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Original Contribution

EFFECT OF SELENIUM ON LYSOZYME CONCENTRATION IN BOAR SPERM

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ABSTRACT

We used 12 inbred boars from the English Large White breed in this study. The animals were reared in individual boxes. Selenium (Se) in the forage was provided from a preparation of Sel-Plex® (Alltech Inc., USA) to a concentration of 0.3 ppm/kg. Semen for analysis was obtained prior to Sel-Plex® administration, and by days 50 and 100 after the supplementation. It was found that during the two tests, lysozyme levels were significantly higher than baseline levels, probably due to the Sel-Plex® treatment ($p < 0,01 – 0,001$). When blood serum lysozyme concentrations were compared with sperm levels from all three tests, it could be seen that the latter were considerably higher than the results of the first testing and almost equal for the other two tests. In conclusion, it could be presumed that the trace element selenium influenced considerably the concentration of lysozyme in boar sperm.

Key words: lysozyme, Sel-Plex®, sperm, boars.

INTRODUCTION

Lysozyme was discovered in 1922 by Fleming. Its properties differ according to its origin Hence it is often more correct to call it lysozymes instead of lysozyme. This term is used to name a large group of enzymes breaking up β-(1-4), and probably β-(1-2) glycoside bonds (1). In animals and humans, lysozyme could be detected in saliva, tears, heart, muscle, spleen, lymph nodes, kidneys, lungs, cartilage, and amniotic fluid; in lesser amounts in skeletal muscles, brain, cerebrospinal fluid and the urine (2). Via the method of tissue cultures, a high concentration of this enzyme was detected in granulocytes, macrophages and Panet's cells (3, 4, 5, 6, 7). A significant content of muramidase is also found in the zones of active proliferation of cells (8). Tzirkin and Zabirov (9) observed high levels of the enzyme in human sperm and prostatic secretion. This fact is confirmed by Kuzmin et al. (10), by observing a highly significant correlation between the concentration of this enzyme and the motility of spermatozoa. This allowed recommending a method for treatment of male infertility (11). A number of factors influence the lysozyme content in sperm –adequate protein and trace elements levels in the diet, etc. (12).

The lack of information about lysozyme concentration in boar sperm and the effect of selenium (Se) on this parameter motivated the present investigation.

MATERIALS AND METHODS

In this experiment, 12 inbred boars from the English Large White breed were used. The animals were 2 years old, clinically healthy and were reared in individual boxes and given a ration whose composition is given on **Table 1**. As a source of Se, the preparation of Sel-Plex® (Alltech Inc., USA) was added at a concentration of 0.3 ppm/kg to the forage. Semen for analysis was obtained prior to Sel-Plex® administration, and by days 50 and 100 after the supplementation. For comparative purposes, blood for analysis was sampled by post supplementation day 100. Sperm and serum lysozyme concentrations were determined according to the method of Lie (13), after centrifugation for 10 min at 2000 g.

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Briefly 20 ml of 2 % agarose (ICN, UK, Lot 2050), dissolved in sodium phosphate buffer (pH = 6.2) was mixed with 20 ml suspension of 24 hours culture of *Micrococcus lysodeicticus* at 67°C. This mixture was poured out in Petri's dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microlitres of undiluted sera and sperm supernatants were poured in each well. Eight standard dilutions (from 0,025 to 3,125 μg/ml) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured. Final lysozyme concentrations were calculated by using special computer program developed in Trakia University and expressed as μg/ml.

Table 1. *Forage mixture given to the boars in this experiment*

Forages and additives%	
Maize	15
Wheat	15
Barley	40
Soyabean of meal (44 % DP)	4
Sunflower of meal (37 % DP)	3
Wheat bran	19,81
Synthetic L-lysin	0,15
Synthetic Treonin	0,09
Salt	0,27
Dical. phosphate	0,94
VMP AGREX P_{2005}	0,5

Contents of energy and nutritious ingredients in forage mixture for boars.

Moisture, %	12,5
DE, MJ/kg	12,5
DE, kcal/kg	2986
EE, MJ/kg	12,0
EE, kcal/kg	2870
Crude protein, %	13,6
Crude fibres, %	5,7
Crude fats, %	2,6

DE – digestible energy
DP – digestible protein
EE – exchange energy
CP – crude protein
VMP – vitamins, minerals and protein

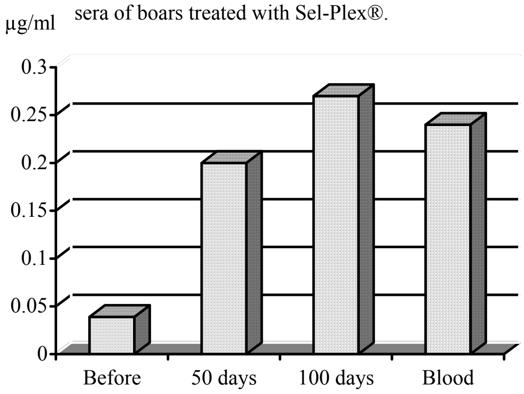
Statistical analysis:

Data were analysed using the fixed effect ANOVA model (Program STATISTICA, StatSoft, Inc., USA).

RESULTS AND DISCUSSION

The graph shows considerable changes in lysozyme concentrations in boar sperm. Throughout the first (pre-treatment) testing, the average concentration of the enzyme was very low **(Fig. 1)**

Fig. 1. Lysozyme concentrations in sperm and blood sera of boars treated with Sel-Plex®.



During the following two tests (by days 50 and 100 respectively), lysozyme levels were significantly higher than baseline levels, probably due to the Sel-Plex® treatment ($p < 0,01 – 0,001$). When blood serum lysozyme concentrations were compared to sperm levels from all three tests, it could be seen that the latter were considerably higher than the results of the first testing and almost equal for the other two tests. The cause for the initial low sperm lysozyme activity in our opinion was the significant level of inbreeding in boars. This assumption is also supported by comparing the results of the present experiment with the average concentrations for this breed, observed by Sotirov (14) – 3,65 ± 0,20 μg/ml. The negative impact of inbreeding upon blood serum lysozyme concentrations was evidenced by the studies of Tanchev (15). This author found significant effect of the level of inbreeding on lysozyme concentration in blood sera (*Fx=0,25* – 0,41 ± 0,09 μg/ml; *Fx=0,125* – 0,52 ± 0,03 μg/ml; *Fx=0* – 2,35 ± 0,16 μg/ml). Against this background, the positive effect of Se on sperm lysozyme levels of studied boars was even more evident. Practically, the supplementation of this trace element restores the presence of lysozyme in semen. This is very important because, according to Xu et al. (16, 17), there is a significant positive correlation ($r = 0.50$, $p < 0.01$) between sperm Se concentration and each of ejaculate volume, semen density and spermatozoa counts. These positive outcomes resulted in improved fertility in studied men. Similar results are reported by Sotirov et al. (12) in the feeding of gobblers with diets containing more protein. The studies of

Kuzmin et al. (10, 11,) proved that when lysozyme is absent or at low concentrations, the fertility of human semen was low. When semen with low spermatozoa motility was supplemented with lysozyme, the normal motility was restored and over 50% fertility rate was achieved in couples participating in this experiment. All these studies emphasize the importance of these two factors (Se and lysozyme) for the normal reproductive ability of male individuals. Probably, the higher content of Se in semen leads to increased sperm lysozyme concentration and consequently, to a higher fertility of spermatozoa. According to unpublished data of Sotirov et al., the supplementation of sows' diet with Sel-Plex® (as a source of Se) increased blood serum lysozyme activities in both dams and suckling piglets.

In conclusion, it would be suggested that the trace element Selenium influenced considerably the concentration of lysozyme in boar sperm.

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