generally accepted methodology. The results are expressed as a mean ± standard error of mean with the determination of significance between the control and experimental lots.

RESULTS AND DISCUSSIONS

Glutathione plays an essential role in maintaining cellular health and oxidative homeostasis and is one of the most important antioxidants in the body. It helps neutralize ROS generated during cellular metabolism, thus preventing oxidative stress that can negatively affect the reproductive health of roosters. Moreover, adequate glutathione levels are essential for the optimal functioning of the testes and sperm production, and also have a significant role in regulating metabolism and supporting the immune system essential for the overall health of breeding roosters and for reproductive success.

Monitoring glutathione levels and ensuring optimal conditions for its production can help improve the reproductive performance of birds. Therefore, initially the value of glutathione concentration indices in the blood serum of breeding roosters was investigated. The results of the research are presented in Table 1.

The data in Table 1 show significant changes in gamma-glutamyltranspeptidase (G-GTP) in the experimental batch compared to the reference batch. The G-GTP content is 11.79±0.59 u/L for the experimental batch compared to 9.33±0.40 u/L in the control batch ($P<0.05$), which demonstrates the influence of the polyphenol extract compounds on these indices.

Table 1. Content of gamma-glutamyltranspeptidase and glutathione transferase in the blood serum of breeding roosters

Groups	G-GTP, u/L	GST, nM/Sl
Experimental	11.79±0.59*	33.00±0.56*
Control	9.33±0.40	19.21±0.38

Note:
*The differences are statistically true between the experimental and control group ($P<0.05$);
G-GTP - gamma-glutamyltranspeptidase;
GST - glutathione transferase.

As a result of the fact that G-GTP catalyzes the transfer of the γ–glutamyl group of peptides and its major content at the level of the cytoplasmic membrane of cells, it is worth mentioning that the increase in its content beneficially influences the oxidative metabolic processes inside the cell. At the same time, the luminous surface of cells with secretory and absorbent functions is especially rich in G-GTP, which influences the extracellular oxidative metabolism of the cell. At the same time, G-GTP is the only enzyme that cleaves significant amounts of GSH and GSH conjugates within the γ-glutamyl cycle (GSH is transported to the extracellular surface of the membrane, where it is separated by G-GTP into cysteinyl-glycine and γ-glutamyl residues, which are transferred to other amino acids) (Deponte, 2013). An important property of glutathione refers to the direct blocking of ROS and intervenes as a redox cofactor for various antioxidant enzymes (glutaredoxins, glutathione peroxidases, glutathione reductase and glutathione S-transferase).

Another studied derivative of glutathione is GSH also because its reactivity with proteins, molecular structures and xenobiotics *in vivo* can be decreased (Deponte, 2017), and the development of GSH-dependent reactions are accelerated by glutaredoxine enzymes that can influence oxidation processes.

In this context, the data in Table 1 show a significant increase in glutathione transferase (GST) content. The value of this index was 33.00±0.56 nM/sL and 19.21±0.38 nM/sL, corresponding in the experimental and reference group ($P<0.05$). Therefore, the increase in the amount of GST in the experimental batch can be correlated with the fact that GST catalyzes conjugation reactions with metabolites, chemicals and metals, or if GST was not depleted during the isomerization and dehalogenation processes, as well as in reactions where GSH can be oxidized by GSH-dependent peroxidases, -thiol transferase, -dehydroascorbate reductase (Deponte, 2013; Deponte, 2017). Glutathione transferases also participate in the binding and transport processes of molecules due to their non-catalytic ligand properties (Schwartz et al., 2018), as well as interacting with proteins, modifying their activity through glutationylation or swallowing (Kalinina & Novichkova, 2021; Sing & Reindi, 2021).

Regarding the interpretation of the results obtained of the researched compound, it is possible to mention that glutathione is maintained in the mitochondria in its reduced form (10-15% of the total cellular GSH) in a concentration of 5-10 mM (Kojer et al., 2012). At the same time, mitochondria do not contain the enzymes necessary for GSH biosynthesis and mitochondrial GSH, respectively, must be imported from the cytoplasm (Scire et al., 2019). Moreover, glutathione has a negative charge at physiological pH and cannot freely cross the lipid bilayer, therefore the outer and inner mitochondrial membranes are provided with vector transporters and channels for GSH absorption. The outer mitochondrial membrane is rich in pores, which form channels through the lipid bilayer and allow diffusion between the intermembrane space and the cytosol of molecules smaller than ~5 kDa, including glutathione (Scire et al., 2019). Kojer demonstrated that glutathione bases in the intermembrane space and cytosol interact through porins (Kojer et al., 2012). This metabolic pathway is widespread in all biological systems and is involved in the catalyzation of the conversion of 2-oxaldehyde to 2-hydroxy acid by interaction with S-2-hydroxyacylglutathione, as well as in the cellular detoxification of α-cetoldehydes produced as a result of glycolysis contributing to the synthesis and metabolism of complex biological and chemical molecules.

The mitochondrial content of GSH is maintained in constant concentration as a result of its transport from the cytosol through two systems, one by increased manifestation produced by adenosine triphosphate (ATP), the other by low influence by ATP and adenosine diphosphate (ADP) with involvement in processes of reduction of oxidative components and in the regulation of the redox state of the cell (Martensson et al., 1990). Regarding other intracellular organelles (endoplasmic reticulum) it has been demonstrated the existence of a transport system that allows selective permeability and transformation of GSH into oxidized form of glutathione (GSSG) (Chakravarthi & Bulleid, 2004) and subsequently at this level GSH participates in the reduction of the activity of isomerases responsible for catalyzing the constitution of disulfide bonds in proteins (Chakravarthi & Bulleid, 2004). Therefore, in the lumen of the endoplasmic reticulum a constant production of GSSG takes place under the influence of the use of GSH that maintains oxidoreductases in their reduced form. Subsequently, through the protein-conducting channels, GSSG by facilitating diffusion is transported to the cytosol (Ponsero et al., 2017), where it undergoes reduction under the action of the enzyme glutathione reductase (Lu, 2013; Chakravarthi & Bulleid, 2004; Ponsero et al., 2017). This process plays an essential role in maintaining cellular redox balance and protecting cells against oxidative stress. Specifically, when cells are subjected to oxidative stress, GSH is oxidized to form GSSG and then GSSG can be reduced back to GSH by the enzyme glutathione reductase, using nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme.

The study continued by determining the content of free amino acids in the reproductive material of birds that contribute to the metabolism and presence of glutathione in optimal concentrations in this biological fluid. In particular, for preservation and detoxification with a strategic importance for maintaining life and preserving genetic potential. The research

involved the seminal plasma and reproductive cells of breeding roosters. The results of the research are presented in Table 2.

Table 2. Free amino acid content in seminal plasma and reproductive cells of breeding roosters

Amino acids	Seminal plasma, (mcm/100 ml)	Reproductive cells, (mcm/100 g)
Cysteic acid	22.04±0.34	0.66± 0.04
Glutamic acid	207.54±17.36	7.57±0.22
Glutamine	757.26±42.11	21.75±0.18
Glycine	37.56±2.01	8.88±0.08
Cysteine	4.37±1.04	1.56±0.07
Isoleucine	10.98±1.58	1.55±0.11
Leucine	16.64±2.80	2.25±0.14
Proline	56.90±3.58	6.66±0.02

Table data show that 8 free amino acids have been determined. The results correlate with the existing ones that for the seminal fluid of the rooster it is characteristic that the largest share of the content is represented by glutamine which constitutes 757.26±42.11 mcm/100 ml. Also, the presence of glutamic acid (207.54±17.36 mcm/100 ml) was also determined in major quantities. The content of these two components prevails 6.5 times the total amount of all other amino acids investigated. The content of the latter is increased in the range from 4.37± 1.04 mcm/100 ml for cysteine to 56.90± 3.58 mcm/100 ml for proline.

In the reproductive cells, the same amino acids were determined, but in a significantly smaller amount. The data in the table show that the highest share in spermatozoa is also held by glutamine with a concentration of 21.75± 0.18 mcm/100 g, followed by compounds with practically identical amounts (glycine 8.88±0.08 mcm/100 g, glutamic acid 7.57± 0.22 mcm/100 g and proline 6.66± 0.02 mcm/100 g).

Free amino acids, especially those with reactive functional groups (containing the thiol-SH group) act as antioxidants against ROS and participate in repair and detoxification, and their interaction with glutathione in the oxidation process is essential for cellular protection against oxidative stress. Glutathione reduces reactive oxygen species or other dangerous molecules, becoming GSSG and in this process, the thiol (-SH) group of GSH plays a central role. Furthermore, free amino acids, especially those with thiol groups, participate in reduction reactions or can contribute to the regeneration of GSH from GSSG and thus maintaining redox balance.

Some glutathione restoration mechanisms are maintained by the activity of glutathione-dependent enzymes by releasing cysteinyl-glycine dipeptide that undergoes cleavage into cysteine and glycine by specific Cys-Gly peptidases and 5-oxoproline that is converted to glutamate by ATP-dependent 5-oxoprolinase (Bachhawat & Yadav, 2018). Further the Cys-Gly dipeptide is cleaved by dipeptidase in Cys and Gly with emigration to the cytosol where it participates in the synthesis of proteins and GSH (Bachhawat & Yadav, 2018), and the amide combination between glutamine γ-carboxyl and amino cysteine units prevents the degradation of GSH by circulating serum and cellular peptidases (Lieberman et al., 1996). The process continues with the cellular absorption of Cys, Gly and glutamate units that serve as precursors for the intracellular synthesis of GSH.

GSH in subcellular structures is distributed as follows, in the cytosol (80-85%), mitochondria (10-15%) and endoplasmic reticulum (5-10%) (Giustarini et al., 2015). There is research that has shown that in the endoplasmic reticulum the total GSH content sometimes exceeds the total cellular GSH content (Birk et al., 2013), but these concentrations of GSH, including GSSG depend on the cell type, the subcellular compartment and the individuality of the organism. Consequently, the total glutathione concentration and the ratio of GSH to GSSG are directly proportional to the redox capacity of the variable system of these compounds in tissues and organisms (Kojer et al., 2012). At the same time, cytoplasmic GSH influences its diffusion through nuclear pores (Schafer & Buettner, 2001), where it signals oxidation at the proliferative, epigenetic and cell cycle levels (Markovic et al., 2007).

In the context of the above, the interaction between free amino acids and glutathione plays an essential role in the protection of cells against oxidative stress. Free amino acids,

especially those with reactive functional groups, participate in ROS neutralization reactions, helping to maintain redox balance, and GSH acts as a primary antioxidant, reducing ROS and regenerating other antioxidant molecules. This interaction is essential for detoxifying free radicals and preventing damage to cellular components, such as lipids, proteins, and DNA. In addition, free amino acids can be involved in the synthesis and regeneration of glutathione, thus facilitating the endogenous antioxidant system. Therefore, the close collaboration between free amino acids and glutathione is fundamental for maintaining oxidative balance, preventing oxidative stress and supporting cellular health.

CONCLUSIONS

Glutathione is an essential antioxidant in the body of roosters, having a fundamental role in protecting cells against oxidative stress, which can lead to damage to DNA, proteins and lipids, and by neutralizing ROS, glutathione contributes to maintaining the integrity and optimal function of tissues, including the reproductive system.

The interaction between free amino acids and glutathione helps prevent protein, lipid and DNA damage caused by free radicals, contributing to the maintenance of cellular health, and in particular, free amino acids with thiol groups, actively participate in the processes of glutathione reduction and regeneration, facilitating the neutralization of oxidative stress and maintaining the redox balance in the cell. This interaction is fundamental to the body's antioxidant defense mechanisms.

Adequate levels of glutathione ensure the health of the testicles and sperm, and glutathione's antioxidant activity is significant for increasing fertility, reducing oxidative stress, and promoting efficient and sustainable reproduction in roosters.

Polyphenol extract significantly influences the content of free amino acids in seminal plasma compared to their content in reproductive cells, which denotes about maintaining optimal metabolism with multiple evolutionary functionalities of reproductive cells at all stages of spermatogenesis, including maturation, empowerment and achievement of the ultimate goal of egg fertilization.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Moldova State University, Institute of Physiology and Sanocreatology and was financed from the research Sub-Programme 011001 “Mechanisms for regulating the organism's homeostasis and health and the elaboration of procedures and measures to maintain it”.

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