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Impact of antioxidants on enzym activities of glutatione system of bulls bodies antioxidant defense under acute nitrate and nitrite toxicity

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The article presented the results of studies of the effect of sodium nitrate on indexes of glutatione system of antioxidant defense system in young cattle, such as the activity of glutathionereductase, glutathioneperoxidase and glucose-6-phosphate dehydrogenase. It was founded that feeding bulls with the toxicants at a dose 0,45 NO_3^- / kg enzyme activity in the blood of experimental animals throughout the experiment decreased. After using the nitrate load of young cattle it was used drugs Ursovit-ADES and sodium selenite. It was founded stimulatory effects on activity of glutathione system of antioxidant defense. Specifically,it was founded significant activity increase of glutathionereductase, glutathioneperoxidase and glucose-6-phosphatedehydrogenase. in the blood of young cattle, which were conducted with nitrate loading.

Key words: *nitrates, bulls, antioxidants, toxicology, antioxidant system.*

Вплив антиоксидантів на активність ензимів глутатіонової системи антиоксидантного захисту організму бичків за гострого нітратно-нітритного токсикозу

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У статті наведено результати досліджень впливу нітрату натрію на показники глутатіонової системи антиоксидантного захисту організму молодняку великої рогатої худоби. Досліди проводились на бичках шестимісячного віку, чорно-рябої породи. Активність глутатіонпероксидази (К.Ф.1.11.1.9.) та глутатіонредуктази (К.Ф.1.6.4.2.) визначали за методом В.В. Лемешко і співавт., активність глюкозо-6-фосфатдегідрогенази (К.Ф.1.1.1.49.) – за методом N.Z. Baquezetal. Венозну кров відбирали на початку досліду та через 3 години після згодовування бичкам нітрату натрію, а також через 1, 2, 3, 6, 9, 12 годин після введення вітамінних препаратів.

Встановлено, що за умов згодовування бичкам нітрату натрію у дозі 0,45 NO_3^- /кг маси тіла активність ензимів глутатіонової системи крові тварин упродовж усього досліду знижувалась. За умов нітратного навантаження, молодняку великої рогатої худоби застосовували препарати урсовіт АДЕС та селеніт натрію. Застосування препаратів-антиоксидантів за умов розвитку гострого нітратно-нітритного токсикозу у бичків сприяли підвищенню активності ензимної ланки глутатіонової системи антиоксидантного захисту (глутатіонредуктази, глутатіонпероксидази, глюкозо-6-фосфатдегідрогенази) у крові дослідних тварин. Сукупне введення урсовіту-АДЕС та селеніту натрію проявляло кращу дію на глутатіонову систему антиоксидантного захисту організму бичків ніж застосування лише урсовіту-АДЕС.

Ключові слова: *нітрати, бички, антиоксиданти, токсикологія, антиоксидантна система.*

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Introduction

In modern toxicology an activation of lipid peroxidation is taken as a universal response of a living organism to the action of extreme factors (Baglaj et al., 2010; Staryk et al., 2012; Gutyj, 2013; Guberuk et al., 205; Nazaruk et al., 2016; Gutyj et al., 2017). Overall, prooxidant and antioxidant status of animals reflects a balance between two oppositely directed actions in the body, namely antioxidant properties (defense) and the formation of free radicals (damaging). The influence of extreme factors including toxins, leads to an imbalance between them to prooxidant direction and develops so-called «oxidative stress» (Antonjak et al., 2000; Nazaruk et al., 2012; Nazaruk et al., 2015; Martyshuk et al., 2016; Khariv et al., 2016; Khariv and Gutyj, 2017).

Environmental contaminations with nitrates and nitrites and their negative impact on the animals make the problem of studying the mechanism of nitrate and nitrite toxicity on farm animals particularly relevant, which has theoretical and practical importance.

Aaccording to published scientific papers about the study of nitrate-nitrite toxicity in farm animals there are some reports that claim that the toxic effect of nitrates is manifested in two interrelated ways. The first step gets methemohlobinmaking and activation of free radicals which initiate the second phase of lipid peroxidation (Gunchak et al., 2010; Guberuk et al., 2012). The intensity of free radical peroxidation in animals depends on the concentration of oxygen in the tissues, and the activity of the enzyme and nonenzyme systems.

Therefore, the aim of our research was to study the effect of Ursovit-ADES and sodium selenite on activity of the enzymatic antioxidant protecting system of young cattle bodies under acute nitrate and nitrite toxicity.

Material and methods

Experiments were conducted on six- month old bulls, black and white breed, they were formed into 3 groups, 5 animals each.

Bulls in control group were fed with food once with sodium nitrate in a dose 0.45 NO_3^- / kg of body weight. Bulls in the first experimental group were fed once with sodium nitrate in a dose 0.45 NO_3^- / Kg and in three hours later they were injected intramuscularly with Ursovit-ADES at a dose of 4 ml / animal. Bulls in the second experimental group were fed with sodium nitrate in a dose 0.45 NO_3^- / Kg and in three hours later they were injected intramuscularly with Ursovit -ADES at a dose of 4 ml and 0.5% solution of sodium selenite at a dose of 1 ml. Venous blood were taken at the beginning of the experiment and in 3 hours after feeding bulls with sodium nitrate, and in 1, 2, 3, 6, 9, 12 hours after administration of vitamin preparations.

The activity of glutathione peroxidase (GP) (K.F.1.11.1.9.) and glutathionereductase (GR) (K.F.1.6.4.2.) it was determined by the method of V. Lemeshko et al. (Lemeshko et al., 1985), the activity of glucose-6-phosphatedehydrogenase (G-6-FDG) (K.F.1.1.1.49.) – method of N.Z. Baquezetal (Baquezetal et al., 1967).

Results and discussion

In the pathogenesis of various toxicosis it was involved lipid peroxidation and antioxidant system in physiological conditions, maintain a system of regulation of cellular homeostasis (Hutyi, 2005; Hutyi and Hufrii, 2005). So, under acute nitrate and nitrite toxicosis we used drugs that have both direct and indirect antioxidant effects on reactive oxygen species and lipid peroxidation products.

An important antioxidant defense system is glutatione system that is represented with enzyme system: glututionreduktase, glutathioneperoxidase, glucose-6-phosphatedehydrogenase.

The activity changes of glututionreduktase under acute nitrate and nitrite toxicosis of young cattle and influence of vitamin drug and sodium selenite are given in Table 1.

At the beginning of the experiment, the activity of GH in bulls' serum were within physiological values. After feeding sodium nitrate the activity of this enzyme in the control group began to fluctuate within 1.71 ± 0.045 – 1.20 ± 0.033 nmol NADPH / min. to 1 mg of protein. Only in three hours it was marked the activation of the enzyme, which compared with control values, the enzyme activity increased to 9.6, 6.3 and 4.3%.

After administration of drugs in the patient bulls enzyme activity GH in serum increased. When using vitamin-drug Ursovit-ADES animal group D1 in two hours of experiment GR activity increased by 4%, while in six hours it increased respectively by 22%. In eight hours GH activity reached physiological limits values. When combined use of Ursovit-ADES and sodium selenite GR enzyme activity was within the values of 1.62 ± 0.048 – 1.51 ± 0.035 nmol NADPH / min. to 1 mg of protein during the experiment.

The second important enzyme of glutatione system is a glutatioperoksydasa that catalyzes the schedule by a moderate form of lipid hydroperoxides using glutathione. The activity of this enzyme in serum of bulls blood after application of Ursovit-ADES and sodium selenite under acute nitrate and nitrite toxicity is given in Table 2.

Before feeding with sodium nitrate GP activity in blood serum of experimental groups of animals was within 37.10 ± 1.1 – 37.18 ± 1.2 nmol NADPH / min. to 1 mg of protein. After feeding bulls with sodium nitrate, we have noted in three hours of the experiment, a slight increase, but in the control group of animals GP activity further decreased to 28.25 ± 1.1 nmol NADPH / min. to 1 mg of protein. After introduction of the drug and vitamin sodium selenite in bulls research groups, enzyme activity was increased comparing to control group. Thus, in the experimental group D1 bulls enzyme activity in three hours of the experiment increased to 19%, in six hours – to 28%, while the experimental group D2 activity grew to 24 and 31% relative values of the control group animals.

Table 1

Glutathionereductase activity in serum of bulls blood after application Ursovit ADES and sodium selenite under acute nitrate-nitrite toxicity, nmol NADPH / min. to 1 mg protein; (M ± m, n = 5)

Indicators of animal blood (hours)	Groups of animals		
	Control	Experimental 1	Experimental 2
	Before feeding with msodium nitrate		
Control	1.56 ± 0.050	1.58 ± 0.06	1.60 ± 0.056
	After feeding with sodium nitrate		
In three hours	1.71 ± 0.045	1.68 ± 0.057	1.67 ± 0.035
	After introduction of antioxidants		
	—	Ursovit ADES	Ursovit ADES + 0.5% sodium selenite solution
In one hour	1.57 ± 0.065	1.53 ± 0.045	1.62 ± 0.048
In two hours	1.48 ± 0.048	1.54 ± 0.042*	1.59 ± 0.045*
In three hours	1.23 ± 0.024	1.47 ± 0.037*	1.54 ± 0.035**
In six hours	1.20 ± 0.033	1.47 ± 0.031**	1.53 ± 0.025**
In eight hours	1.25 ± 0.026	1.50 ± 0.034**	1.57 ± 0.035**

Note. The degree of probability compared with the control group data – P < 0.05–, P < 0.001–***

Table 2

The activity of glutathioneperoxidase in serum of bulls blood after application Ursovit-ADES and sodium selenite under acute nitrate-nitrite toxicity, nmol NADPH / min. to 1 mg protein; (M ± m, n = 5)

Indicators of animal blood (hours)	Groups of animals		
	Control	Experimental 1	Experimental 2
	Before feeding with sodium nitrate		
Control	37.17 ± 1.2	37.18 ± 1.2	37,10 ± 1.1
	After feeding with sodium nitrate		
In three hours	38.10 ± 1.1	38.15 ± 1.3	38.04 ± 1.1
	After introduction of antioxidants		
	—	Ursovit-ADES	Ursovit-ADES + 0.5% sodium selenite solution
In one hour	34.98 ± 1.0	35.42 ± 1.3	35.67 ± 1.4
In two hours	32.46 ± 1.1	34.32 ± 1.2	35.96 ± 2.5*
In three hours	29.43 ± 1.0	35.06 ± 1.1**	36.59 ± 1.2**
In six hours	28.25 ± 1.1	36.18 ± 1.4**	36.87 ± 1.3**
In eight hours	31.68 ± 1.2	36.44 ± 1.5*	37.05 ± 1.4*

*Note. The degree of probability compared with the control group data – P < 0,05 – *, P < 0,001 – ***

Thus, the use of vitamin-drug Ursovit ADES and sodium selenite helped to normalize the activity of glutathioneperoxidase better than the use of Ursovit-ADES, possible due to the fact that sodium selenite includes important element of antioxidant selenium, and it was a part of the enzyme that was investigated.

The activity of glucose-6-phosphatedehydrogenase under acute nitrate and nitrite toxicity and the use of antioxidants is given in Table. 3. Before feeding bulls with sodium nitrate, the activity values of G-6-FDG was within 0.72 ± 0.024 – 0.73 ± 0.024 nmol NADPH / min. to 1 mg of protein.

Table 3

The activity of glucose-6-phosphatedehydrogenase in serum of bulls blood after application Ursovit-ADES and sodium selenite under acute nitrate and nitrite toxicity, nmol NADPH / min. to 1 mg protein; (M ± m, n = 5)

Indicators of animal blood (hours)	Groups of animals		
	Control	Experimental 1	Experimental 2
	Before feeding with sodium nitrate		
Control	0.72 ± 0.025	0.73 ± 0.024	0.72 ± 0.024
	After feeding with sodium nitrate		
In three hours	0.83 ± 0.035	0.86 ± 0.031	0.85 ± 0.032
	After introduction of antioxidants		
	—	Ursovit-ADES	Ursovit-ADES + 0.5% sodium selenite solution
In one hour	0.56 ± 0.021	0.61 ± 0.022	0.63 ± 0.021
In two hours	0.52 ± 0.021	0.56 ± 0.020	0.61 ± 0.020*
In three hours	0.50 ± 0.017	0.57 ± 0.018*	0.64 ± 0.023**
In six hours	0.44 ± 0.016	0.53 ± 0.015*	0.69 ± 0.021**
In eight hours	0,53 ± 0.013	0.57 ± 0.021**	0.73 ± 0.019**

Note. The degree of probability compared with the control group data – P < 0.05–, P<0.001–***

After feeding the bulls with sodium nitrate forage at a dose of 0.45 g NO_3^-/ kg body weight, enzyme activity began to change. In particular, in three hours of the experiment, the activity of G-6-FDG was 0.83 ± 0.035 – 0.86 ± 0.031 nmol NADPH / min. to 1 mg of protein. In the control group of bulls that were not fed with antioxidants enzyme activity continued to decline in four hours of the experiment respectively decreased to 22% comparing to the initial values.

Introduction of Ursovit-ADES and sodium selenite in the patient bulls, accompanied with an increase in the activity of G-6-FDG in their blood. The most likely enzyme changes were noted in six hours of experiment where results of D1 experimental group was lower by 20% and D2 group – 57%.

Thus, the combined administration Ursovit-ADES and sodium selenite promoted better normalization of enzyme, which was associated with synergistic properties of these drugs; they increase and continue to force each other activity.

Conclusions

1. The use of antioxidant drugs iunder conditions of acute nitrate and nitrite toxicity of bulls have improved enzymatic level of glutathione system of antioxidant protection (glutathionereductase, glutathioneperoxidase, glucose-6-phosphate-dehydrogenase) in the blood of experimental animals.

2. Cumulative input of Ursovit-ADES and sodium selenite showed better effect on glutathione sstem of antioxidant defense of the bulls' bodies than applying only Ursovit-ADES.

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