THE EFFECT OF THE ESTROGENIC MYCOTOXIN ZEARALENONE ON BOAR REPRODUCTIVE POTENTIAL AND THE DYNAMIC OF ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE LEVELS IN THE BOAR BLOOD SERUM

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Summary. This study was carried out to assess the impact of the feedstuff contaminated with mycotoxin zearalenone on boar sperm quality, testicle tissues and the amounts change of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The boars were fed with feedstuff naturally contaminated with mycotoxin zearalenone (1 ppm) for two months period, no testicle changes or negative impact on their sperm quality was observed. Prominently higher levels (by 7.45 ± 2.86 TV/L) of the enzyme alanine aminotransferase (ALT) were observed in the blood serum of experimental boars

Key words: zearalenone, boar, semen quality, aspartate aminotransferase, alanine aminotransferase.

MIKOTOKSINO ZEARALENONO POVEIKIS KUILIŲ REPRODUKCINĖMS SAVYBĖMS, ASPARTATO AMINOTRANSFERAZĖS IR ALANINĖS AMINOTRANSFERAZĖS KIEKIO KAITAI KUILIŲ KRAUJO SERUME

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Santrauka. Šio bandymo tikslas buvo išsiaiškinti užteršto mikotoksinu zearalenonu pašaro poveikį kuiļuī spermų kokybei, kuiļuī sēkļuī audiniam. Beširdžių aspartato aminotransferazės (AST) ir alanino aminotransferazės (ALT) kiekio kaitai kraujo serume. Kuiļus šeriant natūraliai mikotoksinu zearalenonu (1 ppm) užterštu pašaru du mėnesius, spermų kokybės ir sēkļuī sēklinių morfologinių pokyčių nepastebėta. Ženkliai pakito fermento alanino aminotransferazės (ALT) kiekis, turinčios grupės kuiļų kraujo serume jo buvo 7,45±2,86 TV/L daugiau (p<0,001) už kontrolinės grupės. Tyrimų rezultatai parodė, kad 1 ppm zearalenono pašare per du mėnesius įtakos subrendusių kuiļų reprodukcinėms savybėms nedaro, bet kepenų metaboliniai procesai intensyvėja.

Raktažodžiai: zearalenonas, kuiļys, spermų kokybė, alanino aminotransferazė, aspartato aminotransferazė.
which involves primarily all genital system. The mycotoxin produces a well characterized estrogenic syndrome, especially in prepubertal gilts. Various estrogenic effects such as decreased fertility, increased embryolethal defects, proximal and distal cytoplasmic droplets, loose heads, acrosome defects, pouch formations, abnormal midpieces and the incidences of tail abnormalities were determined in wet preparations (an aliquot of semen was fixed in buffered formol-saline solution) under the phase-contrast microscope at 400 × magnification. Sperm head defects (pear shape, narrow at base, abnormal countour, undeveloped, loose abnormal head, narrow, big, little normal, short – broad and abaxial) were determined in dry preparations, stained according to Williams (Williams and Savage, 1925). Sperm concentration (density) was assessed in blood cell counting (Goriajev) chamber. Spermatozoa viability was determined with the hypoosmotic test (HOT) (Vasquez et al., 1997) and staining spermatozoa with eosin-nigrosin (EN) (Dott and Foster, 1972).

Blood serum analysis: After every sperm sampling blood samples for biochemical analysis were taken from the ear vein with intravenous catheter into glass tubes without coagulant. The separated blood serum was centrifuged at 3000 rpm for 5 minutes. 2 ml of the centrifuged blood serum were transferred to Eppendorf-type 2-ml capped test tube, using 1 ml Pasteur pipette (Einweg-Pasteurpipetten, Carl Roth GmbH, Germany). The tubes with the serum were frozen and stored at -20°C until the experiment. The blood analysis was done with a computerized biochemical analyser Hitachi 705 (IFCC, 1986).

The levels of aspartate aminotransferase and alanine aminotransferase (AST) and alanine aminotransferase (ALT) in blood serum were estimated in blood serum. The analysis of variance revealed no significant differences between experimental and control groups by period interaction for any of the measured parameters (Table 1). Nevertheless 7.89 % by HOT and 1.69 % by semen analysis was recorded and freshly ejaculated semen was extended in the BTS/Androhep (v/v) extender. The semen analysis was made in the Animal Reproduction Laboratory, LVA. Motility of spermatozoa was examined subjectively at 37°C under phase-contrast microscope Olympus BH2 with a prewarmed 37°C stage (Olympus Optical Co., Ltd., Japan) using a 400 × magnification. Motility was analyzed on 5-μl aliquots of fresh semen. Sperm tail defects, proximal and distal cytoplasmic droplets, loose heads, acrosome defects, pouch formations, abnormal midpieces and the incidences of tail abnormalities were determined in wet preparations (an aliquot of semen was fixed in buffered formol-saline solution) under the phase-contrast microscope at 400 × magnification. Sperm head defects (pear shape, narrow at base, abnormal countour, undeveloped, loose abnormal head, narrow, big, little normal, short – broad and abaxial) were determined in dry preparations, stained according to Williams (Williams and Savage, 1925). Sperm concentration (density) was assessed in blood cell counting (Goriajev) chamber. Spermatozoa viability was determined with the hypoosmotic test (HOT) (Vasquez et al., 1997) and staining spermatozoa with eosin-nigrosin (EN) (Dott and Foster, 1972).

Analysis of mycotoxin: Mycotoxical feedstuff contamination was tested using a method of thin-layer chromatography in the Mycotoxicology Laboratory, LVA. Mycotoxin Zearalenone concentration measures were ppm (mg/kg⁻¹).

Semen analysis: Ejaculates were collected by gloved-hand technique. The volume of ejaculate (ml) was measured and freshly ejaculated semen was extended in the BTS/Androhep (v/v) extender. The semen analysis was made in the Animal Reproduction Laboratory, LVA. Motility of spermatozoa was examined subjectively at 37°C under phase-contrast microscope Olympus BH2 with a prewarmed 37°C stage (Olympus Optical Co., Ltd., Japan) using a 400 × magnification. Motility was analyzed on 5-μl aliquots of fresh semen. Sperm tail defects, proximal and distal cytoplasmic droplets, loose heads, acrosome defects, pouch formations, abnormal midpieces and the incidences of tail abnormalities were determined in wet preparations (an aliquot of semen was fixed in buffered formol-saline solution) under the phase-contrast microscope at 400 × magnification. Sperm head defects (pear shape, narrow at base, abnormal countour, undeveloped, loose abnormal head, narrow, big, little normal, short – broad and abaxial) were determined in dry preparations, stained according to Williams (Williams and Savage, 1925). Sperm concentration (density) was assessed in blood cell counting (Goriajev) chamber. Spermatozoa viability was determined with the hypoosmotic test (HOT) (Vasquez et al., 1997) and staining spermatozoa with eosin-nigrosin (EN) (Dott and Foster, 1972).

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mental boars was found. In control group boars average weight of left testis was 498.33 ± 2.89 g, in experimental boars - 496.64 ± 5.77 g (p>0.05). In control group boars average weight of right testis was 491.67 ± 2.89 g, in experimental boars - 446.67 ± 83.86 g (p>0.05). There were no pathological changes in testis.

Table 1. Semen production and sperm quality from control and experimental boars

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Boar group</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>No of boars</td>
<td>3</td>
</tr>
<tr>
<td>No of ejaculates collected</td>
<td>36</td>
</tr>
<tr>
<td>Subjective motility, %</td>
<td>61.81 ± 7.09</td>
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<tr>
<td>Sperm concentration, x10⁹/ml</td>
<td>0.32 ± 0.11</td>
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<tr>
<td>Volume of ejaculate, ml</td>
<td>235.0 ± 74.05</td>
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<tr>
<td>Sperm viability, % (HOT)</td>
<td>48.88 ± 16.04</td>
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<tr>
<td>Sperm viability, % (EN)</td>
<td>94.08 ± 3.2</td>
</tr>
<tr>
<td>Total sperm defects, %</td>
<td>10.43 ± 7.29</td>
</tr>
</tbody>
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X ± SD – mean and standart deviation

In the trial we analysed the amounts of aspartate aminotransferase and alanine aminotransferase in boar blood serum. It was found that the level of aspartate aminotransferase in blood serum in both groups vary similar over experimental period (Fig.1). The average level in blood serum of aspartate aminotransferase was 26.22 ± 6.23 TV/L in the control and 28.24 ± 7.63 TV/L in experimental boars group.

Fig. 1. Aspartate aminotransferase levels in the control and the experimental group boar blood serum during the experimental period

The results showed that the concentrations of liver enzymes aspartate aminotransferase and alanine aminotransferase did not exceed the physiological norms, but the amounts of alanine aminotransferase in the experimental group’s boar blood serum were statistically significantly higher (p<0.05) (Fig. 2). The average level in blood serum of alanine aminotransferase was 46.28 ± 8.24 TV/L, in the control and 55.73 ± 11.1 TV/L in experimental boars group.

**Discussion.** Zearalenone is one of the most common fusariotoxins in Lithuania (Bakutis et al., 2006). It causes animal reproductive disorders also. Of all animals swine and young gilts are the most sensitive to zearalenone (Bakutis, 2004). The concentration as low as 0.5–1 ppm can cause pseudo-estrus, and vaginal or rectal prolapse (Lawlor and Lynch, 2001). Pre-pubertal gilts are affected at 5 mg of zearalenone/kg of feed (5 ppm) (Ruhr et al., 1983). The reports about its effects on adult boars are quite controversial. We experimented with mature boars and achieved the results, confirming the opinion that mature boars are not sensitive to zearalenone as long as the daily concentration in feedstuff is no more than 200 ppm (Ruhr et al., 1983; Osweiler, 1992; Carlson and Ensley, 2002).

In the present study we have used naturally contaminated feed at 1 mg of zearalenone/kg of feed. Contaminated feed with zearalenone was administered through a complete spermatogenic cycle, and time of sperm epididymal transit. Significant changes in the various measures of reproductive potential were not detected. Young and King (1986) report that feedstuff containing less than 9 ppm had no negative effect on boar ejaculate volume and sperm motility.
In our experiments no significant difference was established between the experimental group and the control group boars when the variation of the number of live spermatozoa during the trial was estimated. Nevertheless 1.69 % - 7.89 % more viable spermatozoa (depending from the method) were found in the control group ejaculates.

Zearalenone and $\alpha$-zearalenol reduce spermatozoa viability in vitro, but the report emphasizes that the effect of the toxin depends on the time period and dose (Tsaksmakidis et al., 2006).

Morphological sperm examination revealed no statistically significant correlation in the number of pathological spermatozoa between the control and the experimental group boar ejaculates. Nevertheless 1.51 % less sperm defects were found in the control group ejaculates.

Similar results were reported by other authors, stating that zearalenone does not interfere with mature boar spermatogenesis and does not affect the sperm quality if the concentration in feedstuff is less than 60 ppm (Diekman and Green, 1992; Patience et al., 1995).

The results of the control and the experimental group boar testicles showed no statistically significant differences of testicles and epididymides weight between the groups. It concurs with the reports of Young and King (1986), stating that zearalenone does not affect adult boar testicles and epididymides if the doses are not high.

Metabolism of zearalenone takes place in liver (Malekinejad et al., 2005). The toxin suppresses metabolism of blood serum protein albumin, bile colours and cholesterol synthesis (Sutkевичius et al., 2000). Zearalenone toxicity to the liver can be estimated by examining it histomorphologically or by analysing the blood serum enzyme content. In the trial we analysed the amounts of aspartate aminotransferase and alanine aminotransferase in boar blood serum. The results showed that the concentrations of liver enzymes aspartate aminotransferase and alanine aminotransferase did not exceed the physiological norms, but the amounts of alanine aminotransferase in the experimental group’s boar blood serum were statistically significantly higher. This implies that mycotoxin zearalenone might have an effect on boar organism (p<0.05). Reports of experiments with rats also mention the increased levels of alanine aminotransferase and aspartate aminotransferase in the blood serum of animals, directly injected with synthetic mycotoxin zearalenone (Maaroufi et al., 1996).

The results showed that feedstuff’s mycotoxin zearalenone (1 ppm) has no negative impact to mature boar testicle tissues and the sperm qualitative and quantitative traits. Zearalenone, at levels normally found in contaminated feeds and capable of causing severe reproductive problems in female swine (<200 ppm) does not appear to adversely affect the reproductive potential of mature boars, but it stimulates liver metabolic processes.

**Conclusions**

Feeding of diets containing 1ppm of zearalenone for 55 days did not significantly affect the sperm quality parameters and testicles in mature boars. Zearalenone at this level did not permanently blocked or adversely affected spermatogenesis in mature boars, but stimulated liver metabolic processes.

**References**

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