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Influence of Organic Selenium Application in Duck Concentrate Mixtures on Glutation Peroxidase Activity in Duck Blood Plasma

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ABSTRACT: *Different forms of glutathione peroxidase are distributed differently in the body. Each of them shows affinity for a particular tissue, e.g., GPx1 is widespread, GPx2 is present almost exclusively in the gastrointestinal tract, GPx3 is mainly present in tissues in contact with tissue fluids while GPx4 concentration is highest in the testes (sperm). In addition to the tissue hierarchy in cases of selenium deficiency, it has been proven that there is a hierarchy among individual selenoproteins. Compared to the concentration of other selenoproteins, the concentration of the enzyme glutathione peroxidase decreases much faster and more drastically in cases of selenium deficiency. The aim of this study was to investigate the effect of the addition of different amounts of organic selenium (ALKOSEL® R397) in concentrate mixtures on the activity of glutathione peroxidase in the blood plasma of ducks.*

KEYWORDS: *glutathione peroxidase, organic selenium, blood plasma*

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I. INTRODUCTION

The production of duck meat in the world is constantly increasing. It increased by one million tons from 2000 to 2010, so that in 2010 it was over four million tons in the world. Duck meat production is growing at an annual growth rate of 3.3% worldwide, and in Asia, where production of this type of meat is highest (83.5% of world production), the annual growth rate is 3.8%. The largest producer of duck meat in Asia and worldwide is China, followed by India, Vietnam, Thailand and the Republic of Korea. In Europe, the largest producer of duck meat is France (300 thousand tons), followed by Ukraine, Germany, Hungary and the United Kingdom. Over half of the production (52%) of duck meat in Europe is produced in France.

Poultry meat, including duck meat, has a high nutritional value (over 20% protein and less than 5% fat in the meat of breasts and drumsticks with carbata). These data refer to hybrid duck lines intended for fattening.

Modern production of duck meat implies intensive fattening in a period of 49 days with the correct selection of hybrids, arrangement and control of zoohygienic and microclimatic conditions in facilities and the use of optimized feed mixtures. Consumption of duck meat in Bosnia and Herzegovina is in third place among poultry meat and is constantly increasing, which is a good basis for the development of this branch of animal husbandry. Current consumption is provided partly from imports and from domestic production, which takes place exclusively on family farms. Taking into account the interest of consumers, it is very important that fattened ducklings have good yields as well as favorable carcass conformation, which is influenced by: hybrid, sex, age, health, diet, live weight, length of fattening and conditions and ways of keeping ducklings in fattening. In intensive fattening of ducklings, the genetic basis can be manifested in certain conditions such as: ambient conditions in the facility for fattening ducklings, production technology, diet, health, prevention and other factors that affect the growth and development of animals. The duck keeping system, as one of the important factors of duck meat technology, determines the production indicators of the success of intensive poultry production in general and has great economic significance.

Today, it is a well-known fact in the world that animal nutrition can affect the nutritional value of meat, milk, eggs, and that it is one of the ways to obtain food with special properties known as functional food. Selenium-enriched foods can also be considered functional foods.

Selenium performs its biological role in the body through the enzyme glutathione peroxidase (GPx), in the active site of which this element is located. Plasma GPx activity is a reliable indicator of selenium status in animals, but only at suboptimal and optimal selenium levels. However, high levels of selenium do not lead to a proportional increase in selenium enzymes. The dependence of GPx activity on the levels of supplemented selenium has been established in numerous studies in many animal species. The activity of this enzyme varies considerably depending on the types of tissues, cells and subcellular fractions. The level of enzyme activity varies considerably depending on the type and status of selenium in animals. Data from the literature related to GPx activity were obtained using different analytical methods and expressed in different units, which makes

considerable difficulties in comparing the results. Studies in chickens and turkeys have shown that plasma GPx activity is highly correlated with selenium intake.

The Beijing, Muscovy and several hybrid line genotypes are most commonly used to produce duck meat, the most famous of which is the Cherry Valley hybrid. It is understood that in different countries of Asia there are several indigenous genotypes of ducks that are bred primarily for meat production, but there are also genotypes for egg production. Different production systems are used in duck breeding.

Intensive duck breeding systems require, in addition to appropriate housing conditions, that animals be provided with all the necessary ingredients that enable the full use of their genetic potential. One of the most widespread breeds of ducks in the world is the Peking duck, and duck meat production is mainly based on commercial crosses of different Peking species (Anas platyrynchos) (Pingel, 1997; Zejidler, 1998). It originated in China, from where it spread to Europe and America. The most famous is the American and German strain, both white in color. Males reach a weight of about 4-6 kg, and females 3-4 kg.

To date, five selenium-containing glutathione peroxidases (GPx) have been described: cellular or classical GPx, plasma or extracellular GPx, phospholipid hydrosiperoxide GPx, gastrointestinal GPx, and olfactory GPx. Although each of these glutathione peroxidases is a different selenoprotein, they are all antioxidant enzymes that reduce potentially harmful reactive oxygen free radicals, such as hydrogen peroxide and lipid hydroperoxide to harmless products such as water and alcohols, with reduction accompanied by glutathione oxidation (Schedrina et al., 2010; Kryukov et al., 2003; Rotruck et al., 1973).

Cytosolic glutathione peroxidase (GPx1) is the first selenoprotein identified and described in mammals. GPx1 is the most abundant enzyme in the selenoprotein family and plays a significant role in protecting cells from oxidative damage by catalyzing the reduction of large numbers of hydroxyperoxides while using glutathione as the reducing substrate.

In addition to the tissue hierarchy in cases of selenium deficiency, it has been proven that there is a hierarchy among individual selenoproteins (Behne et al., 1988; Behne and Kyriakopoulos, 1993). All glutathione peroxidases contain residues of tryptophan and glutamine, which once again proves that it is a family of enzymes (Maiorino et al., 1998).

GPx indicates selenium status in animals (Ruck et al., 1997), but high selenium levels do not mean a proportional increase in selenoenzyme activity, which is referred to as the plateau effect (Raisbeck, 2000; Joksimović-Todorović Mirjana et al., 2006; Drljačić, 2013; Duan and Akesson, 2004).

Selenium can affect meat quality by reducing fat peroxidation during meat storage and preservation. It appears that in different species of poultry, there are specific differences in selenium metabolism in muscle. For example, a comparison of GPx activity in the muscles of chickens, turkeys, and ducks that ingest similar amounts of selenium shows that GPx activity in duck muscle is several times higher than in chickens or turkeys (Down and Akesson, 2004). Duck muscle tissue is characterized by an increased content of selenium, with differences in the concentration of selenium in the muscles of ducks and chickens ranging from 19 to 37% (Down and Akesson, 2004).

II. EXPERIMENTAL SETUP

The study of the influence of organic selenium on the production results of fattening ducks, carcass meat parameters, meat quality and selenium content in the meat and internal organs of fattened ducklings was conducted on a total of 240 one - day - old ducklings. Breeding eggs were imported from Hungary and hatched in the hatchery "HA Company" in Gracanica. The first move (I. repetition) of one-day-old ducklings into the prepared facility was on March 27, 2014. In the next two weeks, new ducklings (repetitions II and III) were made, on April 3 and 10, 2014.

Upon arrival at the immigration facility, and before being placed in the box, the one-day-old ducklings were weighed on a digital scale with a tolerance of ± 1 g and each individual was marked with a ring on its leg with its own number. In all repetitions, the ducklings were placed in special cardboard boxes for the first week for easier temperature control. Immediately during the weighing, groups of 20 ducklings were randomly formed and arranged in prepared and marked boxes. Ducklings were randomly divided into 4 experimental groups (K0, K1, K2 and K3). There were 60 one-day-old ducklings in each experimental group, and fattening was performed in three repetitions of 20 ducklings. The research plan is shown in Table 1.

Table 1. Research implementation plan

	Experimental groups			
	K0	**K1**	**K2**	**K3**
	Number of ducklings			
According to repetitions				
I - V1	20	20	20	20
II - V2	20	20	20	20
III- V3	20	20	20	20
Total	**60**	**60**	**60**	**60**

Duckling diet

The ducklings are in two phases of feeding duration in fattening, fed with two and nutritionally different concentrate feed mixtures: starter (from 1st to 14th day) and finisher (from 15th to 49th day of fattening).

The first, the control group of ducklings (K0) during fattening received food without added selenium in both phases of fattening.

The second group of ducklings (K1) was fed with food in both phases of fattening as well as the control group, but with the addition of 0.2 mg / kg of organic selenium (commercial preparation, Alkosel R 397, France).

The third group of ducks (K2) used food with 0.4 mg / kg of organic selenium in fattening, and the fourth group of ducks (K3) with 0.6 mg / kg of organic selenium.

The experiment lasted 7 weeks (49 days), and the food recipe for individual stages of fattening was adjusted to the selected duck hybrid.

At the beginning of fattening, the chemical composition of the concentrate mixtures as well as the selenium content were determined (to determine the total selenium content, i.e. basal derived from food and to add organic selenium).

At the beginning of the experiment, the activity of the enzyme glutathione peroxidase (GSH Px) was determined, and the activity of glutathione peroxidase in blood plasma was determined at the end of each phase of fattening.

Complete concentrate feed mixtures were prepared at the Hinus Animal Feed Factory in Srebrenik.

Before the ducklings arrived at the facility, water and food were prepared in shallow drinkers and feeders for tempering. Feeding and feeding from small drinkers and feeders was done in the first week of fattening. After that, the ducklings were fed from hanging feeders, the height of which was regulated according to the age of the ducklings. The feeding of the ducklings in this period was done by means of tin troughs with chains hung on the front part of the box fence, which also had the possibility of regulating the height according to the age of the ducklings. The ducklings had free access to food and water (ad libitum), and the facility was lit for 24 hours. The raw material compositions of the mixtures used in duck fattening (starter and finisher) are shown in Tables 2 and 3.

Table 2. Raw material composition of starter fattening concentrate mixture

Raw material (%)	**Concentrated starter mixture (1st to 15th day)**			
	Experimental groups			
	K0	**K1**	**K2**	**K3**
Corn	54,83	54,63	54,43	54,23
Soybean semolina	18,00	18,00	18,00	18,00
Soybean meal	16,00	16,00	16,00	16,00
Soy protein concentrate	5,00	5,00	5,00	5,00
Alcoholic yeast	2,50	2,50	2,50	2,50
Mono-Ca-phosphate	1,30	1,30	1,30	1,30
Premix for fattening ducks I	1,00	1,00	1,00	1,00
Livestock chalk	0,90	0,90	0,90	0,90
Fodder salt	0,35	0,35	0,35	0,35
Dl-Methiomin	0,12	0,12	0,12	0,12
Organic selenium (Se)	-	0,20	0,40	0,60
Σ	**100,00**	**100,00**	**100,00**	**100,00**

Tabela 3 Raw material composition of finisher fattening concentrate mixture

Raw material (%)	**Concentrated finisher mixture (15th to 49th day)**			
	Experimental groups			
	K0	**K1**	**K2**	**K3**
Corn	72,02	71,82	71,62	71,42
Soybean semolina	11,00	11,00	11,00	11,00
Soybean meal	9,00	9,00	9,00	9,00
Soy protein concentrate	2,50	2,50	2,50	2,50
Alcoholic yeast	2,00	2,00	2,00	2,00
Mono-Ca-phosphate	1,20	1,20	1,20	1,20
Premix for fattening ducks I	1,00	1,00	1,00	1,00
Livestock chalk	0,90	0,90	0,90	0,90
Fodder salt	0,30	0,30	0,30	0,30
Dl-Methiomin	0,08	0,08	0,08	0,08
Organic selenium (Se)	-	0,20	0,40	0,60
Σ	**100,00**	**100,00**	**100,00**	**100,00**

The calculated chemical composition of the mixtures used in duck fattening is shown in Tables 4 and 5.

Table 4. Calculative chemical composition of concentrate mixture starter for duck fattening

Name	Unit of measure	Starter concentrate mixture (1st to 15th day)			
		Experimental groups			
		K0	K1	K2	K3
Dry matter	%	88,06	88,07	88,08	88,08
Crude proteins	%	22,16	22,16	22,13	22,10
Crude fat	%	5,73	5,72	5,72	5,71
Crude fiber	%	3,34	3,34	3,33	3,33
Ca	%	0,65	0,63	0,63	0,63
Na	%	0,14	0,14	0,14	0,14
Cl	%	0,20	0,20	0,20	0,20
P	%	0,75	0,75	0,75	0,75
Lysine	%	1,41	1,41	1,41	1,41
Methionine	%	0,51	0,51	0,51	0,51
A	IJ/kg	10,00	10,00	10,00	10,00
D3	IJ/kg	2,00	2,00	2,00	2,00
E	mg/kg	30,00	30,00	30,00	30,00
ME poultry	MJ/kg	13,19	13,16	13,13	13,10

Table 4. Calculative chemical composition of concentrate mixture finisher for duck fattening

Name	Unit of measure	Finisher concentrate mixture (15th to 49th day)			
		Experimental groups			
		K0	K1	K2	K3
Dry matter	%	87,31	87,32	87,32	87,33
Crude proteins	%	16,09	16,08	16,08	16,05
Crude fat	%	4,52	4,51	4,51	4,50
Crude fiber	%	2,87	2,87	2,86	2,88
Ca	%	0,58	0,58	0,58	0,58
Na	%	0,12	0,12	0,12	0,12
Cl	%	0,17	0,17	0,17	0,17
P	%	0,67	0,67	0,67	0,67
Lysine	%	0,96	0,96	0,96	0,96
Methionine	%	0,41	0,41	0,41	0,41
A	IJ/kg	10,00	10,00	10,00	10,00
D3	IJ/kg	2,00	2,00	2,00	2,00
E	mg/kg	30,00	30,00	30,00	30,00
ME poultry	MJ/kg	13,38	13,35	13,32	13,28

Methods for determining GSH-Px activity (E.C.1.11.9.)

The method described by Gunzler et al. was used to determine the activity of glutathione peroxidase (GSH-Px). (1974), and is based on spectrophotometric recording of NADPH consumption in a coupled enzyme system.

Glutathione peroxidase (GSH-Px) activity was measured by a coupled assay (Gunzler et al., 1974) on a Gilford water bath spectrophotometer and a constant temperature thermostat at a measurement of 37 ºC. The measurement principle is based on spectrophotometric recording of NADPH consumption in the coupled enzyme system. The difference in NADPH consumption rate between sample and blank represents GSH-Px activity.

The necessary GR, GSH and NADPH (Sigma-Aldrich) solutions were always freshly prepared using redistilled water as solvent, and the low concentration of TBH-tertiary butyl hydroperoxide (<2.32 mmol) used in this method allowed only selenium-dependent glutathione peroxidase activity (Burk et al., 1978).

III. RESULTS AND DISCUSSION

The average activity of GPx in the blood plasma of the control and experimental groups of ducks during the experiment was examined on the 1st, 14th and 49th day of the experiment. The average activity of GPx in the blood plasma of the control and experimental groups of ducks during the experiment is shown in Table 6.

Table 6. Average activity of GPx in the blood plasma of control and experimental groups of ducks during the experiment (μkat / L)

Group	Days of experiment ($\overline{X}$ ±Sd)		
	1.	14	49
	14,40±0,69	-	-
K0	-	$7,22^{ABC}$±0,83	$11,06^{AB}$±0,99

K1	-	$13,54^{ADE}$±1,13	$12,13^{CD}$±0,45
K2	-	$15,77^{BD}$±1,10	$14,97^{ACE}$±0,84
K3	-	$16,05^{CE}$±0,94	$16,71^{BDE}$±0,54

Legend: same words A, B, C, D, E– p<0,01

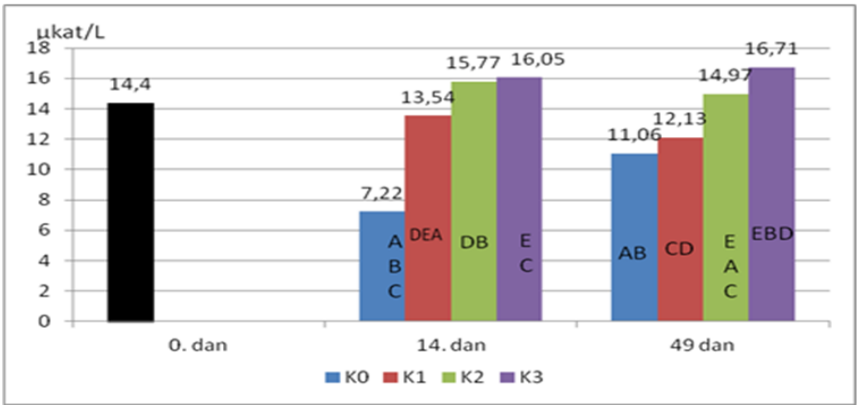
The most important function of selenoproteins is in their antioxidant ability, ie the ability to eliminate free radicals (eg hydrogen peroxide) (Shchedrina et al., 2010). Free radicals react with unsaturated fatty acids, which leads to damage to the cell membrane. So far, five selenoproteins, ie glutathione peroxidases, as well as thioredoxin reductases have been identified, which are important in preventing the negative effects of free oxygen radicals.

The importance of selenium in animal nutrition is most often studied through GPx activity. Selenium is present in the active site of this enzyme, so GPx activity is an indicator of selenium status in animals (Rotruck et al., 1973). It was found that with increasing selenium content in blood plasma, GPx activity also increases, but only at suboptimal and optimal selenium levels. Namely, high levels of selenium do not mean a proportional increase in the activity of selenoenzymes (Raisbeck, 2000). GPx activity can be determined by different analytical procedures and is also expressed in different units. This creates difficulties in comparing GPx activity in different studies.

On the first (zero day) day of the experiment, the activity of GPx in the blood plasma of ducks was 14.40 ± 0.69 μkat/L. In the second measurement, ie14. On the day of the experiment, the activity of GPx in the blood plasma of ducks of the control group was 7.22 ± 0.83 μcat / L and was statistically significantly lower (p <0.01) than the activity of GPx in the blood plasma of the experimental groups of ducks.

The activity of GPx in the blood plasma of the experimental group K1 was 13.54 ± 1.13 μkat/L and was statistically significantly lower (P <0.01) than the activity of GPx in the blood plasma of the experimental groups K2 and K3. No statistically significant difference was found between the activity of GPx in the blood plasma of ducks of the experimental group K2 (15.77 ± 1.10 μkat/L) and K3 16.05 ± 0.94 μkat/L.

At the end of the experiment, ie on day 49, the activity of GPx in the blood plasma of the control group of ducks K0 was 11.06 ± 0.99 μkat/L and was statistically significantly lower (p <0.01) than the activity of GPx in the blood plasma of experimental ducks. group: K2 (14.97 ± 0.84 μkat/L) and K3 (16.71 ± 0.54 μkat / L), but did not differ statistically significantly from the activity of GPx in the blood plasma of the experimental group K1 (12.13 ± 0.45 μkat/L). The activity of GPx in the blood plasma of K1 group ducks was statistically significantly lower (p <0.01) than the activity of GPx in the blood plasma of K2 and K3 group ducks. The activity of GPx in the blood plasma of K2 group ducks was found to be statistically significantly lower (p <0.01) than the activity of this enzyme in the blood plasma of the K3 group (Graph 1).

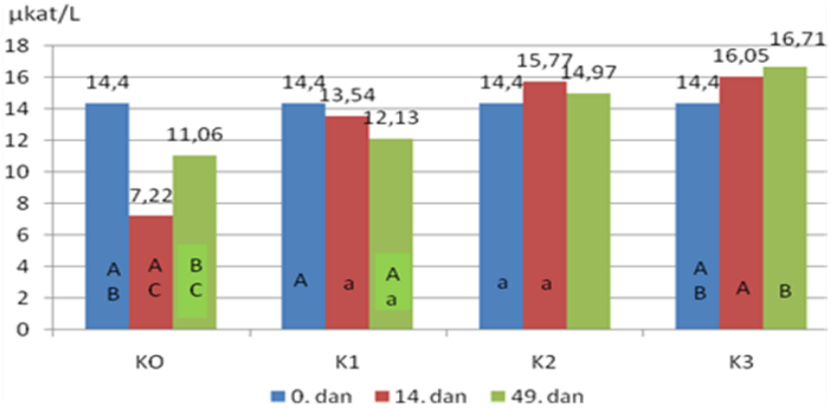


Graph 1. GPx activity in blood plasma of control and experimental groups of ducks

In the control K0 group of ducks, the activity of GPx in blood plasma was statistically significantly different (p <0.01) between all days of the study (days 1, 14 and 49). The activity of GPx in the blood plasma of ducks of the experimental group K1 was statistically significantly higher (p <0.01) on day 1 compared to day 49, but no statistically significant difference was found between the activity of GPx in blood plasma on days 1 and 14. It was found that the activity of GPx in the blood plasma of ducks on day 14 of the experiment was statistically significantly higher (p <0.05) than the activity of GPx in the blood plasma of ducks on day 49.

The activity of GPx in the blood plasma on the 14th day of the experiment in the blood plasma of the K2 group of ducks was statistically significantly higher than the activity of GPx in the blood plasma on the 1st day of the experiment. In other cases of comparison, no statistically significant difference was found in the activity of GPx in the blood plasma of the examined groups of ducks. In the blood plasma of ducks of group K3, the activity of GPx on the 1st day of the study was statistically significantly lower (p <0.01) than the activity of

this enzyme of this group of ducks on the 14th and 49th day, respectively. No statistically significant difference was found between the activity of GPx in the blood plasma of K3 group ducks on days 14 and 49, which can be seen in Graph 2.



Graph 2. GPx activity in blood plasma of control and experimental groups of ducks (µkat/L)

The activity of GPx in duck meat is many times higher than the activity of this enzyme in turkey broiler meat, as well as in lamb meat. Higher GPx activity was observed in duck breast meat compared to karabakat drumstick meat. In broiler and turkey meat, GPx activity was higher in carabak drumsticks than in breast meat. This is the result of differences in muscle type. The pectoral muscles of broiler turkeys belong to the glycolytic type of muscle (white meat), which contains less mitochondria and less myoglobin, and the muscles of the thighs with carabatka belong to the oxidative type of muscle (red meat, more mitochondria and more myoglobin). The oxidative type of muscle mainly uses fatty acids as an energy substrate and has less ATPase and phosphorylase activity, and the glycolytic type of muscle uses mainly glycogen as energy. In ducks, the pectoral musculature belongs to the oxidative, and the musculature of the thighs with the carabatic glycolytic type of muscle, which is the opposite in relation to broilers, ie turkeys (Down and Akesson, 2004).

IV. CONCLUSION

1. The average activity of GPx in the blood plasma of one-day-old ducklings was 14.40 µkat / L and in the K0 group, which was fed without the addition of organic selenium, it was twice lower on day 14 (7.22 µkat / L) and on day 49 was 11.06 µkat / L
   .
2. The average activity of GPx in ducks on day 14 was 13.54 µkat / L, and on day 49 12.13 µkat / L, which is less than the average activity of GPx ducks on the first day of the experiment. In ducks of K2 and K3 groups, on day 14 and 49, respectively, GPx activity was higher than GPx activity recorded on the first day of the experiment, so that on day 14 it was 15.77 µkat / L (K2 group) and 16.05 µkat / L (K3 group), and on the 49th day 14.97 µkat / L (K2 group) and 16.71 µkat / L (K3 group).

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