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Review

PREVALENCE, TOXICOKINETICS AND CLINICAL SIGNS OF ZEARALENONE MYCOTOXICOSIS IN PIGS - AN OVERVIEW

N. Nikolov, R. Binev*

Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The contamination of feeds and foods with mycotoxins is a global ecological and public health issue. The effects of fungi growing on feeds and of mycotoxins produced by them are outlined with the fact that even minimum concentrations, especially in cases of potentiated synergism and continuous exposure, may cause substantial harm to health manifested with immunosuppression, reproductive disorders in farm animals and hence, reduced productive performance and great economic losses. By affecting numerous economic sectors and the food industry in particular, mycotoxins pose a huge threat to animal and human health. The Food and Agriculture Organization (FAO) reports that 25% of global food crops used as primary feed ingredients in animal and human nutrition are affected by mycotoxins and contaminated as early as during the stages of plant growth, harvesting, transportation or storage.

The most important mycotoxins, subject to extensive research and of enormous economic relevance in modern livestock husbandry, pig farming in particular, are deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2), fumonisin B_1 (FB_1), ochratoxin A (OTA) and aflatoxin B_1 (AFB_1). The present review describes zearalenone (ZEA) as one of the main pig feed contaminants, its prevalence, toxicokinetics, toxicodynamics and clinical signs.

Key words: zearalenone (ZEA), prevalence, toxicity, metabolism, pigs.

INTRODUCTION

Zearalenone (ZEA), also known as F-2 toxin, is produced by different fungi from the *Fusarium species* - *F. graminearum* (Gibberella zeae), *Gibberella fujikuroi*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. semitectum and F. crookwellense*. Among 40 isolated Gibberella fujikuroi strains in Bulgarian feeds for pigs and chicks, 10 were found to produce ZEA, whereas the mean contamination levels of ZEA in the same feeds were reported to be 133 ppb for 2006 and 107 ppb for 2007. ZEA was also found in the serum and urine of the same pigs (2). The members of *Fusarium spp*. are among the most commonly encountered fungi in cereal crops – corn, barley, wheat, rice, sorghum and soybean, cultivated in both moderate and warmer climatic zones (1-3).

***Correspondence to:** *Rumen Binev, Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria, E-mail: rumen.binev@trakia-uni.bg*

The first reports about disease or intoxication, accompanied with vulvar oedema and reddening in growing female piglets and preputial swelling in boars date back to 1927. In all clinical cases, a common etiological factor was a history of feeding *Fusarium graminearum (F. roseum)*-contaminated feeds (mainly corn) to animals. The fungi from genus *Fusarium*, spread in different regions over the world: Europe, the USA, Canada, Australia, India, Japan, the Republic of South Africa and Oceania, exhibit the best growth and toxin production in wet climatic conditions, with moderate or high ambient temperature, especially at the time of plants' flowering (3).

In Bulgaria, investigations on the prevalence of ZEA-producing *Fusarium* spp. fungi are reported from the second half of the past century (1, 3-5). According to these researchers, *F. graminearum* is the main ZEA producer, and almost always present contaminant during the production of pig

compound feeds and undoubtedly involved in farm animals' pathology, especially in pigs (2).

By its chemical structure, the toxin is a non-steroidal estrogenic secondary metabolite, a resorcylic acid lactone (6- (10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone; C18H22O5; MW: 318.36; CAS 17924–92-4) **(Figure 1).** ZEA is a stable compound that is not degraded at high temperature and during feed processing. It is a whitish crystalline toxin with a melting point of 164°-165 °C. ZEA is water-insoluble, soluble in fats, alkalis and different organic solvents (3).

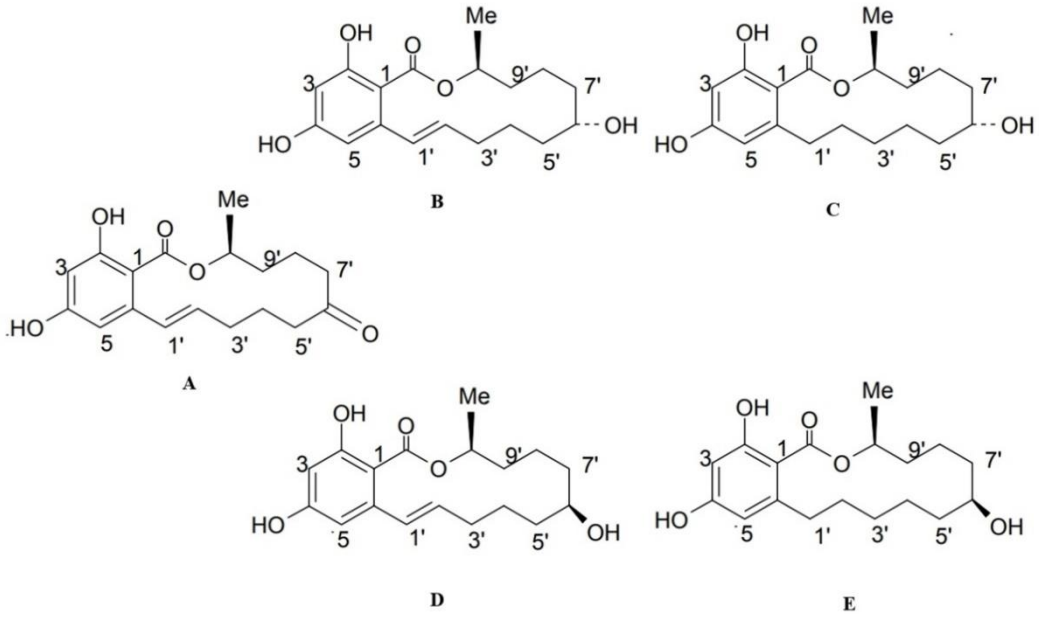

Figure 1. Chemical structure of zearalenone (A) and its main metabolites: (B) alpha- zearalenol, (C) alpha-zearalanol, (D) beta-zearalenol, (F) beta-zearalanol.

Toxicokinetics

Absorption

Men and animals ingest mycotoxins and ZEA in particular, by two main pathways: through ZEA-contaminated food or feed, and in humans – through intake of ZEA, already converted in the animal body to α- and β-zearalenol with milk, placenta and other biological fluids.

After oral administration to susceptible animal species, ZEA is rapidly absorbed in the proximal intestinal tract through the enterocytes' cellular membranes (6, 7). After intake of a single oral dose of 10 mg/kg body weight in pigs, high serum concentrations are measured within 30 minutes with an uptake of 80-85%. The ZEA biotransformation in mammals occurs mainly in the liver, but also in other organs – intestines, kidneys and lungs (8).

Distribution

The plasma elimination half-life is 87 hours both after two intravenous applications and after a single oral dose (8). According to the authors, the impairment of the enterohepatic cycling via bile duct ligation decreases the half-life to approximately 3 hours, evidencing the important role of the bile in the excretion of glucuronidated metabolites followed by partial reabsorption at the end of the enterohepatic circulation. The distribution of ZEA includes tissues positive for estrogen receptors (ER) such as the uterus, ovarian follicles, adipose tissue and testes (9). It is reported that 4 weeks following application of ZEA at a high dose (40 mg/kg feed), the amount of liver metabolites ranged between 78 and 128 μg/kg (10). Another author (11) reported that liver samples from pigs, fed ZEA-contaminated oats, contained predominantly the metabolite α-zearalenol (α-ZOL) and at a lesser extent, β-zearalenol (β-ZOL) and the original form (ZEA). The authors affirmed that ZEA is widely spread in the body and is slowly eliminated from tissues, probably by reason of enterohepatic recycling of ZEA and its metabolites.

The studies of another author (12) indicated two main pathways of ZEA biotransformation in animals **(Figure 2).**

The first pathway occurs through hydroxylation and formation of α- and β-zearalenol (α- and β-ZOL), and is possibly catalysed by 3α- and 3β-

hydroxysteroid dehydrogenases (HDS). The estrogenic potential of metabolites formed through this pathway is different. The affinity of α-ZOL to estrogen receptors is much higher, so the resulting metabolite is far more toxic than the original ZEA, whereas the β-ZOL form has a lower affinity to estrogen receptors and is practically harmless (13).

The second metabolic pathway is based on the conjugation of ZEA and its reduced metabolites with glucuronic acid, catalyzed by uridine diphosphate glucuronic acid (UDPGA) (14-16).

Research data demonstrate that the first stage of ZEA biotransformation in mammals with the formation of α- and β-ZOL, may occur in the liver of many animal species (17), in the porcine intestinal mucosa (8) and granulosa cells of cattle and pigs (15, 17).

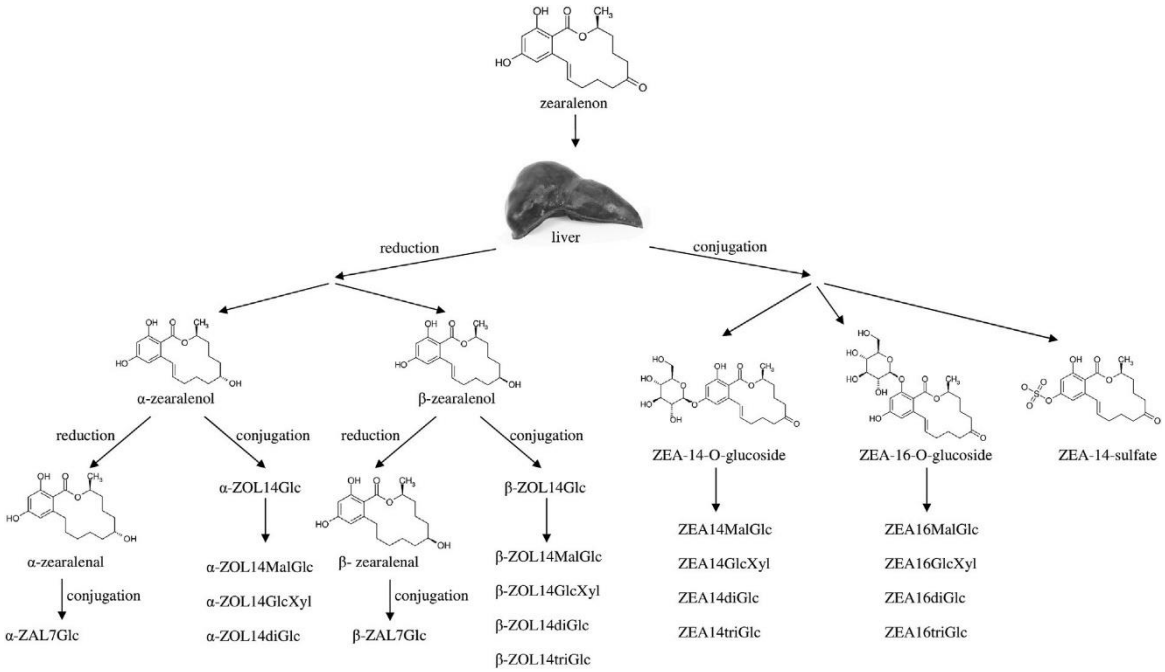

Figure 2. Major ZEA biotransformation pathways in animals (18)

It has been found out that in pigs, ZEA is converted mainly into α-form, whereas in cattle, the β-form is the main liver metabolite (17). The data of other authors showed that α-ZOL is the main metabolite produced by pig, rat, rabbit, cow and murine hepatocyte cultures (19). Another author (20) evaluated ZEA metabolism in two-month-old female pigs after intravenous and oral administration. The prevailing detected metabolite was α-ZOL, whereas the levels of β-ZOL were insignificant. The further conversion of ZEA and its metabolites to glucuronide conjugates occurs in the second stage of biotransformation (20). Earlier, ZEA conjugates were detected in different tissues and body fluids, e.g. urine, faeces, liver tissue (11). These compounds are effectively secreted in the bile. Thus, conjugation facilitates the elimination of toxins from the body, for instance with urine or faeces.

Clinical signs

ZEA has a resorcylic acid lactone structure and may pass through cellular membranes, binding to cytosol 17β-estradiol (E2) receptors and forming ZEA-E2 R complex. Alpha-hydroxylation increases estrogenic activity, explaining the species-specific sensitivity to ZEA intoxication, whereas glucuronidation capacity inactivates ZEA. Compared to other species, the glucuronidation capacity of pigs is low, therefore this animal species is the most ZEA-sensitive (14, 21, 22), through the compound's hyperestrogenic effect.

1. Acute intoxication

ZEA exhibits a relatively low acute toxicity (oral LD_{50}>2000-20000 mg/kg body weight) after oral administration to mice, rats and guinea pigs (23). According to the author, the mycotoxin is more toxic after being intraperitoneally injected.

Table 1. Summarises the results from some studies on ZEA acute toxicity (LD_{50}) in various animal species.

Table 1. *Acute toxicity (LD_{50}) of ZEA in different animal species (24).*

Species	Sex	Exposure route	LD_{50} (mg/kg body weight)	References
Mice	M/F	Oral	>2000	NTP (1982)
Mice	F	Oral	>20000	Hidy et al. (1977)
Mice	F	Intraperitoneal	>500	Hidy et al. (1977)
Rat	M/F	Oral	>4000	NTP (1982)
Rat	M/F	Oral	>10000	Hidy et al. (1977)
Rat	M	Intraperitoneal	5500	Hidy et al. (1977)
Guinea pig	F	Oral	>5000	Hidy et al. (1977)
Guinea pig	F	Intraperitoneal	2500	Hidy et al. (1977)

2. Chronic intoxication

Among the estrogenic effects of ZEA, the most pronounced include: increased embryo lethal resorptions, decreased fertility, and reduced litter size, change in serum levels of progesterone and teratogenic effects in pigs and sheep (25). At higher doses, ZEA can disrupt ovulation, implantation, fetal development and the viability of newborn animals (15), and even to induce oxidative stress, nausea or diarrhea in addition to its estrogenic activity (26). The young female pigs were found to be the most sensitive ones to ZEA, having some characteristic clinical symptoms such as swelling of the vulva and mammary glands, prolonged estrus intervals, vulvovaginitis, stillbirth, vaginal and/or rectal prolapse, ovarian atrophy, abortion and infertility (21). In male pigs, ZEA provokes feminization and a decrease in spermatogenesis, testicular weight, libido, and testosterone levels (15).

The hyperestrogenism in female pigs (vulvovaginitis or rectal and vaginal prolapses), which is provoked by the high feed concentrations of ZEA was seen only after a prolonged exposure to the same mycotoxin (around a month) in the practice, whereas the first clinical symptoms of exposure to feed contaminated with *Fusarium graminearum* are usually connected with some target toxic effects of another mycotoxin DON produced by the same fungus, e.g. cytotoxic (degenerative changes in internal organs and gastrointestinal tract), emetic (gastrointestinal damages), neurotropic (paresis of the limbs) and immunosuppressive (secondary bacterial infections) effect (27). The maximum permitted level of ZEA in feedstuffs for piglets and gilts according to EC regulations is 100 ppb, whereas for sows and fattening pigs is 250 ppb (27).

The experimental application of ZEA or ZEA-contaminated feed at relatively low doses of 1.5-2 ppm to young sows resulted in oedema and thickening of vaginal and vulvar walls, increased uterus weight and ovarian atrophy, with growing female pigs being more susceptible than adult cycling and lactating sows (22, 28). The clinical signs appear three to seven days following the first application and disappear 14 days after elimination of the etiological factor (29). For sows, far higher doses are needed (64 ppm) to produce similar symptoms (30). ZEA may also induce early puberty in 70-day-old gilts if they ingest 2 ppm of the toxin over 45 to 90 days (30). The last studies demonstrated that the oocyte quality of female pigs fed feeds containing low ZEA concentrations (from 0.235 to 0.358 ppm) is significantly reduced (30, 31). Level of contamination induces dose-dependent specific effects on granulosa cells, steroidogenesis and gene expression (32-34). In cycling sows, 5 to 10 ppm dietary ZEA results in prolonged cycle or anestrus after weaning (30, 34). Authors (30) proved a linear relationship between ZEA concentration (in ppm) and anestrus period length (in days).

According to authors (30), litter size in pregnant gilts and sows, fed ZEA-contaminated feed at levels exceeding 2.8-3.0 ppm, especially during early gestation, was reduced. The ZEA-induced premature estrogenic stimulation impairs the proper secretory response of the endometrium to progesterone (P4) at the time of embryos implantation by the 11-12th day after the mating. Exposure to higher ZEA doses

(>25 ppm) resulted in stillbirths, neonatal mortality and mummification of foetuses. After farrowing induction programme using PGF2α at the end of the gestational period, dystocia accompanied with splay leg deformities of newborn piglets and neonatal estrogenic syndrome are reported (35). According to the author, clinical signs were also accompanied by marked vulvar swelling in day-old female piglets. It is assumed that the most critical period of ZEA application is between gestational days 7 and 10, as confirmed by the higher embryonic death rate compared to ZEA intake prior to or after that period (36).

Higher levels of ZEA contamination (64 ppm) may cause the death of the entire litter (36), whereas moderate levels (up to 60 ppm) lead to birth of less vital piglets or litter of smaller size (28). The neonates may be exposed to the effect of ZEA in utero, as well as through the sow milk. The typical clinical signs indicating exposure to ZEA are swelling and reddening of the vulva (Fig. 3) and teats, enlargement of mammary glands, hypertrophy of the vulva and the uterus, rectal prolapse. Similar signs associated with enlarged mammary glands may be observed also in males. The onset and severity of symptoms are dependent on period and duration of ZEA exposure in pregnant sows, the period between weaning the offspring, and the absolute amount of ingested toxin (8, 29, 30).

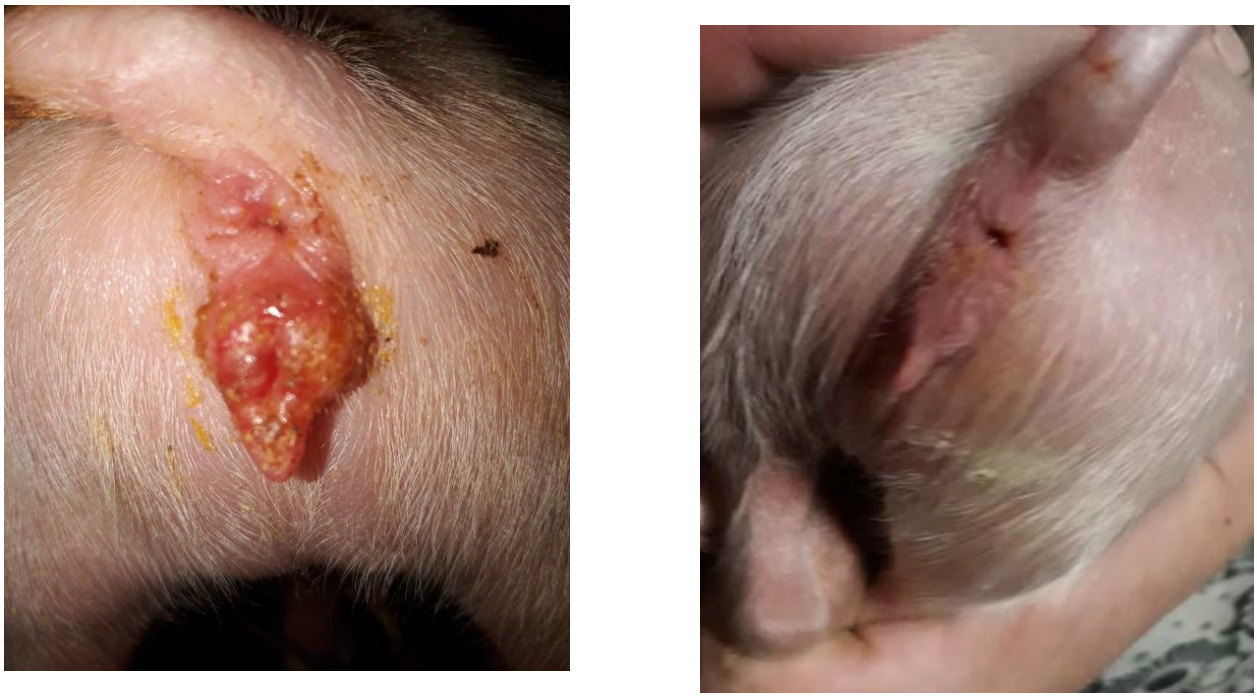

Figure 3. Reddening and vulvar oedema in a neonate piglet fed ZEA contaminated feed (left) (original). Vulva in a healthy pig (right).

REFERENCES

1. Borisova, L., Tacheva, T., Baykushev, R., 2000. Mycotoxicological investigahtions of wheat from two grain-producing areas in Bulgaria, *Vet. Meditsina*, 2-3:29–31, 2000.
2. Stoev, S.D., Dutton, M., Njobeh, P., Mosonik, J., Steenkamp, P., Mycotoxic nephropathy in Bulgarian pigs and chickens: complex aetiology and similarity to Balkan Enedemic Nephropathy. *Food Addit. Contam*. A. 27, 72-88, 2010.
3. EFSA (European Food Safety Authority), Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed, *The EFSA Journal*, 89:1–35, 2004.
4. Stankushev, H., Alexandrov, M. : On some lesions in pigs and sows after feeding fusarium grain. In Gastrointestinal Diseases in Pigs. *Shumen*, November 1-2:140–142, 1977.
5. Beev G, Denev S, Bakalova D. Zearalenone - producing activity of *Fusarium graminearum* and *Fusarium oxysporum* isolated from Bulgarian wheat. *Bulg J Agric Sci*, 19(2):255–259, 2013.
6. Hueza, I.M., Raspantini, P.C.F., Raspantini, L.E.R., Latorre, A.O., Górniak, S.L,: Zearalenone, an estrogenic mycotoxin, is an

immunotoxic compound. *Toxins* 6:1080–1095, 2014.
7. Kowalska, K., Habrowska-Górczynska, D.E., Piastowska-Ciesielska, A.W., :Zearalenone as an endocrine disruptor in humans, *Environ. Toxicol. Pharmacol*, 48:141–149, 2016.
8. Biehl, M.L., Prelusky, D.B., Koritz, G.D., Hartin, K.E., Buck, W.B., Trenholm, H.L., Biliary-excretion and enterohepatic cycling of zearalenone in immature pigs. *Toxicol. Appl. Pharmacol*. 121:152–159, 1993.
9. Kuiper-Goodman, T. Uncertainties in the risk assessment of three mycotoxins: Aflatoxin, ochratoxin, and zearalenone, *Can. J. Physiol. Pharmacol.*, 68:1017–1024, 1990.
10. James, L.J. and Smith, T.K.,: Effect of dietary alfalfa on zearalenone toxicity and metabolism in rats and swine, *J. Anim. Sci. 55*:110–118, 1982.
11. Zöllner, P., Jodlbauer, J., Kleinova, M., Kahlbacher, H., Kuhn, T., Hochsteiner, W., Lindner, W.,: Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats. *J. Agric. Food Chem.* 50:2494–250, 2002.
12. Olsen, M., Pettersson, H., Kiessling, K.H.,: Reduction of zearalenone to zearalenol in female rat liver by 3a-hydroxysteroid dehydrogenase. Acta Pharmacology and Toxicology (Copenhagen) 48:157–161, 1981.
13. Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., Walczak, J., Buszewski, B.,Microbiology neutralization of zearalenone using Lactococcus lactis and Bifidobacterium sp. *Anal. Bioanal. Chem*. 410:943–952, 2018.
14. Malekinejad H., Maas-Bakker R.F., Fink-Gremmels J. : Bioactivation of zearalenone by porcine hepatic biotransformation, *Veterinary Research*, 36:799–810., 2005.
15. Zinedine, A., Soriano, J.M., Molto, J.C., Manes, J.,: Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin, *Food Chem. Toxicol*. 45:1–18, 2007.
16. Videmann, B., Mazallon, M., Tep, J., Lecoeur, S., Metabolism and transfer of the mycotoxin zearalenone in human intestinal Caco-2 cells. Food Chem. Toxicol. 46:3279–3286, 2008.
17. Malekinejad, H, R. Maas-Bakker, J. Fink-Gremmels.: Species differences in the hepatic biotransformation of zearalenone, *Vet. J, Jul*; 172 (1):96- 102, 2006.
18. Rogowskaa, P. Pomastowskia, G. Sagandykovaa, B. Buszewskia. Zearalenone and its metabolites: Effect on human health, metabolism and neutralisation methods, *Toxicon* 162:46–56, 2019.
19. Dänicke, S., Swiech, E., Buraczewska, L. and Ueberschär, K.-H., 2005. Kinetics and metabolism of zearalenone in young female pigs., *Journal of Animal Physiology and Animal Nutrition*, 89:268–276, 2005.
20. Fleck S.C., Churchwell, M.I., Doerge, D.R.: Metabolism and pharmacokinetics of zearalenone following oral and intravenous administration in juvenile female pigs., *Food Chem. Toxicol*. 106:193–201, 2017.
21. Fink-Gremmels J., Malekinejad H. : Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone, *Animal Feed Science and Technology*, 137: 326–341, 2007.
22. Andretta I, Lovatto PA, Hauschild L, Dilkin P, Garcia GG, Lanferdini E, et al. Feeding of pre-pubertal gilts with diets containing zearalenone, *Arq Bras Med Vet Zootec*, 60:1227–33, 2008.
23. Flannigan, B.,: Mycotoxins. In: D'Mello, J.P.F., Duffus, C.M. and Duffus, J.H. (eds.) Toxic substances in crop plants. *The Royal Society of Chemistry, Cambridge*, UK, pp. 226–257, 1991.
24. WHO, Zearalenone. Safety evaluation of certain food additives and contaminants. Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series 44, *World Health Organization*, Geneva, Switzerland: 393–482, 2000.
25. Gajęcki M. Zearalenone - undesirable substances in feed. *Polish Journal of Veterinary Sciences*; 5:117-122, 2002.
26. Neme, K., & Mohammed, A. T. Mycotoxin occurrence in grains and the role of postharvest management as mitigation strategies: a review. *Food Control*, 78, 412-425, 2017.
27. Stoev, S. D. Foodborne mycotoxicoses, risk assessment and underestimated hazard of masked mycotoxins and joint mycotoxin effects or interaction, *Environmental*

Toxicology and Pharmacology, 9, 794–809, 2015.
28. Obremski, K., Gajecki, M., Zwierzchowski, W., Bakula, T., Apoznaniski, J., Wojciechoeski, J.,: The level of zearalenone and α-zearalenol in the blood of gilts with clinical symptoms of toxicosis, fed diets with a low zearalenone content. *J. Anim. Feed Sci*. 12:529–538, 2003.
29. Kordic, B., Pribicevic, S.,: Muntanol-Cvetkovic, M., Nicolic, P., Nicolic, B., 1992. Experimental studies of the effects of known quantities of zearalenone on swine reproduction, *J. Environ. Pathol. Toxicol. Oncol*., 11:53–55, 1992.
30. Kanora , D. Maes. The role of mycotoxins in pig reproduction: a review, *Veterinarni Medicina*, 54(12):565–576, 2009.
31. Alm H., Brussow K.-P., Torner H., Vanselow J., Tomek W., Danicke S., Tiemann U., Influence of Fusarium – toxin contaminated feed on initial quality and meiotic competence of gilts oocytes., *Reproductive Toxicology*, 22, 44–50, 2006.
32. Malekinejad H., Schoevers E.J., Daemen I.J.J.M., Zijstra C., Colenbrander B., Fink-Gremmels J., Roelen B.A.J. Exposure of oocytes to the Fusarium toxins zearalenone and deoxynivalenol causes aneuploidy and abnormal embryo development in pigs, *Biology of Reproduction*, 77:840–847, 2007.
33. Ranzenigo G., Caloni F., Crernonesi F., Aad P.Y., Spicer L.J.: Effects of Fusarium mycotoxins on steroid production by porcine granulosa cells, *Animal Reproduction Science*, 107:115–130, 2008.
34. Meyer K., Usleber E., Martlbauer E., Bauer L.: Occurence of zearalenone, alpha and beta – zearalenone in bile of breeding sows in relation to reproductive performances, *Berliner und Munchener Tierartzliche Wochenschrift*, 113:374–379, 2000.
35. Alexopoulos C., Association of Fusarium mycotoxicosis with failure in applying an induction of parturition program with PGF2alpha and oxytocin in sows. *Theriogenology*, 55:1745–1757, 2001.
36. Long, G.G., M. Diekman, J.F. Tuite, G.M. Shannon, R.F. Vesonder. Effect of Fusarium roseum corn culture containing zearalenone on early pregnancy in swine, *Am. J. Vet. Res*, Sep; 43(9) : 1599-603, 1982.