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Influence of vitamins on the prooxidant-antioxidant homeostasis in boars under the conditions of heat stress

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Contents

Abstract

The intensity of reproduction of the live-stock of pigs largely depends on environmental factors, in particular fluctuations in indoor temperature, which is often seasonal. It was found out the fact that in the summer with a long rise in temperature it is worsened the quality of sperm in boars, which is accompanied by a decrease in the fertility of sperm, and in multiplicity and large foetus fertility of sows. The aim of the study was to determine the influence of antioxidant vitamins on the prooxidant-antioxidant homeostasis in blood of boars under the conditions of heat stress. In the study it was used the adult boars of the Large White breed. The duration of the experiment was 120 days, including: preparatory one is 30 days, basic one is 60 days (feeding vitamin A, vitamin E, ascorbic acid) and final one is 30 days. In the main period of the experiment, the diet of animals in the control group remained unchanged, and it was for two experimental ones with the addition of vitamin A, vitamin E and ascorbic acid. The level of biologically active components in the diet of the experimental groups was higher by 10 % and 20 % compared with the control group. In the received blood samples it has been determined the state of prooxidant-antioxidant state. It has been determined that housing boars in conditions of increased temperature is accompanied by the acceleration of peroxidation processes and the depletion of the antioxidant defense system in blood. The introduction of a vitamin supplement in the feed mixture to boars significantly changes the state of prooxidant-antioxidant homeostasis in this tissue depending on the amount of additionally fed vitamins with antioxidant action. The addition of these biologically active substances by 10 % above the norm after 60 days of feeding increases the content of vitamin A ($P < 0.05$), vitamin E ($P < 0.05$), reduced glutathione and slows down the processes of peroxidation – reducing the concentration of diene conjugates ($P < 0.01$) and TBA-active complexes. Feeding vitamins with antioxidant action in the feed mixture is 20 % more than the norm for boars, compared with the control group, after a month of the use, inhibits peroxidation processes. Two months of using these compounds probably reduced the number of diene conjugates ($P < 0.01$) and TBA-active complexes. It is accompanied by an increase in the content of low molecular weight antioxidants – vitamin A ($P < 0.01$), vitamin E ($P < 0.05$) and ascorbic acid, the level of which is maintained for at least a month after their use.

Key words: blood, ascorbic acid, vitamin A, vitamin E, antioxidants, peroxidation.

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1. Introduction

The efficiency of work of industrial enterprises largely depends on the high reproductive capacity of boars. However, it is often the various sexual dysfunctions complicate their rational use in breeding and make it impossible to maintain the structure of the herd.

Among the factors that reduce the intensity of reproduction of pigs, the leading importance belongs to environmental factors, in particular the fluctuation in indoor temperature, which is often seasonal. According to researchers, in the summer with a prolonged rise in temperature it is deteriorated the sperm quality in boars, which is accompanied by a decrease in sperm fertility, and the multiplicity and the large foetus fertility in sows (Topchii, 2009; Narizhnyj et al., 2014; Hyria et al., 2019; Peña et al., 2019).

In reducing the effect of the thermal factor on the body of boars, the considerable attention is paid to the feeding with the quality combined feeds, especially their supply of limiting substances – vitamins with antioxidant action. Traditionally, fat-soluble vitamins are introduced into micropremixes, which must contain antioxidants. Today, as an alternative to traditional compounds of fat-soluble vitamins, it is proposed to use micronutable forms of them, which bind well to water increasing their digestibility.

Among the vitamins that determine the productivity of animals, the leading role belongs to vitamin A, vitamin E and ascorbic acid. These substances regulate the growth,

development and reproduction of pigs (Kołodziej & Jacyno, 2005; Sivertsen et al., 2007). Most of these biological effects of these vitamins are accompanied by changes in prooxidant-antioxidant homeostasis due to their ability to bind reactive oxygen forms, embedding in cell membranes, inhibiting the oxidation of microelements (Echeverria-Alonzo et al., 2009; Colagar et al., 2013; Nowicka-Bauer, & Nixon, 2020).

It has been proved the possibility of improving the quality of sperm production in boars in the direction of increasing ejaculate volume, increasing the concentration, motility and survival of sperm through additional feeding with fat-soluble vitamins with antioxidant action, as well as the direct introduction into ejaculate (Audet et al., 2017; Izquiedo, 2017; Liu et al., 2017; Lugar et al., 2019). Such features of the formation of sexual function are accompanied by profound changes in prooxidant-antioxidant homeostasis (Shostia et al., 2018). In this regard, it is important to study the influence of individual vitamins of antioxidants on the formation of these homeostatic constants in boars when they are in the conditions of elevated temperatures, to avoid the negative effects of external factors.

The aim of the study was to determine the influence of antioxidant vitamins on the prooxidant-antioxidant homeostasis in blood of boars under the conditions of heat stress. To achieve this goal, the following tasks were performed:

- the influence of fed vitamins of antioxidant action on the intensity of peroxidation processes in blood of boars was studied;
- the influence of fed vitamins of antioxidant action on the formation of the system of antioxidant protection in blood of boars was clarified.

2. Materials and methods

In the experiment it was used 9 adult boars of the Large White breed aged from 18 to 36 months. From which three groups-analogues of boars were formed – I (control) and II and III (experimental), three animals in each.

The duration of the experiment was 120 days, including: preparatory one is 30 days, basic one – 60 days (feeding vitamin A, vitamin E, ascorbic acid) and final one – 30 days. In the main period of the experiment, the diet for boars of the control group remained unchanged, and in the experimental one it was added a vitamin supplement to it containing dry microgranular forms of retinol acetate (vitamin A), DL-α-tocopherol polyethylene glycol succinate (vitamin E) and ascorbic acid in crystalline form (vitamin C). These forms of vitamins are highly bioavailable. The level of these biologically active components in the diet of the second and third experimental groups was higher by 10 % and 20 %, respectively, compared with the control group.

The state of prooxidant-antioxidant homeostasis (PAH) in blood was determined by the resistance of erythrocytes to peroxide hemolysis (Kaidashev, 1996), xanthine oxidase activity (Shabunin, 2010), concentrations of diene conjugates – spectrophotometrically (Gavrilov & Melkorudnaja, 1983) (aldehydes and ketones) – photoelectrocolorimetrically (Kaidashev, 1996). The state of the antioxidant defense system was assessed by the activities of superoxide dismutase (Brusov et al., 1976) and catalase (Koroljuk et al., 1988), by the amount of reduced glutathione (Shabunin, 2010), vitamin A and vitamin E (Kovalenko et al., 2005), ascorbic acid and dehydroascorbic acid (Kaidashev, 1996).

The obtained digital material was statistically processed using the program Statistics for Windows XP. After comparing the studied indexes and their intergroup differences it was used Student's t-test, and the result was considered probable after $P < 0.05$.

3. Results and discussion

Results

The obtained data indicate that after feeding vitamins of antioxidant action in the feed mixture to boars of groups II and III led to an increase in the resistance of erythrocytes to peroxide hemolysis, respectively, by 3.7 and 23.1 % (30th day), 30.1 and 36 % (60th day), 11.2 and 36.2 % (final period) compared with the control (table 1). Perhaps such changes are due to a significant decrease in the functional activity of xanthine oxidase, especially in blood of animals of group III at the end of the main period of the experiment and after the cessation of their use, respectively, by 28.5 and 26.7 % relative to intact animals.

Table 1

The influence of antioxidant vitamins on the processes of peroxidation in blood of boars, M ± m, n = 6

Indexes	Groups	Preparatory period	The main period		The final period
			The 30th day	The 60th day	
Peroxide resistance of erythrocytes,%	1	10.52 ± 2.09	13.41 ± 1.95	15.38 ± 2.14	12.76 ± 2.19
	2	9.22 ± 1.51	12.93 ± 1.62	10.65 ± 1.44	11.28 ± 1.06
	3	8.83 ± 1.48	10.41 ± 0.95	9.82 ± 0.53	8.08 ± 4.73
Xanthine oxidase, mccat / sec • l	1	30.42 ± 4.85	25.88 ± 4.24	32.45 ± 5.89	34.17 ± 4.05
	2	32.83 ± 6.54	30.17 ± 3.76	28.64 ± 2.93	27.21 ± 5.32
	3	30.45 ± 4.48	22.83 ± 4.72	23.17 ± 4.94	25.86 ± 5.07
Diene conjugates, μmol / l	1	2.87 ± 0.48	3.74 ± 0.28	5.93 ± 0.41**	4.66 ± 0.37*
	2	3.05 ± 0.49	3.23 ± 0.44	3.48 ± 0.63□□	3.21 ± 0.48
	3	2.76 ± 0.21	2.49 ± 0.23□	2.32 ± 0.38□□	2.11 ± 0.32□□
TBA-active compounds, μmol / l	1	8.23 ± 1.19	12.70 ± 1.98	15.92 ± 1.69*	13.11 ± 2.72
	2	8.92 ± 0.73	10.43 ± 1.97	12.72 ± 1.87	12.91 ± 1.88
	3	9.37 ± 1.92	11.80 ± 1.66	12.11 ± 2.01	9.82 ± 0.78
TBA-active compounds after incubation, μmol / l	1	11.59 ± 1.91	16.47 ± 2.15	22.38 ± 3.19*	14.22 ± 1.97
	2	10.72 ± 0.99	13.38 ± 2.01	15.12 ± 2.79	12.79 ± 2.43
	3	13.50 ± 1.59	12.67 ± 1.62	14.63 ± 1.91	12.08 ± 1.65

Note: * – $P < 0.05$; ** – $P < 0.01$ – compared witrh the preparatory period;
□ – $P < 0.05$; □□ – $P < 0.01$ – compared with the first group (control)

At the end of the second month of heat stress in blood of boars there was an increase in the content of diene conjugates ($P < 0.01$). An additional 10 % increase in the intake of antioxidant vitamins reduced the concentration of primary peroxidation products on the 60th day of the main period relative to the control group by 41.3 % ($P < 0.01$), and at the end of the experiment by 30.9 %. At the same time, with the increase in the amount of fed antioxidant vitamins (20 %) at the end of the first and second months of the main period, there was a decrease in the concentration of diene conjugates by 26.2 ($P < 0.05$) and 56.5 %, $P < 0.01$), and at the end of the experiment by 54.7 % ($P < 0.01$) relative to the control group.

The largest difference in the number of secondary peroxidation products was observed at the end of the second month of consuming the vitamin supplements by boars, where the content of these substances in animals of groups II and III was lower by 20.1 % and 25.2 %, respectively, relative to control. At the end of the experiment, the intergroup difference decreased. It is important to note that after incubation of blood in prooxidant buffer, the content of TBA-active complexes increased significantly in samples of animals of the control group during the study periods by 30.0 % (30th day), 40.9 % (60th day) and 8.4 % (final period), which indicates that during heat stress the peroxidation is accelerated, but at least a month after the action of the negative factor, it is inhibited. Under the condition of maximum consuming the vitamin supplement, the intensity of accumulation of TBA-active compounds was 7.6 and 21.6 %, respectively, after one and two months of consuming, and 22.4 % after the end of the experiment. Thus, additional feeding vitamins with antioxidant action in the amount of 20 % above the norm caused inhibition of peroxidation processes – a decrease in the number of TBA-active complexes, as well as an increase in the total level of the antioxidant defense system.

The level of enzymatic antioxidants in blood of boars during the experimental period varied depending on the dose of fed vitamins of antioxidant action (Table 2). Thus, the activity of superoxide dismutase in this tissue of animals of groups II and III was lower relative to the control, respectively, by 13.7 and 23.5 % on the 60th day of the main period, as well as 8.7 % and 28.3 % at the end of the experiment.

The presence of boars during the month under heat stress was accompanied by an increase in catalase levels. However, in the animals of the II group on the 30th day of the main period the activity of this enzyme was lower by 15.2, and in the III group higher by 19 % than the control group. However, after feeding more than 10 % of the vitamin supplement for 60 days caused an increase in the level of this enzyme by 26.30 %, and in conditions of the consumption of more than 20 % a significant increase by 13.2 %, this trend persisted until the end of the experiment compared with intact animals.

Feeding antioxidant vitamins to boars for 60 days contributed to a significant accumulation of reduced glutathione in blood of animals of group II, where its content decreased by 27.5 %. The most intense oxidation of this substance was observed when using a complex additive by 20 % above the norma, which was accompanied by an increase in the concentration of relatively intact animals by 15.9 (30th day) and 35.0 % (60th day), and the phenomenon after action continued at least a month, but the intergroup difference decreased to 23.8 % ($P < 0.05$). Obviously, this distribution of glutathione concentrations is due to its safety from oxidation and the restoration of antioxidant vitamins.

Table 2

The influence of vitamins with antioxidant action on the system of antioxidant protection in blood of boars, M ± m, n = 6

Indexes	Groups	Preparatory period	The main period		The final period
			30-доба	60-доба	
Superoxide dismutase, u.o./ml	1	0.332 ± 0.076	0.421 ± 0.094	0.512 ± 0.119	0.458 ± 0.053
	2	0.413 ± 0.066	0.417 ± 0.031	0.441 ± 0.071	0.425 ± 0.029
	3	0.367 ± 0.043	0.380 ± 0.073	0.392 ± 0.055	0.328 ± 0.062
Catalase, H_2O_2/min/l	1	113.42 ± 6.56	158.17 ± 12.79*	111.23 ± 10.17	130.44 ± 9.59
	2	125.08 ± 15.63	134.16 ± 12.94	140.40 ± 13.71	138.21 ± 19.88
	3	134.75 ± 12.19	176.87 ± 23.31	180.31 ± 35.74	124.68 ± 14.11
Reduced glutathione, μmol/l	1	0.527 ± 0.079	0.442 ± 0.060	0.401 ± 0.062	0.425 ± 0.031
	2	0.478 ± 0.056	0.408 ± 0.071	0.512 ± 0.037	0.458 ± 0.036
	3	0.492 ± 0.068	0.513 ± 0.049*	0.545 ± 0.106	0.520 ± 0.040□
Ascorbic acid, mmol/l	1	15.12 ±2.99	8.13 ± 1.67	10.77 ± 2.42	12.28 ± 1.79
	2	13.83 ± 2.32	13.52 ± 2.85	14.58 ± 2.81	17.16 ± 2.57
	3	12.45 ± 2.18	14.73 ± 2.54	18.36 ± 2.33	14.88 ± 2.81
Dehydroascorbic acid, mmol/l	1	14.83 ± 2.90	13.32 ± 1.37	17.18 ± 1.36	15.67 ± 2.69
	2	15.42 ± 1.97	18.55 ± 2.58	12.88 ± 1.39	13.53 ± 2.72
	3	12.74 ± 0.82	11.35 ± 1.86	10.12 ± 1.63□	11.12 ± 1.28
Vitamin A mmol / l	1	1.075 ± 0.063	0.917 ± 0.151	0.750 ± 0.109*	0.725 ± 0.139
	2	0.850 ± 0.091	1.106 ± 0.101	1.330 ± 0.118*□	1.05 ± 0.109□□□
	3	0.917 ± 0.122	1.230 ± 0.159	2.083 ± 0.232**□□	1.95 ± 0.245*□□
Vitamin E, mmol / l	1	9.63 ± 1.31	7.13 ± 1.05	6.05 ± 0.76	5.54 ± 0.93
	2	11.20 ± 1.49	9.37 ± 1.68	13.32 ± 2.75	12.58 ± 2.11□
	3	9.42 ± 1.53	11.90 ± 2.37	14.13 ± 2.26□	14.91 ± 2.31□

Note: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ – compared with the preparatory period;
□ – $P < 0.05$; □□ – $P < 0.01$; □□□ – $P < 0.001$ – compared with the first group (control)

Boars fed with a vitamin supplement were characterized by a higher content of ascorbic acid in blood. Thus, during the use of the additive in the samples of this tissue of groups II and III, the content of this acid prevailed compared to the control by 65.8 and 80.6 % after the first, as well as 36.4 and 72.0 % of the second months of the main period, this regularity was maintained until the end of the experiment. It is important to note that animals were characterized with the maximum concentration of ascorbic acid that consumed by 20% more vitamins.

The content of dehydroascorbic acid in animals of experimental groups – II and III was lower than the control by 28.0 and 25.6 % ($P < 0.05$) (60th day) and 13.5 and 40.5 % (the final period experiment).

The addition of a vitamin supplement to the diet of boars significantly influenced on the content of antioxidant vitamins in blood. There was a slight difference between the concentrations of vitamin A in samples of groups I and II, which was observed after the first month of its use, amounting to 23.1 %, the second – 77.3% ($P < 0.05$) and at the end of the experiment – 59.7 % ($P < 0.001$). The most pronounced changes were observed in samples of this tissue after consuming this supplement by animals of the group III, where the amount of vitamin A during the experiment exceeded the control by 35.2 % (30th day), 280 % ($P < 0.01$) (60th day)) and 244.4 % ($P < 0.01$) during the final period.

In blood of boars of groups II and III compared with intact animals, the concentration of vitamin E prevailed respectively in 1.3 and 1.7 (30th day), 2.2 and 2.4 ($P < 0.05$) (60- and day) and 2.3 ($P < 0.05$) and 2.6 ($P < 0.05$) times at the end of the experiment.

Discussion

The results of studies indicate a significant influence of heat stress on the state of PAH in blood of boars, which is manifested in the acceleration of peroxidation and changes in the ratio of low molecular weight antioxidants, the development of this state is accompanied by changes of thiol-disulfide state in other animals, in particular in rats (Gorchakova et al., 2017). Such changes become more noticeable with increasing duration of the heat factor, but after reducing the impact of this stress it is optimizing the processes of generating reactive oxygen forms and increasing the strength of the antioxidant defense system.

The significant influence of vitamins with antioxidant action on the formation of PAH in blood of boars, primarily is to increase the resistance of erythrocytes to peroxide hemolysis. This is apparently due to a decrease in the functional activity of the prooxidant enzyme xanthine oxidase, the stabilization of these cells through the incorporation of fed lipid antioxidants, the saturation of the cytosol with ascorbic acid isomers, and also with the inhibition of the accumulation of primary and secondary oxidation products). The functional activity of inactivation of oxygen radicals and hydrogen peroxide by superoxide dismutase and catalase was insignificant. Of particular note is the predominance of ascorbic and dehydroascorbic acids, which is apparently due to the intensive use of glutathione and vitamin E (Csala et al., 2001; Nardai et al., 2001).

The effect after consuming antioxidant vitamins by boars in the amount of 10 % above the norm was observed after the first month of consuming. The action of these compounds was manifested in a slight inhibition of peroxidation processes - a minimal amount of diene conjugates and TBA-active compounds, with the slightly lower activity of xanthine oxidase, superoxide dismutase and catalase against intact animals. Such changes occurred against the background of a decrease in the intensity of the oxidation of ascorbic acid and glutathione, which coincides with the statement about the synegic effect of the latter on the formation of PAH due to the reduction of dehydroascorbic acid by thiol proteins (Fang et al., 2004; Bánhegyi et al., 2003). This adaptive mechanism under conditions of heat stress prevents the development of cell apoptosis (Kalinina et al., 2014; Linster & Van Schaftingen, 2007). M. Takahashi (Takahashi, 2012) also notes the possible stabilizing effect of these vitamins with antioxidant action on the cell membranes of sperm, oocytes and embryos. This amount of the fed easily digestible vitamin supplement increased the level of vitamin A and vitamin E during the main and decreased during the final periods of the experiment. In this group of animals, a vitamin supplement apparently stimulated the formation of physiologically normal levels of reactive oxygen forms and it is necessary for immune responses (Valko et al., 2007), cellular signaling (Dröge, 2002; Nowicka-Bauer & Nixon, 2020), gamete formation, and their merger (Shestakova et al., 2017).

Increasing the dose of fed vitamins of antioxidant activity to boars by 20 % above the norm significantly strengthened the system of antioxidant defence due to the saturation of blood with vitamin A, vitamin E and ascorbic acid. It is was accompanied by an increase in the concentration of reduced glutathione with a parallel decrease in the functional activity of antioxidant enzymes. Against the background of these changes, the course of peroxidation processes was inhibited, which is confirmed by the minimal amount of diene conjugates and TBA-active substances. These changes in the formation of PAH observed after the intake of vitamin supplements and the deposition of vitamin A and vitamin E and lasted for at least a month after the cessation of feeding. This indicates that the vitamins of antioxidant action coming from the feed to boars, significantly change the processes of formation of PAH, and their degree of influence is determined by the fed doses. The addition of the studied vitamins to the feed at a dose of 10 % above the norm can be used to optimize the processes of PAH during heat stress. Increasing the amount of fed vitamin supplements to 20 % above the norma significantly inhibits the peroxidation processes, increases the strength of antioxidant defence due to the low molecular weight antioxidants. The effect after the action lasts at least a month, which is associated with their deposition. This opens up the possibility of satisfying the growing need for antioxidant vitamins during the maximum physiological loads in the body of this species of animals, especially in the period of heat stress during the mating campaign with seasonal tour technology.

4. Conclusions

1. Housing boars in the conditions of elevated temperature, accompanied by the acceleration of peroxidation processes and the depletion of the antioxidant defense system in blood. The introduction of a vitamin supplement in the feed mixture for boars significantly changes the state of PAH in this tissue depending on the amount of additionally fed vitamins with antioxidant action.

2. The addition of antioxidant vitamins to the diet by 10 % above the norma after 60 days of feeding increases the

content of vitamin A by 77.3 % ($P < 0.05$), vitamin E – 220.0 % ($P < 0.05$), reduced glutathione and slows down the processes of peroxidation – reducing the concentration of diene conjugates – 41.3 % ($P < 0.01$) and TBA-active complexes – 20.1 % in blood.

3. Feeding vitamins with antioxidant action in the feed mixture is 20 % more than the norma for boars, after a month of consuming inhibits peroxidation processes. Two months of consuming these compounds probably reduces the amount of diene conjugates by 56.5 % ($P < 0.01$) and TBA-active complexes by 25.2 %. This is accompanied by an increase in the content of low molecular weight antioxidants – vitamin A by 280 % ($P < 0.01$), vitamin E by 260,0 % ($P < 0.05$) and ascorbic acid by 80.6 %, the level of which lasts at least 30 days after their consuming.

Conflict of interest

The authors declare that there is no conflict of interest.

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