THE EFFECT OF THE ESTROGENIC MYCOTOXIN ZEARALENONE ON BOAR REPRODUCTIVE POTENCIAL 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 ($p \leq 0.001$). The results showed that zearalenone at amounts 1ppm in field exposures for two months did not negatively affect the reproductive potential of mature boars yet it stimulates liver metabolic processes.

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

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

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Santrauka. Šio bandymo tikslas buvo ištirti užterštο mikotoksinu zearalenonu pašaro poveikį kuilių spermos kokybei, kuilių sėklidžių audiniams bei fermentų aspartato aminotransferazės (AST) ir alanino aminotransferazės (ALT) kiekio kaitai kraujo serume.

Kuilius šeriant natūraliai mikotoksinu zearalenonu (1 ppm) užterštu pašaru du mėnesius, spermos kokybės ir sėklidžių morfologinių pokyčių nepastebėta. Ženkliai pakito fermento alanino aminotransferazės kiekis. Tiriamosios grupės kuilių 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 kuilių reprodukcinėms savybėms nedaro, bet kepenų metaboliniai procesai intensyvėja.

Raktažodžiai: zearalenonas, kuilys, spermos kokybė, alanino aminotransferazė, aspartato aminotransferazė.

Introduction. Feedstuff contaminated with mycotoxins is still an important problem in husbandry. Mycotoxins are biological toxins. It was established that mycotoxins have an immunosuppressive, hepatogenic, mutagenic, nephrotoxic, enterotoxic, teratogenic, estrogenic and allergic effect on a living organism. Fusariotoxicoses cause major problem in Lithuania (Bakutis, 2002). Mycotoxin zearalenone, produced by *Fusarium* spp., causes the most extensive damage to animal reproduction, as it directly affects the reproductive system as an estrogen agonist. Zearalenone is a phytohormone, possessing anabolic and strong estrogenic, as well as heamatotoxic and genotoxic properties (Baliukoniene et al., 2003; Cankova et al., 2003). Zearalenones act as estrogen because of phenol ring chemical structure that gives it the possibility on binding with cellular estrogenic receptors (Gajęcki, 2002). Zearalenones for this reason are able to adopt a conformation which sufficiently resembles 17β-estradiol and other natural estrogens to permit binding to the estrogen receptors (Shier, 1998).

It has been long known that sows and gilts are sensitive to this toxin (Long and Diekman, 1984; Osweiler, 1992; Diekman and Green, 1992; Malekinejad et al., 2005). Among large animal species, swine are the most sensitive; nevertheless zearalenone also causes problems in dairy cattle, chicken, turkey, horses and sheep (Baliukoniene et al., 2003; Diekman and Green, 1992). In swine the major problem they cause is vulvovaginitis,

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 receptors, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and estradiol have been observed, and teratogenic effects were found in pigs and sheep (Gajęcki, 2002).

The animals were examined over a two month period. The 8 weeks period allowed exposure to the zearalenone for a complete spermatogenic cycle of 35 days, plus epididymal transit time of 11 days and 10 additional days of exposure (Ruhr et al., 1983).

In boars high concentration of zearalenone in feed may cause reduced libido, reduced testes size and weight (Diekman and Green, 1992). Zearalenone also leads to disorders of spermatogenesis (Baliukoniene et al., 2003) and related testicular development (Carlson and Ensley, 2002). It may cause also preputial and mammary enlargements (Ruhr et al., 1983). Still and all the results are often controversial. A study in prepubertal boars found only reduced plasma testosterone concentration during the zearalenone administration and a subsequent reduction in libido (Ruhr et al., 1983). Young boars may have reduced libido and decreased testicular size but mature boars are unaffected by concentrations of zearalenone as high as 200 ppm (Osweiler, 1992; Carlson and Ensley, 2002). However, little is known about the effect that zearalenone and its metabolites have on the boar reproductive system, and the available results are quite contradictory.

The aim of our study was to determine the effect of the naturally contaminated fed with estrogenic mycotoxin zearalenone at rate 1ppm on boar reproductive potencial: libido, sperm quality parameters, testicle tissues and the amounts change of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood serum.

Materials and methods. *Animals:* In total six mature Danish Landrace boars (three control and three experimental animals) were included into the analysis. The animals were examined over a 2 month period in spring. The mean age of animals was 20.1 ± 2.94 months. All boars were kept in separate stalls. Water was available ad libitum. In the experimental period three boars were fed naturally contaminated commercial swine ration with 1.00 ppm zearalenone, other three as a control boars were fed according to the state norms for AI boars (Jančienė, 2005). Blood and semen were sampling every five days at the time of the trial. After the experimental period the boars were slaughtered and samples of their testicle tissues were taken for histological examination.

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 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, norrow, 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 Paster 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 were estimated in blood serum.

Analysis of testicles: The testicles with epididymis were examined macroscopically, their weight was measured, and the form, position, sectional view's colour and consistency were estimated.

Statistical analysis: Statistical analysis was accomplished using the version of the statistic package *SPSS No. 15* for Windows. Statistical data analysis was done by descriptive statistics and using monofactorial analysis. Comparison among groups was made by *Post Hoc* multiple comparison method. Differences among groups were analyzed by LSD method. The data was considered to be statistically reliable when: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Correlation among dependent variables and strength of the direct relation was evaluated by Pearson correlation matrix.

Results. 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 EN more viable spermatozoa and 1.51 % less sperm defects were found in the control group ejaculates.

Macroscopically, no significant difference of testis and epididymis weight and form in control and experi-

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 experimantal boars**

Measurement	Boar group	
	Control	Experimental
No of boars	3	3
No of ejaculates collected	36	36
Subjective motility, %	61.81 ± 7.09	62.64 ± 7.97
Sperm concentration, $x10^9$/ml	0.32 ± 0.11	0.35 ± 0.14
Volume of ejaculate, ml	235.0 ± 74.05	223.06 ± 62.06
Sperm viability, % (HOT)	48.88 ± 16.04	40.99 ± 19.42
Sperm viability, % (EN)	94.08 ± 3.2	92.39 ± 4.55
Total sperm defects, %	10.43 ± 7.29	11.94 ± 3.94

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.

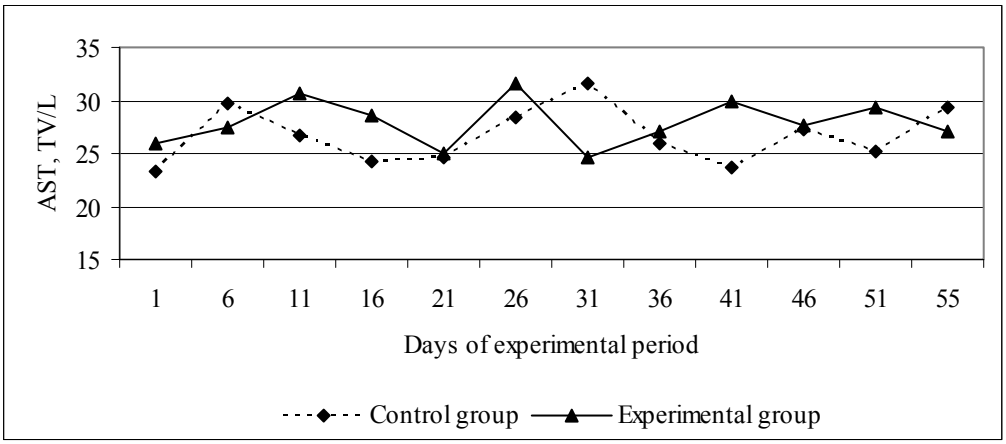

Fig. 1. **Aspartate aminotransferase levels in the control and the experimental group boar blood serum during the experimental period**

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.

Disccusion. 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.

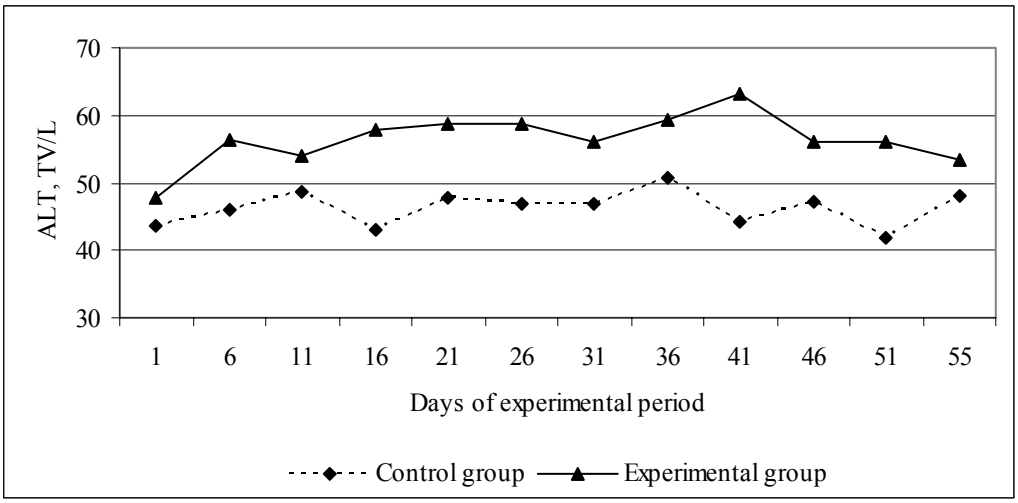

Fig. 2. **Alanine aminotransferase levels in the control and the experimental group boar blood serum during the experimental period**

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 α-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 (Sutkevičius 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 significally affected 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.

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