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The influence of the feed additive “Metisevit” on the activity of the antioxidant defense system of piglets under conditions of nitrate-nitrite load

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The work aimed to investigate the effect of the “Metisevit” feed additive on the activity of the enzymes of the antioxidant system of the blood of piglets under conditions of nitrate-nitrite load. The research was carried out on 60-day-old piglets of the large white breed. For the experiment, 10 clinically healthy piglets were selected, from which 2 groups of five animals were formed. Piglets of the control group were fed sodium nitrate at a dose of 0.3 g NO₃/kg body weight for three months. Piglets of the research group were also fed sodium nitrate at a dose of 0.3 g NO₃/kg of body weight, together with Metisevit at a dose of 0.36 g/kg of feed for three months. The “Metisevit” feed additive was developed at the Department of Pharmacology and Toxicology of the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnology Lviv, which contains vitamin E, selenium, and methiphen (methionine, phenarone, zeolite). Under the conditions of feeding piglets with sodium nitrate feed at a dose of 0.3 g of NO₃/kg, a decrease in the activity of enzymes of the antioxidant defense system was established: superoxide dismutase by 14.8 %, catalase by 20%, glutathione peroxidase by 15.1 %. Metisevit in piglets increased the activity of the enzyme link of the antioxidant protection system, preventing the development of oxidative stress that occurs in nitrate-nitrite toxicosis. During our experiments, it was found that the introduction of Metisevit into the diet of piglets with excessive consumption of nitrates prevented the development of chronic nitrate-nitrite toxicosis. It was established that Metisevit increases the activity of enzymes – superoxide dismutase, catalase, and glutathione peroxidase. These enzymes reduce the high level of lipid peroxidation products in the body of piglets under conditions of nitrate-nitrite load.

Key words: piglets, feed, nitrates, nitrites, antioxidants, enzymes, Metisevit.

Introduction

Pig farming in Ukraine plays a crucial role in the production of most products of animal origin. The promising development of this industry depends on the effective reproduction of the pig population (Povod et al., 2022, 2023; Mykhalko et al., 2023; Khalak et al., 2023).

Modern methods of intensive breeding of pigs in various farms, using highly productive breeds and industrial technologies of maintenance, are significantly different from traditional approaches. However, it is essential to

note that processes such as early weaning of piglets from sows, transportation of animals, and other aspects are significant stressors that can cause disturbances in the protective reactions of the piglets' body (Kramarenko et al., 2019; Martyshuk et al., 2019, 2020, 2021, 2023).

This can cause an imbalance between the activity of the antioxidant system and the intensity of lipid oxidation, causing stress, growth retardation, increased disease susceptibility, and mortality in pigs. It can also affect the reproductive capacity and quality of meat products (Vyslotska et al., 2021; Khalak & Gutyj, 2023).

It is also important to note that the problem of environmental pollution with nitrates and their negative impact on the body of pigs is becoming very relevant in research into the mechanisms of their toxic action. This is of great importance both theoretically and practically (Leskiv et al., 2022; Gutyj et al., 2023).

When nitrates enter the body of animals, they first irritate the intestinal mucosa, which can later lead to inflammation. These substances contribute to the reproduction of pathogenic microflora, which secretes poisonous substances that contribute to autotoxicity, that is, poisoning of the body itself. Nitrates strongly influence the development of cancerous tumors in the digestive tract, as research results show.

After absorption, nitrates enter the blood, disrupting its ion balance, and spread to tissues and organs. The largest nitrate was found in the liver and kidneys, and the smallest – in the muscles. In the blood, nitrates and nitrites cause the main toxic effect; in particular, they contribute to forming methemoglobin (Douglas & Lee, 1970; Aslani & Vojdani, 2007; Gutyj et al., 2017).

The formation of methemoglobin is a process that takes place with the participation of nitrite ions in a free radical process. The toxic metabolites formed contribute to the destruction and decomposition of erythrocyte membranes, leading to the release of hemoglobin. This process leads to increased oxidation of hemoglobin to methemoglobin, which, in turn, causes tissue oxygen starvation or hypoxia (Saito et al., 1996; Leskiv et al., 2020).

Therefore, the study of the antioxidant system in pigs during nitrite intoxication is highly relevant, which will allow the development of an effective scheme of treatment and prevention of nitrate-nitrite toxicosis in pigs, taking into account the antioxidant system.

Aim of the research

The work aimed to investigate the effect of the feed additive “Metisevit” on the enzyme link of the system of antioxidant protection of piglets under conditions of nitrate-nitrite load.

Materials and Methods

The research was carried out on 60-day-old piglets of the large white breed. We strictly followed the mandatory principles in zootechnical research regarding the selection and maintenance of participating animals in the appropriate groups, the technology of care, and the use and accounting of used feed. The animals' diet was balanced regarding nutrients and minerals necessary to meet their basic nutritional needs.

The research aimed to analyze the activity of the antioxidant system and the influence of the Metisevit feed additive on regulating the balance between the antioxidant protection system and the process of lipid oxidation.

For the experiment, ten clinically healthy piglets were selected, from which two groups of five animals were formed:

1 group – control (C), piglets were fed sodium nitrate at a dose of 0.3 g NO₃⁻/kg body weight for three months;

In the 2nd group – experimental (E), the piglets were fed sodium nitrate in a dose of 0.3 g NO₃⁻/kg of body weight together with Metisevit in an amount of 0.36 g/kg of feed for three months.

Test drugs were administered orally. The necessary amount of them weighed for the corresponding group of experimental animals was mixed with compound feed. The feed was repeated every morning for three months.

The “Metisevit” feed additive was developed at the Department of Pharmacology and Toxicology of the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, which contains vitamin E, selenium, and methiphen (methionine, phenarone, zeolite).

Blood for biochemical studies was taken from animals – from the tail vein. The blood was stabilized with heparin. The blood serum was separated from the formed elements by centrifugation for 5–8 min at 3000 rpm.

The activity of glutathione peroxidase (K.F.1.11.1.9) was determined by the method of V. M. Moina (1986), the activity of superoxide dismutase was determined (KF 1.15.1.1.) – according to the method of E. E. Dubinina (1983), catalase activity (KT-K.F. 1.11.1.6.) – according to the method of M. A. Koroliuk et al. (1988) (Vlizlo et al., 2012).

The research results were subjected to biometrical statistical analysis using the student's probability criterion and computer technology. The degree of probability, compared to the control group's data, was – P < 0.05 –*, P < 0.002 –**.

Results and discussion

Nitrates, when they affect animals' bodies, cause methemoglobin formation in their blood. This is a condition where the divalent iron of hemoglobin changes to trivalent iron. This transition occurs due to the interaction of the oxyform of hemoglobin with nitrite ions along a particular path. This process creates a variety of radical metabolites that are potent oxidants for biological substances and can have significant cytotoxic effects. They also enhance the processes of lipid peroxidation. During the oxidation of oxyhemoglobin, active forms of oxygen become participants in the main stages of the process, forming toxic hydrogen peroxide, which also participates in the oxidation processes of oxyhemoglobin. This leads to the development of oxidative stress.

Oxidative stress occurs when the balance between the intensity of free radical oxidation and the antioxidant defense system is disturbed. In the case of nitrate-nitrite toxicosis in piglets, the leading radical that triggers lipid peroxidation processes is the superoxide radical. Therefore, the study of superoxide dismutase, which converts this radical into less toxic hydrogen peroxide, is important. This enzyme is key in the anti-radical protection system and belongs to the direct-acting antioxidants.

During the study of chronic nitrate-nitrite toxicosis in piglets, a decrease in the activity of this enzyme was found from the 10th day of the experiment, when it was 31.10 ± 0.18 IU/min x mg of protein (Table 1). On the 30th day of the experiment, the activity of SOD decreased by 11.1 % compared to the initial values. On the 60th day

of the experiment, the lowest activity of the enzyme was found, where, accordingly, it decreased to 28.48 ± 0.17 UA/min x mg of protein.

Metisevit in piglets, which were simultaneously administered sodium nitrate at a dose of $0.3 \text{ gNO}_3^-/\text{kg}$ of

body weight, prevented the depletion of superoxide dismutase enzyme activity, as indicated by the results of studies presented in [Table 1](#).

Table 1

The effect of Metisevit on the activity of superoxide dismutase in the blood of piglets with chronic nitrate-nitrite toxicosis, UO/min x mg of protein ($M \pm m$, $n = 5$)

Periods of the research	Groups	
	Control	Experimental
At the beginning	33.42 ± 0.12	33.79 ± 0.13
10 th day	31.10 ± 0.18	32.45 ± 0.10
30 th day	29.72 ± 0.15	$32.86 \pm 0.12^{**}$
60 th day	28.48 ± 0.17	$32.93 \pm 0.12^{**}$
90 th day	30.05 ± 0.16	$33.54 \pm 0.09^{**}$

When Metisevit was fed to experimental piglets together with sodium nitrate, the activity of superoxide dismutase on the 10th day of the experiment was 32.45 ± 0.10 U/min x mg of protein, respectively. On the 30th day of the experiment, the activity of this enzyme in the experimental group increased by 10.6 %. On the 60th day of the experiment, even more, significant changes in the activity of this enzyme were found; in the experimental group, it was 32.93 ± 0.12 IU/min x mg of protein, and in the control group of animals, this activity was 28.48 ± 0.17 IU/min x mg of protein.

Therefore, the use of Metisevit led to an increase in superoxide dismutase activity in the blood of piglets exposed to nitrate and nitrite loading. This may be due to the direct participation of this drug in neutralizing free radicals and lipid oxidation products.

It is important to note that the balance between superoxide dismutase (SOD) and catalase is crucial for the functioning of the enzyme system of the body's antioxidant protection since catalase performs a catalytic role in the decomposition of hydrogen peroxide, turning it into water and oxygen.

Catalase activity in the blood of piglets during the development of chronic nitrate-nitrite toxicosis is presented in [Table 2](#). As this table shows, on the 10th day of the experiment, catalase activity slightly decreased compared to the initial values. On the 30th day of the experiment, the activity of this enzyme decreased by 14.4 %, and on the 60th day of the experiment, respectively, by 20 % from the initial values taken at the beginning of the study.

Table 2

The effect of Metisevit on the activity of catalase in the blood of piglets with chronic nitrate-nitrite toxicosis, nmol/min x mg of protein ($M \pm m$, $n = 5$)

Periods of the research	Groups	
	Control	Experimental
At the beginning	1.25 ± 0.05	1.24 ± 0.06
10 th day	1.15 ± 0.04	$1.26 \pm 0.07^*$
30 th day	1.07 ± 0.06	$1.24 \pm 0.04^{**}$
60 th day	1.00 ± 0.10	$1.22 \pm 0.03^{**}$
90 th day	1.05 ± 0.07	$1.25 \pm 0.06^*$

When studying the activity of catalase in the blood of the experimental group of piglets, it was established that feeding Metisevit contributed to a probable increase in this enzyme's activity, starting from the 10th day of the experiment. In the indicated period of the experiment, an increase in catalase activity by 9.6 % compared to the indicators of the control group was established. On the 30th day of the experiment, catalase activity fluctuated within 1.24 ± 0.04 nmol/min x mg of protein, while in control – 1.07 ± 0.06 nmol/min x mg of protein. A more likely increase in enzyme activity was established on the 60th day of the experiment, where it increased by 22 %, following the control group.

The increased catalase activity in the blood of the research group's piglets indicates a high activity of oxida-

tion and reduction processes in piglets fed the antioxidant drug Metisevit.

Another critical component of the antioxidant system is the glutathione system, which has both enzymatic and non-enzymatic components. An essential enzyme in this system is glutathione peroxidase, which protects the animal body against oxidative damage. This enzyme actively catalyzes the reduction of lipid peroxides and the conversion of hydrogen peroxide into water. The activity level of this enzyme in the blood of animals is an essential indicator of the state of the body's glutathione system of antioxidant protection.

During the development of chronic nitrate-nitrite toxicosis, a decrease in glutathione peroxidase activity is observed in the blood of piglets ([Table 3](#)). At the beginning of the experiment, the activity of glutathione peroxi-

dase in the blood of piglets in the control and experimental groups was within $35.52 \pm 0.13 - 35.54 \pm 0.10$ nmol/min x mg of protein. On the 10th day of the experiment, the activity of this enzyme in the blood of the control group of animals decreased by 6.7 %, on the 30th day of the experiment, respectively, by 11.2 % compared to the initial values. On the 60th day of the experiment, the activity of glutathione peroxidase in the blood of animals was the lowest, amounting to 30.16 ± 0.10 nmol/min x mg of protein. On the 90th day of the experiment, an increase in the activity of this enzyme was noted, which may be related to the body's ability to adapt to prolonged exposure to sodium nitrate.

Feeding the “Metisevit” drug to the experimental group's piglets increased the glutathione peroxidase activ-

ity in their blood. Thus, the enzyme's activity on the 10th day of the experiment was 36.14 ± 0.12 nmol/min x mg of protein. On the 30th day of the experiment, the enzyme activity in this experimental group of animals increased by 12.3 % compared to the indicators of the control group. The highest activity of glutathione peroxidase was in the blood of the experimental group on the 30th, 60th, and 90th days of the experiment. Thus, on the 60th day of the experiment, the activity of the enzyme in the blood of the experimental group of animals was 35.38 ± 0.18 nmol/min x mg of protein, and on the 90th – 35.53 ± 0.15 nmol/min x mg of protein, where, compared to the control, the activity increased by 17.3 and 6.1 %, respectively.

Table 3

The effect of Metisevit on the activity of glutathione peroxidase in the blood of piglets with chronic nitrate-nitrite toxicosis, nmol/min x mg of protein ($M \pm m$, $n = 5$)

Periods of the research	Groups	
	Control	Experimental
At the beginning	35.52 ± 0.13	35.54 ± 0.10
10 th day	33.15 ± 0.09	$36.14 \pm 0.12^*$
30 th day	31.54 ± 0.12	$35.42 \pm 0.17^{**}$
60 th day	30.16 ± 0.10	$35.38 \pm 0.18^{**}$
90 th day	33.48 ± 0.19	$35.53 \pm 0.15^*$

Therefore, using Metisevit in piglets increased the activity of the enzyme link of the antioxidant protection system, preventing the development of oxidative stress that occurs in nitrate-nitrite toxicosis.

Conclusions

When sodium nitrate is added to the feed of piglets at a dose of 0.3 g per kilogram of weight, it suppresses the activity of enzymes that protect the body from oxidative processes. A decrease in superoxide dismutase, catalase, and glutathione peroxidase activity indicates this. On the 60th day of the experiment, the lowest indicators of the activity of these antioxidant enzymes were noted.

The use of Metisevit in piglets during nitrate and nitrite loading strengthened the body's antioxidant protection, which was confirmed by an increase in the activity of enzymes of the antioxidant system in the blood of these piglets.

Conflict of interest

The authors declare that there is no conflict of interest.

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