XXIV INTERNATIONAL
ECO-CONFERENCE® 2020
23–25th SEPTEMBER

XI SAFE FOOD

PROCEEDINGS

NOVI SAD, SERBIA

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SAFE FOOD

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2020.

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– Ecological Movement of Novi Sad

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– Russian State Agrarian University–MTAA, Moscow, Russian Federation
– International Independent Ecological–Politicology University in Moscow, Russian Federation
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THE ECOLOGICAL MOVEMENT OF THE CITY OF NOVI SAD: AN IMPORTANT DECISION OF ITS PROGRAMME COUNCIL

Since 1995, the Ecological Movement of the City of Novi Sad organizes "Eco-Conference® on Environmental Protection of Urban and Suburban Areas", with international participation. Seven biennial conferences have been held so far (in 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013 and 2015.). Their programs included the following environmental topics:

Session 1: Environmental spheres: a) air, b) water, c) soil, d) biosphere
Session 2: Technical and technological aspects of environmental protection
Session 3: Sociological, health, cultural, educational and recreational aspects of environmental protection
Session 4: Economic aspects of environmental protection
Session 5: Legal aspects of environmental protection
Session 6: Ecological system projecting (informatics and computer applications in the field of integrated protection)
Session 7: Sustainable development of urban and suburban settlements-ecological aspects.

Conference participants have commended the scientific and organizational levels of the conferences. Conference evaluations have indicated that some aspects are missing in the conference program. In addition, since a team of conference organizers was completed, each even year between the conferences started to be viewed as an unnecessary lag in activity.

Eco-Conference® on Safe Food

With the above deliberations in mind, a decision was made that the Ecological Movement of the City of Novi Sad should embark on another project – the organization of Eco-Conferences® on Safe Food. These Conferences were planned to take place in each even year. Preparations for the first Eco-Conferences® on safe food started after the successful completion of the Eco-Conference® '99.

So far four Eco-Conferences® have been held (in 2000, 2002, 2004, 2006, 2008, 2010, 2012 and 2014.) focusing this general theme.

Theme of the Eco-Conference®

By organizing the Eco-Conference® on Safe Food, the organizer wishes to cover all factors that affect the quality of human living. Exchange of opinions and practical experiences should help in identifying and resolving the various problems associated with the production of safe food.

Since 2007 Eco-Conference gained patronship from UNESCO and became purely scientific Conference.

Objectives of the Eco-Conference®

– To acquaint participants with current problems in the production of safe food.

– To make realistic assessments of the causes of ecological imbalance in the conventional agricultural production and the impact of various pollution sources on the current agricultural production.

– Based on an exchange of opinions and available research data, to make long-term strategic programs of developing an industrialized, controlled, integral, alternative and sustainable agriculture capable of supplying sufficient quantities of quality food, free of negative side effects on human health and the environment.

Basic Topics of the Eco-Conference®

Basic topics should cover all relevant aspects of the production of safe food.

When defining the basic topics, the intention was itemize the segments of the production of safe food as well as the related factors that may affect or that already have already been identified as detrimental for food safety and quality. The topics include ecological factors of safe food production, correct choice of seed (genetic) material, status and preparation of soil as the basic substrate for the production of food and feed, use of fertilizers and pesticides in integrated plant protection, use of biologicals, food processing technology, economic aspects, marketing and packaging of safe food.

To paraphrase, the envisaged topics cover the production of safe food on the whole, individual aspects of the production and their mutual relations, and impact on food quality and safety.

Sessions of the Eco-Conference®

1. Climate and production of safe food.
2. Soil and water as the basis of agricultural production.
3. Genetics, genetic resources, breeding and genetic engineering in the function of producing safe food.
4. Fertilizers and fertilization practice in the function of producing safe food.
5. Integrated pest management and use of biologicals.

ECO-CONFERENCE® 2020
ECOLOGICAL MOVEMENT OF NOVI SAD

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EFFECT OF MYCOTOXINS ON PORCINE SEMEN QUALITY IN ARTIFICIAL INSEMINATION CENTERS

Abstract

This paper describes escalation of sudden drop in semen quality in two boars' farm centers affected by high mycotoxin level in forage (primarily by deoxynivalenol-DON). Barley and wheat harvested in 2019 was generally indicated as most risky grains for animal health and production and main source of DON. DON dominantly affected sperm total and progressive motility (agglutination and astenospermia) as well as sperm chromatin structure, while acrosome status and semen morphology were less sensitive to these changes. Bacterial control indicated increase of bacterial presence. After forage replacement a period of 1-2 months is essential for recovery of semen production, supported with prolonged vitamins and antibiotic treatment.

Routine control of forage for high producing animals is essential. Sperm production is affected with DON level when is even below recommended standards for sows feed (cumulative effect), and no clinical signs may appear. Commercial mycotoxin absorbers are not efficient for such production.

Key words: *mycotoxins, DON, boar, semen quality*

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by certain fungi belonging predominantly to the *Aspergillus, Penicillium* and *Fusarium* genera, which can cause a variety of adverse effects on both humans and animals (Prodanov Radulović et al., 2012). It is estimated that 25% of the world‘s crop production is contaminated by mycotoxins during the pre-harvest period, transport, processing or storage (Greinier et al., 2013; Weaver et al., 2013). *Fusarium spp.* is frequently found in the Serbian

climatic area which is suitable for cereal production (Jakšić et al., 2012). Among mycotoxins produced by *Fusarium spp.*, fumonisins are usually present in maize and maize products, while deoxynivalenol (DON) is a common contaminant of wheat. All farm animals can experience a negative impact from a dietary intake of mycotoxins but pigs are one of the species which are highly sensitive (Prodanov Radulović et al., 2012).

A major problem associated with animal feed contaminated with mycotoxins is not acute disease, but rather the ingestion of low levels of toxins, which may cause an array of metabolic, physiologic and immunologic disturbances (Stojanov et al., 2013; Waśkiewicz et al., 2014). The manifestation of acute DON toxicity in animals is reflected in feed rejection, vomiting, diarrhea and finally, loss of weight. Pigs are particularly sensitive animals, they reject food already at DON concentrations of 1-2 mg/kg of food, while it is minimal emetic dose in these animals 0.05-0.2 mg/kg body weight, if administered orally (Scientific Committee on Food, 1999). There is evidence that DON is an immunosuppressant and immunostimulant depending on the dose and time of exposure (Rotter et al., 1996).

Pigs are considered to be the farm animals which are the most affected by mycotoxins in general (Burel et al., 2013; Wache et al., 2009). Reproductive failure in swine is often a difficult diagnostic problem. Many times, when diagnosis of infectious disease or management problems is not obtained, feed quality and safety may be questioned. Mycotoxins are often present in swine feed in amount that can have detrimental impact on production and reproduction in Serbia.

This paper describes escalation of inadequate semen quality in two boars' farm centers affected by high mycotoxin level in forage (primarily by DON) in a similar period (spring-summer 2020).

MATERIAL AND METHODS

Animals: Boars were located at two boars' farm centers with 16 (Farm A) and 65 boars in exploitation (Farm B). Semen was produced just for own farm needs. Centers had high sanitary and epizootic standards, experienced workers and standard equipment for intensive semen production. Boars were imported from SPF (specific pathogen free) farms in Denmark, kept in individual boxes, in climate buildings, allocated from other animals/farms.

Semen control: Semen production was organized 3 times in two weeks. Quality control was carried out trough continuous third party analysis assessment (external laboratory) at two week intervals.

Semen was collected by manual fixation with gloved-hand technique. Each case of sudden drop in semen quality and in cases of constant low quality was subjected to bacterial count estimation (CFU/mL), bacterial typisation and antibiotic sensitivity test, as described in Milovanovic et al., 2012.

Semen quality control was performed at Laboratory for reproduction at the Scientific Veterinary Institute "Novi Sad" and consisted of:

1. CASA (Computer Assisted Sperm Analysis, ISAS, Proiser, Spain) for assessing concentration, total and progressive motility and spermatozoa speed parameters;

2. flow cytometry analyses (Guava Milipore-IMV, USA) for sperm chromatin structure assay – SCSA test (acridine orange, Invitrogen), and test of membrane and acrosome integrity (PNA-FITC/PI, Invitrogen);
3. cyto-morphological examination of stained sperm sample with eosin-nigrosine with phase contrast oil immersion objective, 1000´ magnification (Olympus BX-40, Japan). The spermatozoa morphology was assessed according to Barth and Oko (1989).

Sudden drop of semen quality was noted in spring 2020. Generally, boars were affected with no clinical signs, but one suspected feed batch provoked even vomiting and hair loss at Farm A. Concentrates were analyzed for mycotoxin presence, targeting wheat and barley as a most risky grain during year 2019-2020 (primarily by DON).

Determination of DON content in feed using ELISA method was performed applying ELISA Veratox® for DON5/5(NE), Art No. R8331NE (Neogen, USA/Canada). The validation parameters were in accordance with recommendations given in EU Regulation 2006/401 (EC 2006). Feed samples were collected directly from pig farms.

For statistivc means, a t-test for independent samples and descriptive statistic was used (Statistica 8; Stat Soft, Inc., Tula, USA).

RESULTS

Different feed batches were analyzed 4-5 times in a row from March to June 2020 on both farms and DON presence was between 492µg/kg to 1160 µg/kg (averaging at 721 µg/kg).

Table 1. Contents of DON in pig feed and feedstuffs samples

Feed (Farm A & B)	MPL* (µg/kg)	Min (µg/kg)	Max (µg/kg)	Average (µg/kg)
Complete feedmix for boars	900	492	1160	721
Complete feedmix for sows	900	556	869	612
Barley	8000	1535	1865	1650

* MAL – maximum allowed level (Pravilnik (2014)

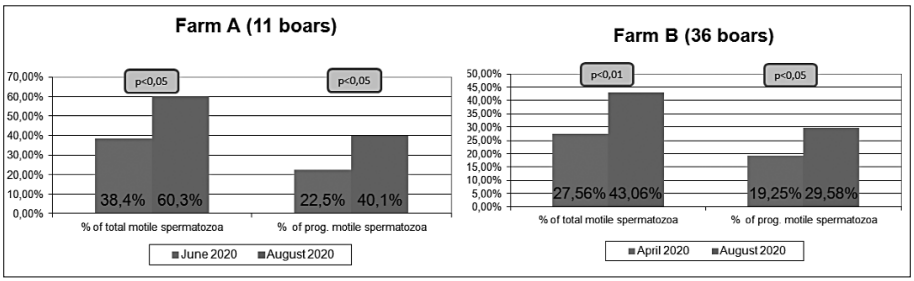
The measured value of DON of barley was up to 3.820 µg/kg. Also, zearalenone was noted at level of 101 µg/kg and fumonisine at 541 µg/kg.

Semen quality was compared between peak of its lowest average quality (April for Farm A and June for Farm B) during mycotoxin exposure and after concentrate replacement (August 2020). Only boars that underwere control on two same specific times of analyses were included in statistic.

Comparable changes on semen quality were seen on booth farms, indicating similar DON mode of action. A high motility depression was noticed (asthenospermia – complete absence of sperm motility was noted on Farm A at 6/11 boars (54,54%) and Farm B at 20/36 boars (54,55%), respectivelly).

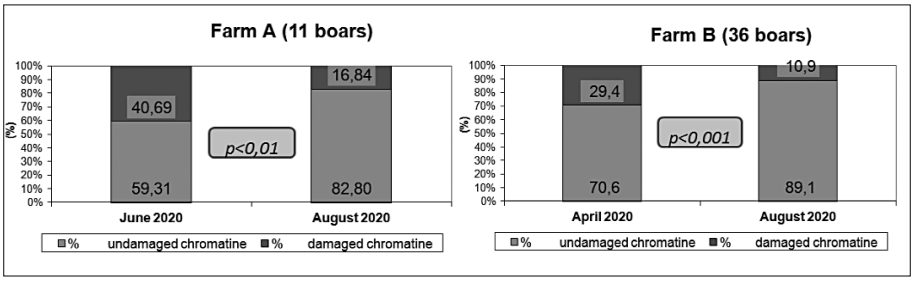
Total spermatozoa motility at DON exposure for Farm A and B were 38,4±33,7% and 27,6±22,7% and for progressive motility only 22,5±23,8% and 29,6±20,1%, respecitivelly. Values for total motility were significantly improved after food change to 69,0±23,9% and 43,1±24,0% ($p<0,05$), as well as progressive motility: 45,9±19,8% and 29,6±20,1% ($p<0,05$), respectivelly.

Graph 1. Total and progressive sperm motility (CASA) during DON consumption period and afer, with observed statistical significance



The percentage of chromosome damage was unnaturaly high (40,7±22,48% and 29,4±13,22% - grade "*Out of class*"), but, after recovery it stabilized at 16,8±13,68% ($p<0,01$) and 10,9±8,06% ($p<0,01$) – grade "*I class*"), respectivelly.

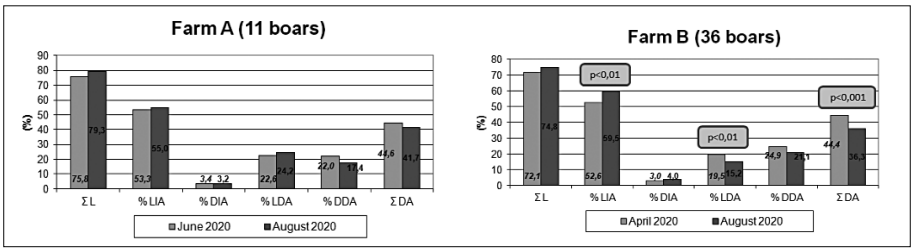
Graph 2. Percent of sperm with fragmented DNA according to SCSA test during DON consuming period and after



Legend: Total live (å L); Live with intact acrosome (LIA); Dead with intact acrosome (DIA); Live with demaged acrosome (LDA); Dead with demaged acrosome (DDA).

Sperm membarane test and acrosome tests (PNA-FITC/PI) indicate their increased senzitivity, dependent of time exposure to DON. Thus, Farm B had longer DON exposition and damaged were more prominent (statistic significant for LIA, LDA and å DA) in comparision to Farm B where similar pattern of defects was noted, but diferences remained only numerical (*Graph 3*).

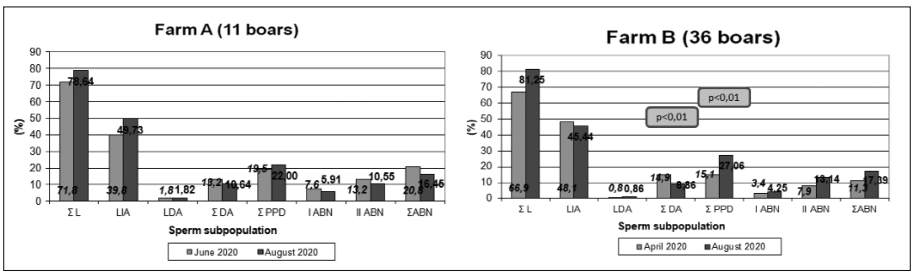
Graph 3. Membrane and acrosome integrity assay of boars' semen (flow cytometry) during DON consumption period and afer, with observed statistical significance.



Legend: Total live (å L); Live with intact acrosome (LIA); Dead with intact acrosome (DIA); Live with demaged acrosome (LDA); Dead with demaged acrosome (DDA); total damaged acrosome (å DA).

Similar results were noted on cyto-morphology with statistic significance on DA ($p < 0,01$) and % of protoplasmatic droplet (25,88±19,38%; $p < 0,01$) for Farm B, indicating a longer recovery process. Rate of pathological forms were not crucial for poor quality.

Graph 4. Boars' sperm subpopulation (cyto-morphological smear) during DON consumption period and afer, with observed statistical significance



Legend: (å L=Total live/unstained spz.; LIA=Live spz. with intact acrosome; LDA=Live spz. with damaged acrosome; å DA=Damaged acrosome – total; å PPD=Protoplasmatic droplet-total; I ABN=Primary abnormalities; II ABN= Secondary abnormalities; (å ABN= Total abnormalities).

Bacterial count: all boars had elevated bacterial count during DON exposition in row semen and ranged from 10.000-18.000 CFU/ml, peaking with 120.000-220.000 CFU/ml (*E. coli* and *Ps. aeruginosa*, with or without the presence of saprophytic bacteria *Proteus sp*.). Main reccomandations are that they shud not not exceed 5.000 CFU/ml. In the case of pathogenic *E. coli*, the recommendation is 3.500 CFU/ml (Maroto Martín et al., 2010). After recovery period supported with prolonged vitamins and antibiotic treatment, a bacterial number fall to values of 700-3.400 CFU/ml (1.633±1.050). A period of 1-2 months is essential for recovery of semen production after adequate food change.

DISCUSSION

Deoxynivalenol, although within normal limits according to the food standards, is detrimental at hronic, cumulative exposure of approximately 600 μg/kg in complete feedmix and is probably a source of high sperm chromosome damage in boars. Mycotoxin combinations weaken the immune system, provoking next level of problem-bacterial invasion of opportunistic pathogen and sperm agglutination with no gross lesion on sperm cells. Recovery period was followed by increase of protoplasmic droplets, increased motility and quick recovery of hromatine status.

Sperm DNA fragmentation-degeneration has a negative impact on fertility and the number of offspring in pluriparic animals. This indicator is not related to sperm motility (Evenson et al., 2002). Boe-Hansen et al., (2008) claim that chromosome damage over 2,1% already has negative effects on the number of live-born piglets, while damage of over 20% results in litters with a maximum of 6,4 piglets.

On cyto-morphological analyse a high proportion of protoplasmic droplets (mostly distal PPD indicating a longer recovery process) was dominant sperm subclass compared to pathologic forms.

CONCLUSION

According to our results, the values of mycotoxins in the complete feedmix although within proposed limits can be detrimental for young breeding boars, affecting chromosome status and membrane maturation (agglutination, protoplasmic droplets, astenospermia).

The presence of mycotoxins should be taken extremely seriously because there is a possibility that boars will be disabled for further reproduction due to a long-term infection and low semen quality.

Addition of adsorbents, mycotoxin blockers, is not enough to prevent the effects of mycotoxins on semen, but they can alleviate the clinical picture.

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УТИЦАЈ МИКОТОКСИНА НА КВАЛИТЕТ СЕМЕНА НЕРАСТОВА У ЦЕНТРИМА ЗА ОСЕМЕЊАВАЊЕ

Сажетак

У овом раду описан је случај наглог пада квалитета семена код два нерастовска, фармска репро центра услед повишеног налаза микотоксина у концентрату (првенствено деоксиниваленола (ДОН-а)). Генерално, јечам и пшеница из рода 2019. године су назначени као најризичније категорије зрна за здравље и производњу животиња и главни извор ДОН-а. ДОН је доминантно утицао на укупну и прогресивну покретљивост сперматозоида (аглутинација и астеноспермија), као и на структуру хроматина, док су статус акросома и морфологија сперме били мање осетљиви на ове промене. Бактериолошке претраге указале су на повећано присуства бактерија. За опоравак сперматогенезе након замене концентрата неопходан је период од 1-2 месеца, подржан продуженим лечењем витаминима и антибиотицима.

Рутинска контрола сточне хране за животиње високе продукције је од суштинског значаја. ДОН утиче на производњу сперме код нерастова чак и у концентрацијама испод препоручених стандардом за храну свиња (кумулативни ефекат) и може протицати без појаве клиничких знакова. Такође, комерцијални апсорбенти микотоксина нису ефикасни за ову осетљиву производњу.

Кључне речи: *микотоксини, ДОН, нераст, квалитет семена*