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EFFECT OF SELENIUM, ZINC, VITAMIN C AND E ON BOAR EJACULATE QUALITY AT HEAT STRESS

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Abstract

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The aim of experiment was to test effect of selected antioxidants (selenium, zinc, vitamin C and E) to reduce the impact of heat stress at boars. In the experiment, boars of Duroc breed were tested. The first control group (n = 10) was not supplemented with antioxidants. The second experimental group (n = 10) was supplemented with antioxidants in the following quantities of 0.5 mg of selenium (seleno-methionine), 100 mg of zinc (zinc-methionine), 70 mg of vitamin E (alpha-tocopherol) and 350 mg of vitamin C (ascorbic acid) per kilogram of their feed. The experiment was carried out for 120 days and took place in summer (June to September). During the experiment, average and maximum daily temperatures, where boars were stabled, were monitored. Average daily temperature ranged from 12 to 28 °C. Maximum temperature during the day was from 13 to 32 °C. The evaluation of the semen quality has revealed increased number of abnormal spermatozoa in the control group of boars by 39 % ($P < 0.05$). There were observed no significant changes at other monitored parameters (ejaculate volume, total count of produced sperm, motility and sperm concentration). The results show that the addition of selenium, zinc, vitamin C and E may reduce the effect of heat stress to some extent at breeding boars.

Keywords: antioxidant, boars, ejaculate, heat stress

INTRODUCTION

Heat stress causes great economic losses in livestock reproduction. In males, there is higher production of free oxygen radicals able to damage cells, including animal sperm. In case the temperature exceeds 25 °C, heat stress occurs at boars, causing damage to cell structures (Horký *et al.*, 2015; Hansen, 2009). Stress leads to increased production of free radicals in the body. During the evolution, organism has developed defence mechanisms eliminating the production of free radicals (Horky, 2014; Komínková *et al.*, 2015). Some of the most important antioxidants include selenium, zinc, vitamin C and E. Selenium and zinc are a part of antioxidant enzyme-glutathione peroxidase and superoxide dismutase, respectively (Klusoňová *et al.*, 2015; Horký, 2015). Vitamin E acts against peroxidation of polyenoic acids of biological membranes. Tocopherols have the ability to donate hydrogen. They stop radical chain reactions by transferring hydrogen from the phenolic group of the free radical peroxide. The resulting phenoxy-radical may react with vitamin C. Vitamin C is involved in the antioxidant protection of cells as it reduces tocopherol radical. (Lovercramp *et al.*, 2013; Horký *et al.*, 2013). Mineral and vitamin nutrition becomes significant in reducing heat stress of boars and also maintains their good health (Horky *et al.*, 2016a; Nevrkla *et al.*, 2014; Nevrkla *et al.*, 2012).

The aim of the experiment was to test the effect of selenium, zinc, vitamin C and E to reduce heat stress with the respect of ejaculate quality of breeding boars.

MATERIALS AND METHODS

The experiment was carried out in the insemination centre of boars in Velké Meziříčí (Czech Republic). 20 boars of *Duroc* breed were selected in the experiment. The average age was 2 ± 0.2 years and the average weight of boars was 270 ± 20 kg. The experimental animals were stabled individually (2.5 × 2.5 m) and had ad libitum access to water. All animals were fed with 3.3 kg of basic feed mixture (the Tab. I and II). Content of metabolized energy (MEp) was 12.6 MJ/kg of diet. The basic feed mixture contained 0.02 mg of selenium, 26 mg of zinc, 10 mg of vitamin E a 16 mg of vitamin C per kilogram of feed mixture. The content of selenium and zinc in feed mixture was assessed using atomic absorption spectrophotometry (Lei and Marshall, 1995), vitamin E was determined by method HPLC (Hosmanova and Dousa, 2007), and vitamin C was determined using HPLC (Rudenko and Kartsova, 2010).

Boars were divided into two groups. The first group (n = 10) served as control (selenium, zinc, vitamin C and E came only from native sources). The second experimental group (n = 10) was supplemented with 0.5 mg selenium (selenomethionine), 100 mg of zinc (zinc-methionin), 70 mg of vitamin E (alpha-tocopherol) and 350 mg of vitamin C (ascorbic acid) per kilogram of their diet. Premix of selenium, zinc and vitamin C and E were individually dosed during morning feeding of boars.

I: *Composition of feed mixture of boars*

Component	% in feed mixture
Barley grain	36.00
Wheat grain	20.36
Oat grain	20.00
SBM (soybean meal)	14.50
EKPO T	3.00
BergaFat	2.10
Calcium carbonate	1.50
Monodicalciumphosphate	1.20
Mineral vitamin premix for boars 0.5 %	0.50
Sodium chloride	0.40
Magnesium oxide	0.15
L-Lysine HCl	0.14
L- Threonine	0.09
Methionine DL	0.06

Bergafat (Berg + Schmidt, Germany) – palm oil; EKPO T (Delika – Pet, Czech Republic) – biscuit meal

Experiment was held for 120 days (June–September). Ejaculate was taken from boars once per week. The ejaculate was collected using jump a phantom. During the whole experiment, the temperature was recorded by monitoring the environment where the boars were located. The temperature was monitored by a data logger (Voltcraft DL-121TH, Germany) placed at the height of the animals (1 m above the ground). The data logger recorded the actual temperature in hourly intervals. The average temperature for each day was calculated from the following data. The figure 1A shows average and maximal temperatures during the experiment. The figure 1B shows average and maximum relative humidity of environment where the experiment was carried out.

II: *Composition of premix for boars (0.5%)*

Parameter	Unit	Quantity
Vit. A	U.I.	3,000,000
Vit. D3	U.I.	400,000
Vit. B1	mg	500
Vit. B2	mg	1,200
Vit. B6	mg	800
Vit. B12	mg	6
Vit. K3	mg	600
Biotine	mg	70
Folic acid	mg	200
Niacinamide	mg	8,000
Calcium pantothenate	mg	4,000
Choline chloride	mg	55,200
Betaine	mg	26,500
Butylhydroxi-toluene	mg	400
Ethoxyquin	mg	180
Cu – in the form of copper sulfate pentahydrate	mg	2,883
Mn – in the form of manganese oxide	mg	19,760
Co – in the form of cobalt sulphate heptahydrate	mg	91
Lysine in the form of L-Lysine monohydrochloride	g	226
Carrier ad. – wheat meal, calcium carbonate	kg	1

III: *Numbers of samples in experimental and control groups during the experiment*

	Control group				Experimental group			
Days of experiment	**0–30**	**30–90**	**60–90**	**90–120**	**0–30**	**30–90**	**60–90**	**90–120**
Number of boars	10	10	10	10	10	10	10	10
Number of samples	41	45	41	40	42	43	39	40

Determination of ejaculate volume, total sperm production, sperm concentration and motility, and abnormal sperm percentage

The assessments were carried out according to the methodology of Lovercamp *et al.* (2013). The volume of the ejaculate was determined by weighing each ejaculate using the fact 1 g of semen is equal to 1 mL. The concentrations of sperm were evaluated using a self-calibrating photometer (SpermaCueTM, Minitube of America, Verona, WI). The motility analyses were performed using Sperm VisionTM software (Minitube of America, Verona, WI) on images obtained by a digital camera attached to phase contrast microscope (Olympus microscope IX 71 S8F-3; Tokyo, Japan). Prior to analysis, 500 µL of each sample was diluted in 500 µL of Androhep extender and incubated at 37 °C for 30 minutes. The evaluation of sperm morphology and cellular particles was performed using a phase contrast microscope (Zeiss; West Germany). The subjective analyses were always carried out by the same qualified person. Determination of total sperm production was based on the calculation (sperm concentration x ejaculate volume).

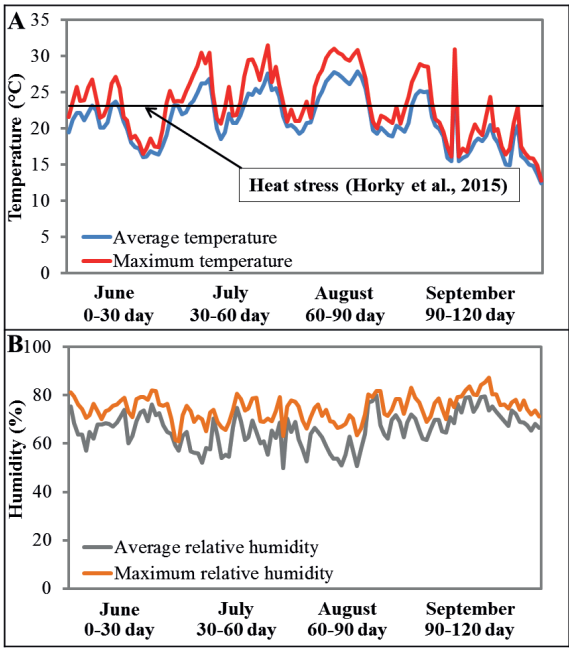
Values of ejaculate volume, motility, sperm concentration, percentage of abnormal sperm, and total count of produced sperm were collected from each group during the experiment. Throughout the experiment, all samples of semen from experimental animals were evaluated. The experiment was divided into four time intervals: the first one (days 0–30), the second one (days 30–90), the third one (days 60–90); and the fourth one (days 90–120). Number of samples in various time intervals is given in the Table III.

Statistics

The data were statistically analyzed using STATISTIKA.CZ version 10.0 (the Czech Republic). Results were expressed as mean ± standard variance. Statistical significance was observed between sampling (the first time interval [days 0-30] was taken as a control one) using ANOVA and Scheffe's test – two-factor analysis (the first factor was animal group, the second one was time interval) for parameters of ejaculate volume, sperm concentration, total count of sperm, motility, percentage of pathological sperm. The difference ($P < 0.05$) was considered as significant.

RESULTS

During the experiment, selected antioxidants (selenium, zinc, vitamins C and E), which shall increase semen quality in summer according to our hypothesis (heat stress elimination), were used. These were chosen as parameters of semen quality: ejaculate volume, percentage of pathological sperms, total count of sperm cells, concentration, and motility. During the experiment, temperature and relative humidity in the environment, where boars were stabled, were monitored (the Figure 1A and 1B). During the experiment, the critical temperature 23 °C was repeatedly exceeded. It is perceived as a borderline for the effect of heat stress of boars. Maximum temperatures exceeded even the limit of 30 °C during the experiment.



1: *Development of average and maximum temperature during the experiment (1A); development of average and maximum relative humidity during the experiment (1B)*

Within the experiment, evaluation of ejaculate did not show any significant difference between groups. The highest volume was achieved in the experimental group of pigs with an increase by 39% from beginning of the experiment. Sperm concentration was balanced in the control group throughout the experiment. Conversely, decrease in sperm concentration was observed in the experimental group. The decrease was 9% at the end of the experiment without statistical significance. In the case of sperm motility, there were not monitored significant changes throughout

IV: *Qualitative and quantitative values of ejaculate at control and experimental groups of boars*

	Control group				Experimental group			
Days of experiment / **Ejaculate**	**0-30**	**30-60**	**60-90**	**90-120**	**0-30**	**30-60**	**60-90**	**90-120**
Ejaculate volume (ml)	136.4	128.7	146.6	158.2	137.3	148.1	160.0	190.6
±	32.6	29.7	33.9	22.1	36.9	35.4	27.4	35.1
Concentration (x103/ mm)	661.4	688.0	676.0	695.7	643,0	630.7	589.5	589.5
±	54.5	75.1	50.6	61.4	43.5	57.5	65.3	65.3
Sperm motility (%)	74.8	72.7	72.8	68.2	73,9	73.4	70.6	70.6
±	2.4	2.6	2.9	2.7	2,9	3.4	2.7	2.7
Abnormal sperm cells (%)	8.9	8.1	14.5*	13.0*	7.6	7.8	7,3	7.3
±	1.1	1.4	2.3	2.0	2.1	1.9	2.1	1.1
Total count of sperm cells (x109)	96.8	84.2	98.5	111.7	84.6	82.8	100.6	100.6
±	10.7	15.3	25.4	15.1	18.8	27.9	17.3	17.3

Symbol * shows a statistical power at the level of $P < 0.05$ compared with the data at the beginning of the experiment within the group

the experiment. In both groups, a linear decrease in sperm motility was observed. The decrease was 10% at the control group. In the experimental group, there was observed decrease in sperm motility by 5% in the last part of the experiment (days 90–120). The increase of abnormal sperm was observed in the control group in the third interval of 60–90 days (39 %; $P < 0.05$) and in the fourth interval of 90 to 120 days (32 %; $P < 0.05$). In the experimental group, there was percentage of abnormal spermatozoa at the same level throughout the experiment. There was not observed any evidence of change within evaluation of total count of sperm. Values of ejaculate volume, percentage of pathological sperms, total count, sperm concentration and motility are shown in the Table IV.

DISCUSSION

Selected antioxidants can positively affect the quality of produced sperm according to available information. These parameters for evaluating the quality of semen were selected: sperm motility, semen volume, total count of sperm, concentration, and the percentage of sperm pathology. Horký *et al.* (2015) found that sperm motility decreased by 8%, the sperm concentration was reduced by about 100 thousand/mm^3, and pathological sperm count increased by 5% during heat stress (temperatures above 23 °C). In our observation, no decline in sperm concentration was observed at the control group of boars. Significant increase in abnormal sperm cells was observed particularly during the last 60 days of the experiment when maximum temperature exceeded 30 °C. Horký *et al.* (2016b; 2016c) found addition of selenium, zinc, vitamin C and E increases antioxidant potential of ejaculate. Conversely, sperm motility decreased significantly in control of group boars. This trend could not be confirmed in our study. Although motility has been declining in our experiment, it could not be verified. Horký *et al.* (2012) observed decrease in sperm concentration within a deficiency of selenium in the diet. In our experiment, we have come to opposite results. The control group of boars showed increased sperm concentration. Moreau *et al.* (2010) added vitamin E in the form of salmon oil (300 mg per kilogram of diet) to diet of boars. The addition of vitamin E reduced the incidence of abnormal sperm cells. In our experiment, we did not observe any reduction of abnormal sperm after the addition of antioxidants (selenium, zinc, vitamin E and C). Contrarily, the control group showed significantly increased percentage of abnormal sperm. One of the studies focused on the evaluation of pathological sperm. In the first half of spring, the incidence of abnormal sperm was 19.4 % and this number rose to 25.0 % in summer (Lipenský *et al.* 2010). In our experiment, we have also observed an increase in abnormal sperm in the period of heat stress. However, our values were not so high compared to those stated by the above-mentioned authors. In a similar experimental observation as ours, there was tested the effect of dietary addition of selenium (0.5 mg/kg of diet) and vitamin E (250 mg/kg of diet) into feed of boars in order to reduce heat stress in the summer period (Echeverria *et*-Alonzo *al.* 2009). During the summer, there were observed higher concentration of sperm, motility, and reduced percent of abnormalities in the experimental group. These authors state the addition of selenium and vitamin E may reduce

the impact of heat stress and thereby enhance the quality of semen. Our results do not correlate with the results of Echeverria-Alonzo, *et al.* (2009), as we have not observed increased concentration, motility, and no reduce in the level of abnormal sperm in the experimental groups.

CONCLUSION

In the feeding experiment, the addition of selenium, zinc, vitamin C and E was tested on ejaculate quality of breeding boars during heat stress. During the experiment, temperature of environment, where boars were stabled, was monitored (the measured maximum was over 30 °C). During the 120 day experiment (June to September), following parameters of semen quality were assessed: volume, concentration, motility, total count of produced sperms and percentage of abnormal sperm cells. Our results indicate lack of antioxidants in combination with heat stress increased the incidence of abnormal sperm ($P < 0.05$) in the control group. Other parameters remained unchanged. As all our results do not agree with other authors completely, it would be suitable to repeat the experiment.

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