UDC: 619: 615,355

Gutyi B., Les'kiv K., Hufriy D., Binkevych V., Farionik T., Binkevych O. ©
Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z Gzhytskyi

METIFEN IMPACT ON THE ANTIOXIDANT PROTECTION OF THE LITTLE PIGS BODIES

The effect of metifen on antioxidant status of the pigs' organisms was studied . Established that piglets nitrate load (0.3 g $NO3^-$ / kg body weight) causes inhibition of enzymes of antioxidant system (glutathione peroxidase, superoxide dismutase, catalase). Feeding pigs a diet consisting of antioxidant metifen had positively influences on the activity of the above enzymes. Increased activity of antioxidant enzymes studied system to the highest rates observed in the applicationof metifeu at a dose of 0.9 mg / kg. w. an ..

Key words: *metifen, antioxidant system, glutathione peroxidase, superoxide dismutase, catalase, nitrate-nitrite load.*

Recently, due to the systematic use of large amounts of nitrogen fertilizer, and the adverse effects of the environment, the impact of nitrates and nitrites on animals, leading to widespread acute and chronic poisoning [3].

Nitrates are characterized by a fairly wide range of toxic effects. This action of nitrates is the development of hypoxia, oxygen starvation tissue that develops as a result of violation of transporting oxygen and blood to inhibition of enzyme systems involved in the above-mentioned diseases. Nowadays it is known important role of pereoxide lipid oxidation (LPO) in the development of many toxicosis, including nitrate-nitrite. One form of respiration is LPO. This process is inherent in normal tissue and is usually in the lipid membrane structures [1].

Effects of nitrite on animals accompanied by the formation of methemoglobin in the blood, where ferrous iron is oxidized to ferric hemoglobin. The process of oxidation of hemoglobin is realized through its interactions with oxide nitrite ion in the chain of path. In the oxidation of hemoglobin a number of radical metabolites are produced that are active oxidants biological substrates, they have pronounced cytotoxic effect, initiate processes pereoxide lipid. During the oxidation of oxyhemoglobin reactive oxygen species are included as direct participants of elementary stages, toxic may be produced with hydrogen peroxide, which are also involved in the oxidation of oxyhemoglobin. Oxidative stress is accompanied by a balance between the intensity of free radical oxidation and antioxidant defense system [2].

At the Department of Pharmacology and Toxicology, Lviv National University of Veterinary Medicine and Biotechnology named after S.Z. Gzhytskyi developed antioxidant product - metifen. Antioxidant in the structure contains fenaron and methionine. Fenaron - a complex of compound consisting 70% of fenozan-acid and 30% of zeolite. Established that fenaron reacts with radicals of fatty acids and prevent the development of oxidative stress chain reaction, reduces oxidation of

phospholipids and forms a biologically inactive compound products from peroxidation of fats. Metifen - antioxidant, it consists in optimal doses introduced methionine (80 mg) fenaron (200 mg) and filler - 1 g drug compatible components on the physico-chemical properties and exhibit in the "Metifen" synergy pharmacological action. Basically, its effect was studied in laboratory on animals and birds bodies, because our aim is to study the antioxidant system of the pigs' bodies nitrate loading [4].

The aim of our study was to determine the impact on the state metifen antioxidant system of piglets with nitrate-nitryt loading.

Materials and methods. The objects of research were 20 pigs of large white breed at the age of three months. The study was conducted in ESPC Komarnivskyi in Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyi. By using group-analogues were formed 4 groups: control and three experimental groups. The scheme of the experiment is shown in Table 1.

Table 1

C_1 Control group	Pigs of the group were fed with general diet of the farm, sodium nitrate at a dose of 0.3 gr NO_3^-/kg of weight once a day along a month.
T_1 Test group	Pigs of the group were fed with general diet of the farm with addition of metifen at a dose 1 mg/kg. an. + sodium nitrate at a dose of grNO_3^-/kg of weight once a day along a month.
T_2 Test group	Pigs of the group were fed with general diet of the farm with addition of metifen at a dose 0.9 mg/kg. an. + sodium nitrate at a dose of 0.3 grNO_3^-/kg of weight once a day along a month
T_3 Test group	Pigs of the group were fed with general diet of the farm with addition of metifen at a dose 0.85 mg/kg. an. + sodium nitrate at a dose of 0.3 grNO_3^-/kg of weight once a day along a month

Blood samples were taken from the cranial vena cava at 1, 3, 6, 9 hours after feeding with sodium nitrate. AOC status in nitrate-nitrite load assessed by activity in blood enzymes: catalase, superoxide dismutase, glutathione peroxidase.

The activity of glutathione peroxidase (GP-K.F.1.11.1.9) was determined by the method of V. Lemeshko, Superoxide dismutase activity was determined by the method of Chevari, the activity of catalase (CT K.F. 1.11.1.6.) - Bach and Zubkov method.

Results and discussion of research.

Activity of the enzyme superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GPO) is one of the key indicators that indicate the state of the antioxidant system.

To avoid various complications in the course of disease can be achieved by blocking the timely trigger of pathology, the decrease in intensity of lipid peroxidation in the body through the use of antioxidants, which prevent the formation of free radicals that can damage cells. Therefore, these studies provide an opportunity to study the protective effect of metifen the state of the antioxidant system in nitrate-nitrite load.

It is known that catalase restores hydrogen pereoxide to water. To the active center of the enzyme include ferric iron protoporphyrin, which reacts with hydrogen

peroxide by catalase, or peroxidase mechanism, depending on the substrate concentration.

The obtained results are shown in the table:

Table 2

The activity of catalase in pig's blood erythrocytes nmol / min x mg protein M ± m, n = 5.

Group	At the begining	Hours			
		1	2	3	9
1 C	1,28 ± 0,08	1,18 ± 0,07	1,21 ± 0,08	1,24 ± 0,08	1,25 ± 0,08
2 T	1,33± 0,05*	1,32±0,02***	1,32 ±0,02**	1,29±0,07*	1,30±0,07***
3 T	1,38 ±0,07*	1,37±0,05***	1,35±0,08***	1,34±0,08***	1,34 ±0,08**
4 T	1,38±0,06*	1,36±0,07***	1,35± 0,08**	1,31 ± 0,05*	1,33±0,05**

*In this and the following tables the degree of probabilit can be compared to control groups: * -p ≤ - 0,05; ** -p ≤ - 0,02; *** -p ≤ 0,001*

Analyzing the results of Table 1, the activity of catalase in animal test group, along with nitrites in the diet antioxidant metifen were administered with values within 1,31-1,38 nmol / min per mg protein. The animals of the control group, were not with metifenfed and catalase activity began to drop do1,18-1,28 nmol / min per mg protein.

On the basis of catalase activity is difficult to draw conclusions about the extent of the negative impact of nitrates and nitrites in the antioxidant defense system of the organism pigs.

It is known that glutathione peroxidase - catalyzed decomposition of lipid hydroperoxides moderate form by using reduced glutathione, namely the collapse of catalyzed hydrogen peroxide and glutathione oxidation. Glutathione peroxidase together with other antioxidants helps to remove primary products partially reduced oxygen.

Table 3

The activity of glutathione peroxidase in pig's blood erythrocytes nmol / min x mg protein, M ± m, n = 5

Group	At the beging	Hours			
		1	3	6	9
1C	34,20 ± 0,18	33,80 ±0,18	31,46 ±0,18	28,21 ± 0,15	29,01 ± 0,11
2 T	35,14±0,16	35,13±0,14*	35,12±0,16**	35,14±0,17**	35,14±0,17**
3 T	35,18 ±0,18	35,18±0,18*	35,19±0,15**	35,20 ±0,17**	35,21±0,18**
4 T	35,21±0,15	35,18±0,18*	35,17±0,15**	35,17±0,15**	35,18±0,18**

SOD - a key enzyme antiradical protection. It transfers superoxidradykal to less toxic hydrogen peroxide. Depending on the trace elements that are in the active center of the enzyme, isolated by Fe-, Zn-Cu- and Mn-dependent SOD. Metals perform catalytic function. They are consistently recovered and oxidized in the active site of the enzyme. Fe-dependent SOD in more located in erythrocytes, Zn-Cu-dependent - in the cytoplasm, and Mn-dependent - in mitochondria.

In a further determination of enzyme activity of glutathione peroxidase and superoxide dismutase, the degree of negative impact of stress on the body nitrate pigs manifested more fully. So in animal research of enzyme activity values were within values: glutathione 35,12-35,21 nmol / min per mg protein, superoxide dismutase

34,17-35,82 AA / min per 1 mg protein. These rates were 1.5 times higher values than the control group.

Table 4

The activity of superoxide dismutase of erythrocytes in pigs' blood nmol / min x mg protein M ± m, n = 5.

Group	At the begining	Hours			
		1	3	6	9
1C	35,39 ± 0,07	34,30 ± 0,9	29,68 ± 1,2	27,10 ± 1,2	30,05 ± 1,1
2 T	35,81 ± 1,8**	34,48±1,2**	34,28±1,6**	34,17±1,5**	34,20±1,21*
3 T	35,80±0,16***	34,44±1,3**	34,30± 1,7**	34,27±1,4**	34,40±1,6**
4 T	35,82 ±0,19**	34,42±1,7**	34,39 ±1,8**	34,22±1,4**	34,24±1,7**

In animal research groups, there was a possible reduction in the activity of enzymes of antioxidant system. It has a positive impact of metifen.

The animals of the control group were founded inhibition of enzymes of antioxidant system: glutathione by 18%, 16% glutathione reductase and catalase by 13%. This indicates a decrease in antioxidant protection.

Conclusions.

1.When piglets were fed with food sodium nitrate at doses of 0.3 g $NO3^-$ / kg found inhibition of enzymes of antioxidant system.

2 When nitrite loading, feeding the diet of pigs antioxidant metifenu positively influences on the activity of enzymes of antioxidant system.

3 When metifen was used in dose of 0.9 mg / kg. m., op they. increased activity of antioxidant enzymes studied systems with the highest performance.

Used Books:

1.Activity of antioxidant enzymes and malondialdehyde content in sows and piglets under the influence of metabolic energy and selenium / V. Danchuk, VV Snitynskyy, OM Buchko, RJ, Spark // scientific-technical. Bull. Inst zemler. and biological. TV. (series - physiological., and Biochem.) Vol. And (3) .- Lviv.- 1999.- S. 18-21.

2.Antioxide defense system (review) IF Byelenichev, EL Levitsky, Y. Gubskiy, C.I. Kovalenko, A. Marchenko // sovr. problems. Toxicological. -2002.- №3.- S.24-31.

3.The impact of fertilizers, methods of harvesting, and storage of fodder for Using Content nytrats and nytryts / I.G. Aristov, N.G. Zolotov, N.G. Tokach et al. // Tez Dokl. Rep. Confit. "The problem nytratov in animal husbandry and veterynaryy" Kiev: Izd USHA, 1990 - C.3.

4.Hunchak V.M. Guidelines for use of the drug "Metifen" // Approved by the State Research Institute for Veterinary drugs and feed additives. - Lviv, 2004 - 2 p.

5 Hufriy D.F. Changes in activity of enzymes in the blood of bulls under the influence of sodium nitrate in different doses // HT Bull. UNDIFIV, Lviv, 1992 - Vol. 14/1. - S.18-20

Рецензент – д.вет.н., професор Гунчак В.М.