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ELIMINATION THE IMPACT OF HEAT STRESS BY SUPPLEMENTATION OF ANTIOXIDANTS INTO DIET OF DUROC BOARS

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Abstract

PŘIBILOVÁ MAGDALENA, HORKÝ PAVEL, NEVRKLA PAVEL, SKLÁDANKA JIŘÍ. 2018. Elimination the Impact of Heat Stress By Supplementation of Antioxidants Into Diet of Duroc Boars. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 66(1): 161–169.

The aim of this experiment was to find out, if is it possible to eliminate the impact of heat stress on quality of Duroc boar ejaculate by the addition of complex of antioxidants (Selenium, Zinc, Vit. C and Vit. E) into the basic diet. The experiment was carried out at the station of insemination in Velke Mezirici (Czech Republic) from May to September. For the study were chosen 12 Duroc boars, of approximately the same age and weight, and were divided into two groups. The control group (n = 6) was fed only by basic diet, the experimental group (n = 6) was fed by basic diet with the supplementation of antioxidants in amount: 0.5 mg selenium (seleno-methionine), 70 mg zinc (zinc-methionine), 70 mg vit. E (alpha-tocopherol) and 350 mg vit. C (L-ascorbic acid) per kilogram of basic diet. Monitored parameters of ejaculate was volume of ejaculate (ml), concentration of sperm (10^6/ml), total rate of sperm (10^9), motility of sperm (%) and amount of morphologically abnormal sperm (%).
The control group reported slight increase in volume of ejaculate (of 19%), which is common in summer months. At the same time was the concentration of sperm unstable, during August there was an increase of 15%, but during September concentration decreased below the initial amount. Motility was around 69% during whole experiment. There was an increase (of 15%) in amount of morphologically abnormal sperm, but the result was insignificant.
The experimental group had a higher volume of ejaculate, but the diagram of volume of ejaculate was almost the same with the control group. Concentration of sperm increased after the supplementation of antioxidants (of 23%) and during the experiment decreased only slightly. Motility was about average amount 70% and was not various during the experiment. In amount of morphologically abnormal sperm there was an increase about 12%.
According to the results, the supplementation of antioxidants had no significant effect on improving of semen quality. But the addition could have a positive effect to stable the parameters of ejaculate.

Keywords: heat stress, quality of ejaculate, antioxidants, boar.

INTRODUCTION

Most of the mammals have their testicles placed in scrotum outside the body cavity, causing that in testicles there is lower temperature than the temperature of the body core (Hansen *et al.*, 2009). Testicles have usually lower temperature than the body core by 2.5 °C. This lower temperature is important for optimal boar fertility (Gadd, 2011), for the correct course of spermiogenesis, storage of ejaculate and to minimize mutations of DNA in gametes. If the testicle tissue is exposed to higher temperatures, it causes a decrease of production

of sperm, decrease of their motility and increase of amount of morphologically abnormal sperm in ejaculate (Hansen *et al.*, 2009; Horky *et al.*, 2015). It is generally stated that the critical value for heat stress in boars is 25 °C (Hajek *et al.*, 1992). At the beginning of the heat stress, sperm changes will not occur immediately, but only after a certain time, when the spermatocytes mature in the sperm and are ejaculated (Hansen *et al.*, 2009). In boars, symptoms of heat stress usually begin to appear after 7 to 14 days, and sperm quality returns to normal within 5 to 8 weeks. Restoration of sperm production in the testicles lasts for 35 days and their ovulation passes on average for an additional 10 days. That is, 45 days must elapse to resume normal sperm ejaculation (Smital, 2001).

Selenium plays an important role in the proper course of physiological functions of the organism and is an important element in the antioxidant chain. Selenium is an important biogenic element for normal sperm development and maturation (Blair, 2007). Lack of selenium results in lower sperm concentration and an increased amount of morphologically abnormal sperm. These are in particular morphological changes of the flagella and the increase in cytoplasmic droplets in sperm. It is part of the enzyme glutathione peroxidase, which destroys peroxides before it damages body tissues (Horky *et al.*, 2012) and is a marker of oxidative stress (Pavlata *et al.*, 2011; Horky 2016a). Together with vit. E increases the concentration of sperm in the ejaculate and their motility (Svoboda, 2011).

Zinc is a component of many enzymes, especially superoxide dismutase, which is important in antioxidant processes. It is an essential element for animal growth, bone metabolism and physiological processes in the skin and skin derivatives. It affects the development of sexual organs and their activity (Jelínek and Koudela, 2003). Lack of Zn in the organism causes insufficient Leydig cell development and reducing susceptibility to luteinizing hormone (Close and Cole, 2000). Zn deficiency causes a reduction in feed intake and a slowing of the growth curve, parakeratosis (skin lesions similar to sputum), diarrhea, vomiting and in rare cases may cause a death (Blair, 2007).

Vitamin E is essential for proper reproduction and growth. The most important natural compound is alpha tocopherol, which can be found in vegetable oils and seeds (Horky *et al.*, 2016a). Its antioxidant function is considered to be its antisteril effect. This is important to protect cells that are easily oxidized under stress. Vitamin E thus inhibits the formation of toxic lipoperoxides (Racek, 2003).

Vitamin C is an important essential ingredient in both human and animal food (Štípek, 2000). L-ascorbic acid is synthesized in pigs organism. However, its synthesis may not be sufficient for piglets at weaning time and also for all categories of pigs during stress, especially heat stress (Zeman *et al.*, 2006). Together with vit. E and selenium helps to protect cells from oxidative damage (Close and Cole, 2000; Horky *et al.*, 2016b), thus positively affecting sperm concentration and decreasing the occurrence of morphologically abnormal sperm in the ejaculate (Svoboda, 2011). Lin *et al.*, (1990) noticed better ejaculate results (especially lower abnormal sperm counts) in boar under heat stress, by addition of 300 mg of vitamin C per boar per day.

MATERIAL AND METHODS

The experiment was carried out at the station of insemination of boars in Velke Mezirici, AGRO Merin (Czech Republic) – (N 49°23.46667′, E 15°52.70135′).

Into the experiment, 12 Duroc boars were chosen (*Sus scrofa domestica*). The average age of boars was 2.5 year ± 0.2 year. The average weight of boars was 275 kg ± 20 kg. Boars were hosed individually in boxes (2.5 × 2.5 m). The boxes were equipped with an automatic drinker with a flow rate of 1 l/min and a feed tray. All animals were fed by 3.3 kg of the basic

I: *Composition of feed mixture*

Component	% of feed mixture
Barley grain	36.00
Wheat grain	20.36
Oat grain	20.00
Soybean meal (SBM)	14.50
EKPO T (biscuit meal)	3.00
BergaFat (palm oil)	2.10
Calcium carbonate	1.50
Monocalciumphosphate	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

feed mixture (Tab. 1). The metabolised energy content (MEp) was 12.6 MJ/kg of diet. The basic feed mixture contained 0.02 mg selenium, 21.5 mg of zinc, 9.9 mg of vitamin E and 16.0 mg of vitamin C per kilogram of feed mixture.

Boars were divided into two groups. First group (n = 6) was the control group, where was no supplementation of antioxidants in basic diet. In second, experimental group (n = 6) there was the basic feed mixture enriched by 0.5 mg selenium, 70 g zinc (zinc-methionine; Alltech CZ), 70 mg vit. E (alpha tocopherol) and 350 mg vit. C (L-ascorbic acid) per kilogram of basic diet. The premix was dosed individually to the boar in the morning feeding time with using an accurate dispenser. The content of selenium, zinc, vitamins C and E in the basic feed dose was calculated on the base of table values (Zeman *et al.*, 2006).

During the whole experiment there was measured temperature and relative humidity in the stable using a datalogger device (Voltcraft DL-121TH, Germany), which was placed in a living animal zone (1 m above the ground). From these determined values were calculated the average temperature and relative humidity in each day. The resulting values of air temperature and relative humidity are shown in Fig. 1 Average and maximum temperature and in Fig. 2 Average and maximum relative humidity.

Ejaculate was taken from boars once a 7–9 days. Boar semen was sourced by a jump to the phantom. In each consumption of ejaculate there was noted the date of picking up the ejaculate, volume of ejaculate, concentration of sperm and the percentage of morphologically abnormal sperm.

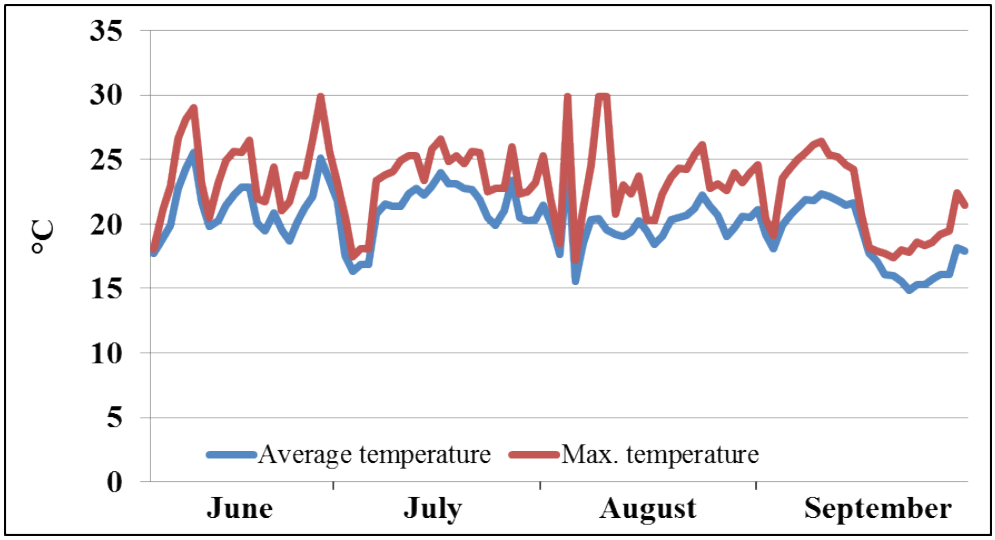
The volume of ejaculate was measured using a measuring cylinder with an accuracy of ± 0.1 ml. The concentration of sperm in the ejaculate was expressed as the number of sperms (10^3) per 1 ml. The concentration was determined photometrically using Spekol 11. Measurement was performed at a wavelength in the range 340–850 nm. By a dispenser for small amount of fluid was picked up in thin walled tubes 9 ml of 1M HCl, using varipipety was added 0.25 ml of the sample of mixed native semen and subsequently mixed. The tube was inserted into the adapter of Spekol 11 and deducting the measured value. According to the calibration table was determined the concentration of sperm.

Total rate of sperm was determined by calculation according to the formula: volume of ejaculate (ml) × concentration of sperm (10^6/ml).

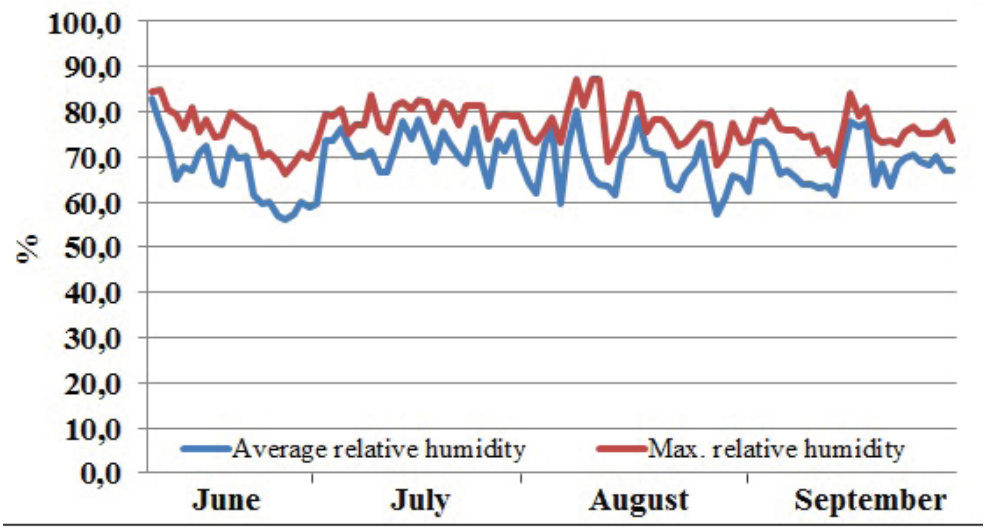
Determination of motility was performed within 15 minutes after collection of boar, microscopically of gently kneaded semen. Semen was taken up with a glass rod, a drop of semen was coated on a preheated microscope slide (about 42 °C) and overlaid with a coverslip. Slides were preheated on preheating table, microscope had also preheating plate. Microscopically determined by a subjective

II: *Composition of premix (0.5 %)*

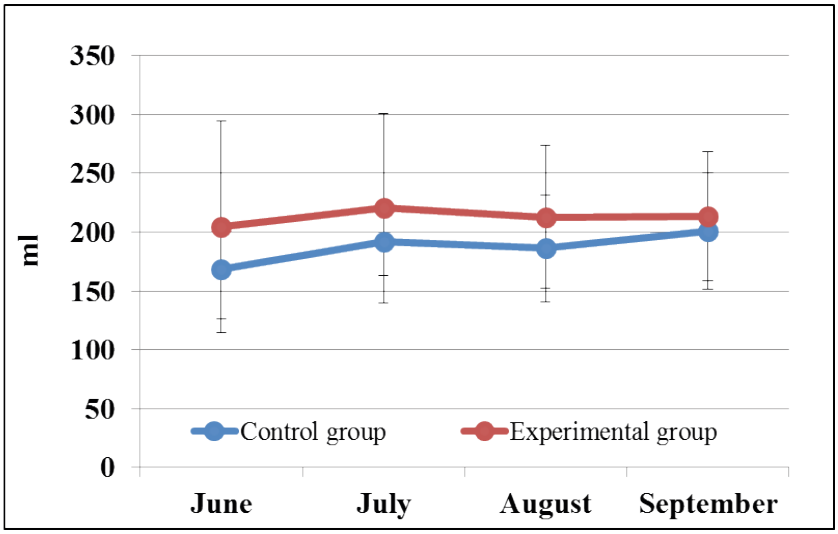
Parameter	Unit	Quantity
Vit.A	U.I.	3 000 000
Vit.D_3	U.I.	400 000
Vit.B_1	mg	500
Vit.B_2	mg	1 200
Vit.B_6	mg	800
Vit.B_{12}	mg	6
Vit.K_3	mg	600
Vit.C	mg	16 000
Biotine	mg	70
Folic acid	mg	200
Niacinamide	mg	8 000
Calcium pantothenate	mg	4 000
Choline chloride	mg	55 200
Betaine	mg	26 500
Lysine in the form of L-Lysine monohydrochloride	g	225.79
Butylhydroxi-toluene	mg	400
Ethoxyquin	mg	179.82
Zn–form of zinc oxide	mg	19 976.02
Mn–form of manganese oxide	mg	19 759.89
Fe–form of iron carbonate	mg	23 624.51
Co–form of cobalt sulphate heptahydrate	mg	91.35
I–form of potassium iodide (KI)	mg	229.20
Carrier ad.–wheat meal and calcium carbonate	kg	1



1: *Maximum and average temperature in the stable*



2: *Maximum and average relative humidity in the stable*



3: *Volume of ejaculate*

estimate of the percentage of sperm with rectilinear motion forward for a head at body temperature and sampled at 1:40. The mobility rate is determined in five ocular fields.

Percentage of morphologically abnormal sperm was determined from the first collection in the month. To prepare the smear: drop of semen was coated with a glass rod on a glass slide; at an angle of 45 ° was spread out over the edge by a grounded smears glass. Morphological assessment (evaluation of abnormal sperm in five ocular fields–all individually numbered with abnormal sperm), staining and evaluation of sperm carried the district veterinarian.

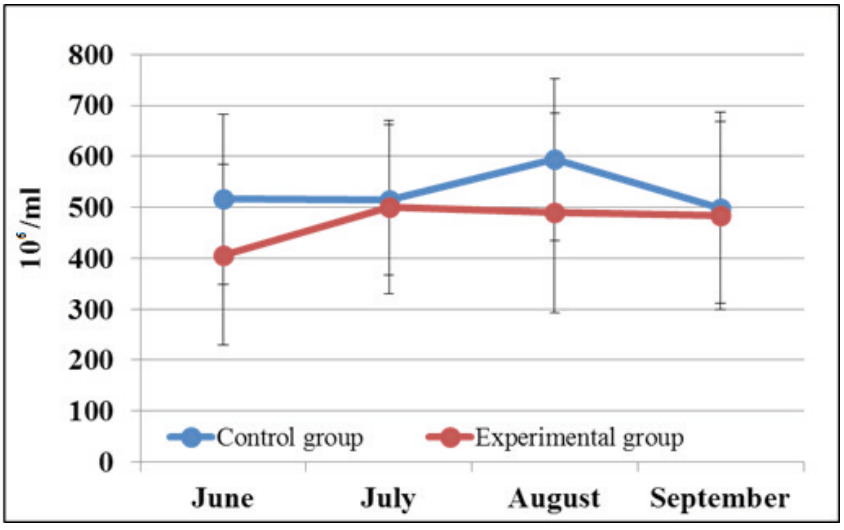
The data were statistically analysed using STATISTICA.CZ version 10.0 (Czech Republic). The results were expressed as the mean ± standard variance. Statistical significance was observed between the groups (the first sampling was taken as a control one) using ANOVA and Scheffe's test-the two-factor analysis (the first factor was the animal group, the second one-the sampling factor) for parameters: volume of ejaculate, sperm concentration, motility and percentage of pathological sperms. The difference ($P < s0.05$) was considered as significant.

RESULTS

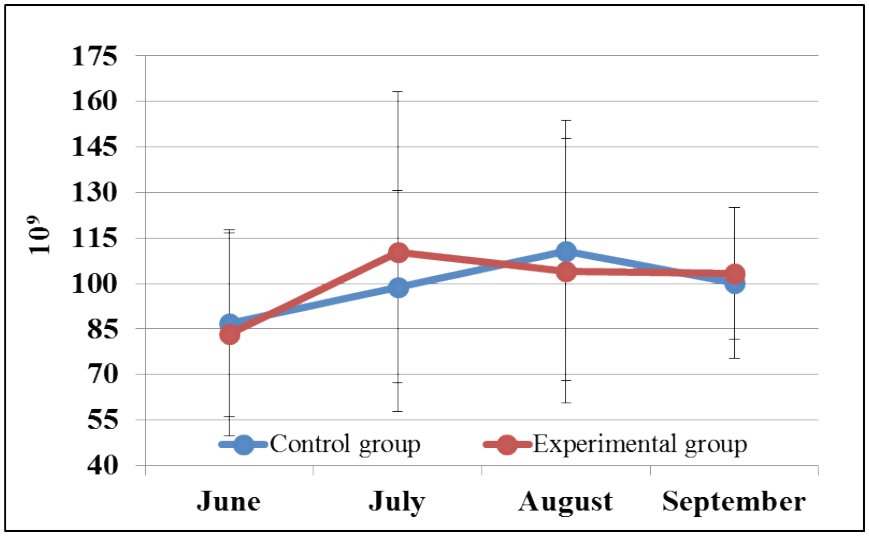
In Fig. 3 there are shown Measured values of volume of ejaculate of both, control and experimental groups. Average volume of ejaculate in experimental group was 212.9 ml and in control group 187.0 ml. During whole experiment the average temperature was 20.2 °C. At the beginning of the experiment, in June, where the average temperature was 21.3 °C, was average volume of experimental group 204.8 ml and of control group 168.3 ml. At the end of the monitoring the volume of ejaculate of experimental group was 213.5 ml and of control group 200.8 ml within the average temperature 18.9 °C. The curve of diagram is very similar for both monitored groups. In this case, boars in the experimental group may have higher volumes due to the added mineral-vitamin supplement. However, heat stress did not show up on the volume of the ejaculate nor in one group.

Fig. 4 shows The concentration of sperm concentration in both groups. At the beginning of the experiment, the average value of the experimental group was 406.9×10^6/ml and the control group 515.8×10^6/ml. In the experimental group, the average values in July increased to 500.8×10^6/ml and then had a slight tendency to decline. In the control group, the average value in July remained almost unchanged, but in August the concentration increased significantly to 594.0×10^6/ml and in September it dropped significantly to 498.7×10^6/ml. In the experimental group, the concentration could be an increase caused by addition of antioxidants. Antioxidants then ensured a constant concentration throughout the monitoring period. In control group experienced a sudden increase in the concentration followed by a fall again. Concentration in the control group is therefore relatively unstable, which is not conducive for the production of insemination doses.

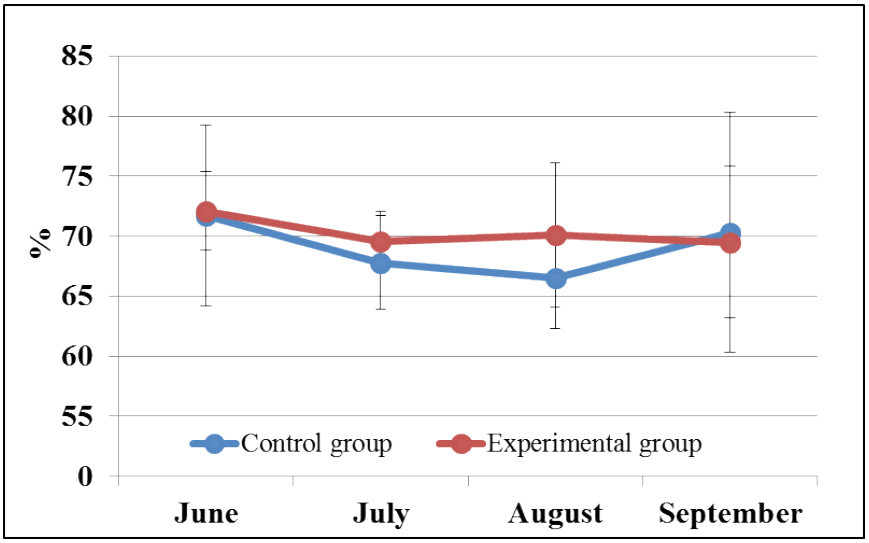
The values of the total number of sperm obtained by the calculation are recorded in Fig. 5. The experimental group experienced a noticeable increase in July from 83.3×10^9 to 110.4×10^9. However, the August dropped slightly and in September the average value was 103.2×10^9. In the control group, the increase in sperm count was gradual. The highest average number was reached in August, when the value was 110.7×10^9, from the initial value 86.3×10^9. In September it decreased to 100.1×10^9. The relatively rapid increase in the experimental group resembles to addition of mineral-vitamin supplement into diet. At the end of the experiment, the values of both groups were again similar to those at the beginning



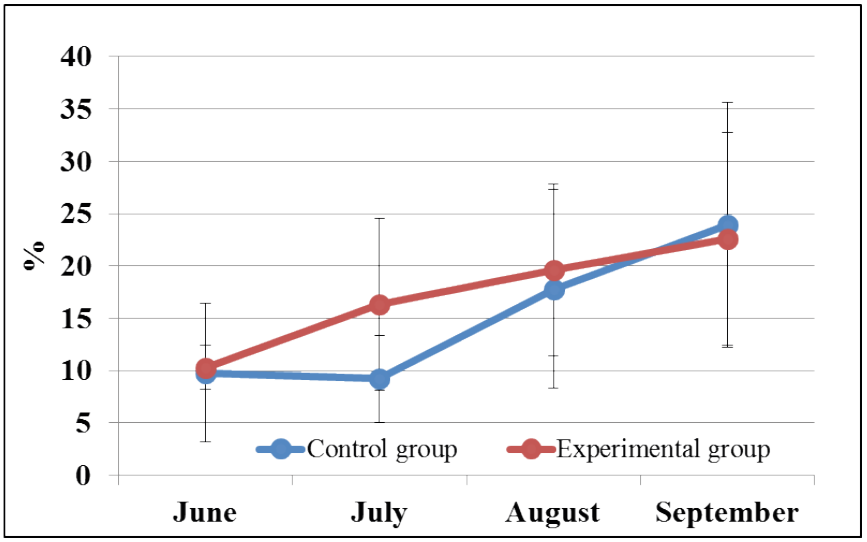
4: *Concentration of sperm*



5: *Total rate of sperm*



6: *Motility of sperm*



7: *Amount of morphologically abnormal sperm*

of the experiment. However, none of the measured values was statistically significant.

Fig. 6 shows Sperm motility in both groups over the monitored period. In the experimental group, the sperm motility value was 72.1% at the beginning of the experiment, 71.7% in the control group. During the experiment the values fluctuated slightly, within the experimental group had overall better results than the control group. In September, motility results were similar to those at the beginning of the experiment. There was a slight decrease in motility in both groups. The experimental group was measured motility at 69.5% and in the control group 70.3%. Because of the statistically inconclusive results, we can say that the heat stress did not affect boar motility. The highest difference in the experimental group was a decrease in average values between June and July, which the difference between month was 2.5%. The control group had the highest difference between August and September, when the motility increased by 3.8%.

Fig. 7 shows how The temperature during the experiment was reflected in the amount of morphologically abnormal sperm. At the beginning of the observed period, the amount of abnormal sperm was almost identical in both groups (experimental group 10.3%, control group 9.8%). However, in the experimental group, abnormal sperm counts increased steadily throughout the period. Monthly average increase in abnormal sperm was about 3%. In the control group, the effect of higher temperatures on sperm abnormality did not show until August. In July, even the percentage of abnormal sperm was lower than in June (0.6%). In August, there was a sharp increase (8.6%) and in September the values even exceeded the experimental group by 1.3% (23.9%). We can therefore conclude that high temperatures and subsequent stress in animals will cause a higher percentage of morphologically abnormal sperm.

DISCUSSION

The positive effect of antioxidants on organism is known for a long time. The effect on quality of semen is still being investigated. Statistically significant results noted Horky *et al.,* (2016a), when they found that the addition of antioxidants to the basic diets of boars does not affect sperm quality, but it stabilizes the quality of the ejaculate. This hypothesis was confirmed in this experiment.

During the monitoring of the qualitative indicators (motility, abnormal sperm), the best results were observed during the winter months when the temperature is more favorable for pigs. On the contrary, during the summer months, the highest value reached in the ejaculate volume (Smital, 2008). In an experiment by Knecht *et al.,* (2014), it has been shown that sperm concentration decreases with a higher volume of ejaculate. We cannot fully confirm this hypothesis in our experiment. In the control group, the sperm concentration increased significantly during August. In the experimental group there was an increase in concentration already in July, followed by a slight decrease during the rest of experiment. The highest concentration of sperm is regularly recorded at the insemination station at the end of autumn months. In the warmest months in the spring and summer, sperm concentration is the lowest, and this is associated with a lower amount of insemination doses (Kunavongkrit *et al*., 2005).

Another observed parameter of quality of ejaculate was motility. The seasons should not affect sperm motility (Knecht *et al.,* 2014). In our experiment, in both groups, sperm motility ranged around 70% throughout the experiment, with daytime peak reaching 28 °C for several days. Barranco *et al.,* (2013) in their work, however, show other results, when the motility in the summer months significantly decreased compared to the other months.

The last observed parameter was the amount of morphologically abnormal sperm. Smital (2008) reports that in the summer, the quality is the lowest due to the ongoing heat stress. We confirmed this hypothesis, however, the increase in morphologically abnormal sperm in both groups was not statistically significant. Cerovský *et al.,* (2005) reported that the effect of heat stress is reflected by the amount of abnormal sperm, with the most frequent abnormality being the occurrence of a protoplasmic drop.

Li *et al.,* (2015) measured the temperature in boar in the rectum and scrotum at heat stress when the outside temperature reached above 28 °C for more than 3 days and found that the percentage of morphologically abnormal sperm had increased, but the function of the additional glands was not impaired. Elevated temperature in the scrotum causes impaired spermiogenesis, which leads to higher incidence of abnormally developed sperm (Ahmad *et al*., 2012).

When comparing values and quality throughout the year, there is a noticeable decrease in the quality of the semen in the summer months. By the end of the winter season, the quality increases and starts to fall slightly during the spring. Due to the higher quality in the winter months, the highest number of piglets was born after insemination with these doses (Steyn *et al*., 2012). If boars and sows are supplemented by 0.15–0.30 mg selenium/kg of feed mixture, the reproduction results in modern genotype pigs are very good (Surai and Ionov, 1992). Hansen (2009) reported that spermiogenesis plays a significant role in the development of gyno – and androspermia. Within higher temperatures more female offspring are born.

CONCLUSION

The results of our experiment show that high temperature causes heat stress in boars, which affects some of the ejaculate parameters. Both groups included boars of the same age, which had very similar ejaculate values at the beginning of the experiment. Thus both groups were fully utilized in the insemination program and were without significant health problems that could distort the results of the experiment.

The high ambient temperature in this experiment only affected the amount of morphologically abnormal sperm, which increased the amount in both groups during the experiment. The experimental group had a steady increase from the beginning of the experiment. The control group had an increase of up to a month later. We can conclude from these results that the addition of antioxidants (especially vitamins) had no effect on the percentage of morphologically abnormal sperm. For the other ejaculate parameters, we found that the experimental group had more stable and balanced ejaculate values than the control group. This is more practical from the insemination point of view, in order to preserve the quality and number of insemination doses. This fact is attributed to the increased antioxidant barrier resulting from the addition of antioxidants to the basic feed. However, the results are not statistically significant.

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