Veterinary Journal of Kastamonu University

Volume 1 (Issue 1) (2022) Pages 42-57

Negative effects of zearalenone on reproductive productivity in dairy cattle

Veysel DOGAN[1,a,*], Sevval Damla DAL[2,b]

[1]Sinop, Türkiye
[2]Kastamonu University, Faculty of Veterinary Medicine, Kastamonu, Türkiye
[a]https://orcid.org/0000-0002-1148-5416; [b]https://orcid.org/0000-0001-7066-4505
*Corresponding author: d_veysel@yahoo.com
Received date: 16.05.2022- Accepted date: 28.06.2022

Abstract: Mycotoxins, which are produced by different fungi reproduced in feed materials worldwide, have adverse effects on animal and human health. Zearalenone (ZEN) is a common mycotoxin, secreted from *Fusarium* spp. that may cause reproductive problems in animal species due to its non-steroidal estrogenic feature. The sensitivity of livestock to ZEN differs based on the species and sexuality. Cows are considered less sensitive to ZEN, however, there is limited information about adverse effects caused by ZEN. In this article, we aimed to review the effects of ZEN on cows in negative energy balance.
Keywords: Zearalenone, α-zearalenol β-zearalenol, negative energy balance, dairy cattle

Zearalenone'nun süt sığırlarındaki üreme verimliliği üzerine olumsuz etkileri

Özet: Dünya genelinde yem materyellerinde yaygın olarak üreme imkanı bulan farklı mantar türleri tarafından üretilen mikotoksinlerin, hayvan ve insan sağlığı üzerinde olmusuz etkileri bulunmaktadır. Zearalenone (ZEN), *Fusarium* türleri tarafından üretilen ve hayvan türlerinde üreme problemlerine neden olabilen non-steroidal östrojen özelliğine sahip yaygın bir mikotoksindir. Çiftlik hayvanlarında bu mikotoksine karşı duyarlılık tür ve cinsiyet bazında farklılık göstermektedir. Süt inekleri, ZEN' e karşı en az hassas hayvanlar olarak düşünülmektedir. Bununla birlikte ZEN tarafından oluşturulan olumsuz etkiler üzerine sınırlı sayıda veriler bulunmaktadır. Bu derlemede, yüksek verimli süt ineklerinde görülen negatif enerji dengesinden ileri gelebilecek üreme kayıplarına, ZEN'un ilave etkisi araştırılmaya odaklanıldı.
Anahtar Kelimeler: Zearalenon, α-zearalenol β-zearalenol, negatif enerji dengesi, süt sığırı

Introduction

Zearalenone (ZEN) is one of the most common mycotoxins that is produced by *Fusarium* spp. mainly *F. graminearum*, *F. culmorum*, and *F. vericillioides* (Ropejko & Twaruzek, 2021). ZEN is a non-steroidal estrogen mycotoxin that contains resorcylic acid lactone and ketone groups in structure and has a close structural relationship with other antibiotic metabolites that are produced by a number of microscopic filamentous fungi (Desjardins & Proctor, 2007). ZEN is a crystalline toxin with a melting point of 159-163 °C (Harcarova et al., 2020).

Approximately 25-50% of crops are contaminated with different mycotoxins exceeding the EU (European Union) and Codex limits worldwide. Actually, this figure greatly undervalues the occurrence above the detectable levels (Ricciardi et al. 2013; Eskola et al., 2020). Suitable conditions for ZEN production by fungi are when humidity is above 20% and temperatures ranging from 20 to 25 °C within a three weeks period. The countries with warm and wet climates are favorable for high levels of ZEN generation in feedstuffs (Mostrom, 2012). A survey study on ZEN contamination of cereals between 1999 and 2008 showed that 83.3% of samples in China at concentrations ranging from 46 to 3079 μg/kg (Li et al., 2021). ZEN and its metabolites are heat stable, and thus thermal breakdown in structure is unlikely to occur during the manufacturing and pelleting of compound feeds. ZEN is stable up to 120 °C, and heating temperatures at 150 °C and 200 °C for 60-min a degradation in ZEN structure occurred 29% and 69%, respectively (Kuiper-Goodman et al., 1987).

Zearalenone has a competition to bind to estrogen receptors due to structural similarities to that of naturally occurring estrogens such as estradiol, estrone, estriol, and 17β-estradiol and exerts estrogenic effects in different animal species (Figure 1) (Metzler et al., 2010; Gromadzka et al., 2008; Edite Bezerra da Rocha et al., 2014; Martins et al., 2020). Additionally, animal species have different sensitivity, including the gender differences, to zearalenone with female pigs being more sensitive than male pigs, followed by sheep, cattle, and poultry (EFSA, 2017). Generally, mycotoxins exert their toxic effects less individually, and the co-occurrence nature of mycotoxins in a feedstuff during harvesting or storage conditions may enhance their toxicity. In a comparison study on the toxic effects of ochratoxin and ZEN although the receptors for these mycotoxins are different, it has been reported that these toxins have mechanistic overlap via the generation of reactive oxygen species, which leads to amplifying cellular toxicity (Li et al., 2014). Antagonistic effects may also occur between toxins. Alassane-Kpembi et al. (2015) observed that deoxynivalenol (DON) and fusarenon-X have an antagonistic impact while nivalenol and fusarenon-X have an agonism. In addition, these agonistic/antagonistic effects between different mycotoxins exist depending on their concentrations. Zheng et al. (2018) reported that ZEN and α-zearalenol was synergistic at high concentrations, whereas they were antagonistic at low concentrations.

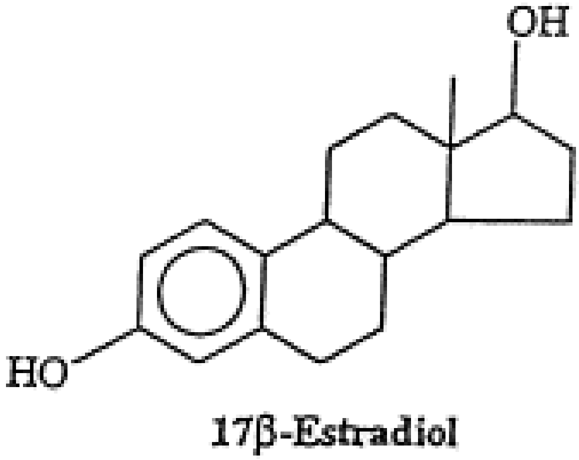

Figure 1: Chemical structure of 17β-estradiol.

Degradation of ZEN in the rumen and other tissues

Ruminants may be exposed to different mycotoxins due to their complex ration contents that originate from different feed materials that include roughage and grains. Unlike monogastric animals, rumen microbiota can degrade some mycotoxins to less toxic or non-toxic metabolites by enzymes that are released by the microorganisms. In the detoxification process, the oxygen in the epoxide group of DON is removed to give a carbon-carbon double bound resulting in non-toxic de-epoxy metabolites (King et al., 1984). The newly formed metabolite is called de-epoxy DON, and the toxicity test showed that de-epoxy DON was 500 times less toxic than the parent metabolite (Schatzmayr et al., 2006). In contrast to DON detoxification and metabolism of ZEN in the rumen fluid by conjugation with glucuronic acid can be converted to toxic metabolites α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), which are 60 times and 0.2 times more toxic, respectively (Figure 2) (Kiessling et al., 1984; Seeling et al., 2006; EFSA, 2016; Mirocha et al., 1978b). α-ZEL exerts a higher estrogenic effect in comparison to the parent compound. Thus, the conversion of ZEN to β-ZEL may be a deactivation process, whereas the conversion to α-ZEL may be an activation process of parent metabolites (Figure 3). This conversion of ZEN to α-ZEL and β-ZEL ratios was observed between 2:1 and 3:1 after an incubation period of 24 h (Valenta & Vemmer, 1996). Danicke et al., (2005) reported that 89% of administrated 0.1 mg ZEN/kg diet was recovered at the proximal duodenum as 30% ZEN, 30% α-ZEL, and 40% β-ZEL. Moreover, α-ZEL and β-ZEL are also produced by *Fusarium* spp. in much lower concentrations in comparison to ZEN production (Zhang et al., 2018). Following absorption from the digestive tract, ZEN is converted to its metabolites in different tissues such as the liver and ovary. In a study conducted by Malekinejad et al. (2006), it has been demonstrated that ZEN is converted to α-ZEL and β-ZEL in porcine and bovine granulosa cells, respectively. In another word, the preferential biotransformation of ZEN to either α-ZEL or β-ZEL in the liver and different tissues may suggest species differences in the sensitivity to the estrogenic effect of ZEN and its metabolites for animal species.

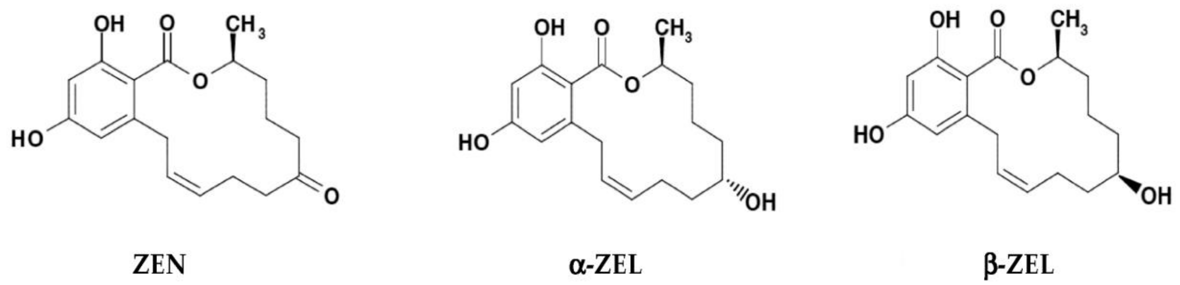

Figure 2: The chemical structure of zearalenone, α-zearalenol, and β-zearalenol (Ropejko and Twaruzek, 2021)

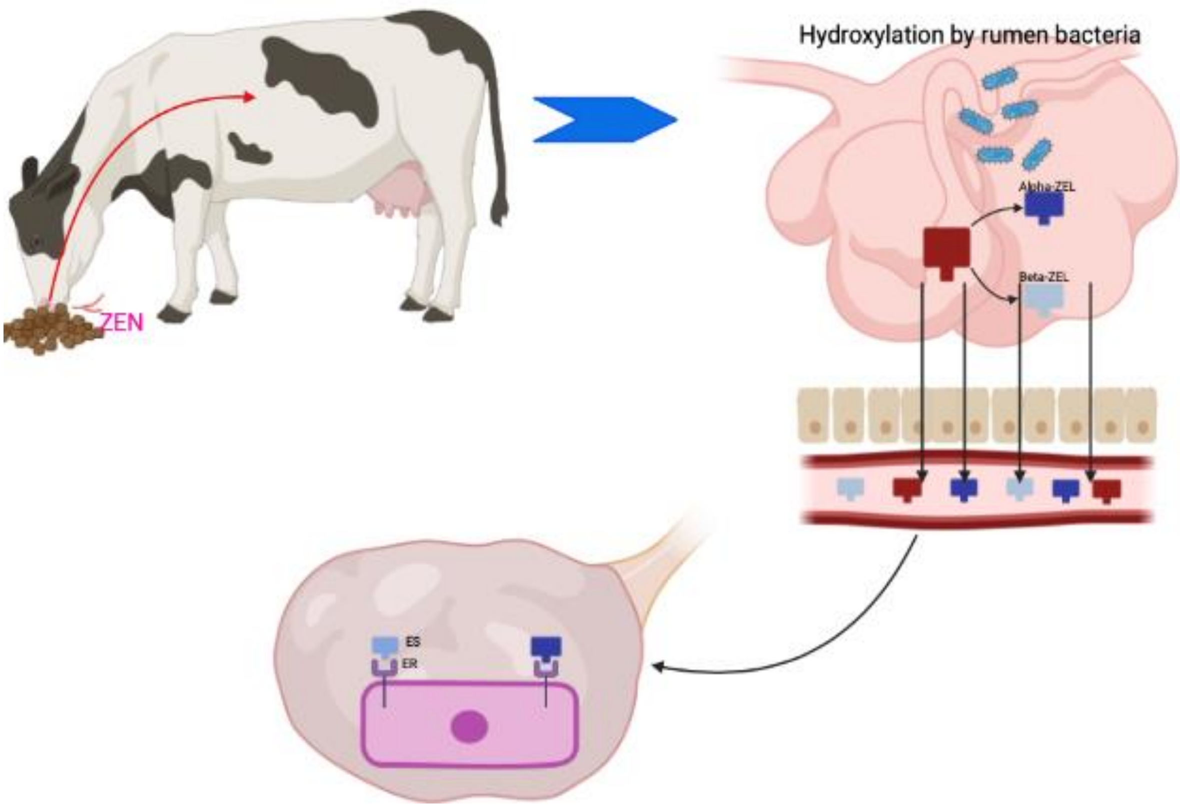

Figure 3: An illustration of ZEN metabolism in the rumen. ZEN and *its* metabolites bind to estrogen receptors and exert estrogenic effects on animals. 17 beta-estradiol and ZEN-and *its* metabolites bind to estrogen receptors

Negative energy balance in dairy cows

Up to date, high-yielding dairy cows are becoming more susceptible to negative energy balance (NEB) and are accompanied by metabolic disorders arising from the significant increase in milk production per lactation as a consequence of genetic selection and improved nutritional management. For dairy cows, the most critical period is the transition period since the energy demand for maintenance and lactation exceeds that provided with dietary energy intake (Bauman & Currie, 1980). During this period, dry matter intake (DMI) is reduced between 20-35% a few days prior to calving and remains low till the lactation peaks (Grummer et al., 2004; Hayirli et al., 2002; Marquardt et al., 1977). During this period body condition score loss occurs that is accompanied by excessive body tissue mobilization to compensate for the energy requirement. Regaining the body mass and the metabolic profile to the normal levels may take up to 20 weeks after lactation onset (Taylor et al., 2003). During the transition period, dairy cows with NEB are vulnerable to perform reproductions problems such as retained fetal membranes, clinical and subclinical metritis, and decreased conception rates (Huzzey et al., 2015; Castro et al., 2012; Moretti et al., 2016; Kumari et al., 2016; Civelek et al., 2011; Shin et al., 2015). In addition to low circulating Insulin-like Growth Factor (IGF)-I after calving, NEB may also influence IGF availability in the oviduct indirectly through changes in specific IGFBP (IGF-Binding Protein) expression. It is possible that the predicted increased signaling by IGF-II may perturb embryo development, contributing to the high rates of embryonic mortality in dairy cows (Fenwick et al., 2008). Plasma insulin concentrations and insulin sensitivity can decrease during the periparturient period, followed by an increase in plasma non-esterified fatty acid concentrations (Wankhade et al., 2017). Kinoshita et al. (2018) have reported that long-term DON consumption (5 mg/kg dry matter) induced mild changes in energy metabolism in lactating dairy cows. For avoiding the detrimental effects of NEB in dairy cows, some strategies have been developed such as enrichment of rations with some ingredients (Overton and Waldron, 2004). Feeding management that is applied to alleviate NEB contains a high level of concentrate feed. In addition, ZEN and other common mycotoxins are produced in numerous substrates including wheat, barley, corn, rice, sorghum, and corn silage more than roughages, and at the same time, concentrated feedstuffs harbor the highest level of mycotoxin (Tola & Kebede, 2016). Thus, consuming a high level of concentrated feedstuffs by transition dairy cows may expose these animals to high levels of mycotoxins including ZEN.

Some substances, which are responsible for the specific moldy odor, may be produced by particular fungi species, and are called microbial volatile organic compounds. Up to date, about 150 volatile compounds have been described (Fiedler et al., 2001). Feeding times are prolonged, and feed intake is reduced in cattle since cattle also dislike the moldy odor when these compounds are present in feedstuffs and total mixed ration (Fink-Gremmels, 2008). During the periparturient period, this moldy scent may further contribute to the NEB in dairy cattle.

Reproduction in dairy cows

Due to its lipophilicity, which is reflected by a relatively high log partition coefficient between octanol and water (log K_{ow}), ZEN is rapidly absorbed in the small intestines following oral exposure. The apparent volume of distribution is large and includes target tissues of ZEN toxic action, such as the uterus, ovarian follicles, and testes (Kuiper-Goodman et al., 1987). Animals eliminate the ZEN via urine, and feces exclusively. In contrast to aflatoxin M1 and DON, ZEN carry-over on milk is not related to the milk yielding (Danicke & Winkler, 2015). As previously mentioned, ZEN has structural similarities to that of naturally occurring estrogens and competition among them, therefore, ZEN plays important role in reproductive disorders in domestic animals, particularly in pigs (Metzler et al., 2010; Gromodzka et al., 2008; EFSA, 2017). Although there are limited publications on cattle sensitivity to ZEN, these experiments have determined infertility, vaginitis, enlarged vulvae, vulvo-vaginitis, reduced milk production, and hyperestrogenism (Coppock et al., 1990; Minervini et al., 2001). Although pigs are considered the most sensitive to ZEN, similar symptoms may be observed in calves that have undeveloped rumen functions, and young heifers (Kallela & Ettala, 1984). In contrast, Silva et al. (2021) have observed no change in morphometric parameters of the genital tract of the beef heifer that consumed a diet contaminated with 300 ppb (~8.82 μM).

Mirocha et al. (1968) have reported that administration of hay containing 14 ppm ZEN caused infertility in cattle. Additionally, cattle and sheep that were grazing in contaminated pastures, have demonstrated infertility (Towers & Sprosen, 1993). In another study conducted by Roine et al. (1971), it has been verified that ZEN-administered cows were infertile. For 42 consecutive days, cows that were administered ZEN at concentrations of 25 or 100 ppm exhibited swollen and hyperemic external genitalia but estrous cycles and ovulations were normal in the trial (Mirocha et al., 1978a). Due to the estrogenic effect, mammary gland enlargement and gland development in heifers occurred in the herds that consume ZEN-contaminated feedstuffs (Coppock et al., 1990; Bloomquist et al., 1983). The artificial insemination index was increased when contaminated feeds were consumed by dairy herds (Kramer, 1997). Moreover, Weaver et al. (1986) have observed a decrease in conception rates in dairy heifers fed 250 mg of ZEN for three estrous cycles.

Following the ingestion of ZEN, parent compounds and their metabolites can be detected in follicular fluids in the ovary in different concentrations (Winkler et al., 2015; Takagi et al., 2008; Malekinejad et al., 2006). In the ovary, ZEN may exert its detrimental effects in different ways in cattle. In an *in vitro* study, it was observed that α-ZEL or ZEN (94μM) inhibited the maturation of oocytes to metaphase II with a significant increase in chromatin abnormalities (Minervini et al., 2001). In contrast, Takagi et al. (2008) have not observed a difference in the occurrence of metaphase II in oocytes that were exposed to ZEN with <0.31 μM. Thus, dose-dependent exposure is important for the maturation rates in cattle oocytes. However, it has been observed that the maturation rates in the oocytes that were exposed to ZEN with 3.1 μM are significantly decreased during the trials. Beef heifers fed a diet contaminated with 300 ppb ZEN had significantly reduced viable oocytes compared with the control group (Silva et al., 2021).
Anti-Müllerian hormone is a glycoprotein produced by the granulosa cells and inhibits the primordial follicles to turn into the growing follicles, in addition, decreases the responsiveness of follicles to follicle-stimulating hormone (FSH). Anti-Müllerian hormone is considered to be a marker of the population of small antral gonadotropin-responsive follicles in cows (Monniaux et al., 20013; Visser and Themmen, 2014; Monniaux et al., 2014; Rico et al., 2009). A study carried out by Fushimi et al. (2015) observed that two different herds with dietary ZEN contamination below the admissible levels showed a significant difference in anti-Müllerian hormone which indicate differences in the antral follicle populations between herds. Thus, ZEN, even at low levels, may alter the ovarian antral follicle populations, but not fertility after the artificial insemination, of post-partum cows.

Zhu et al. (2012) observed apoptosis and necrosis of granulosa cells via a caspase-3 and caspase-9-dependent mitochondrial pathway in a mouse. It suggests that ZEN and its metabolites also may induce atresia in ovarian follicles of dairy cattle. A study carried out by Yang et al. (2019) corroborates the apoptotic effect of ZEN in bovine ovarian granulosa cells. In this study, β-ZEL and HT-2 inhibited cell proliferation in a dose-dependent manner and induced apoptosis in bovine granulosa cells. The individual effect of β-ZEL or α-ZEL on apoptosis in the bovine ovarian granulosa cells requires further investigation.

G protein-coupled estrogen receptor (GPR30) present in cattle gonadotropes is responsible for rapid negative estradiol feedback regulation of GnRH-induced luteinizing hormone (LH) secretion in cattle (Rudolf and Kadokawa, 2013). Nakamura et al., (2015) observed that α-ZEL may suppress LH secretion from the anterior pituitary of cattle via GPR30. In beef heifers offered ZEN-contaminated ration, plasma estrogen concentrations were not affected (Silva et al., 2021). In addition, α-ZEL had no effect on cell proliferation in the presence of IGF-I and FSH, inhibited progesterone and estradiol, whereas decreased cell numbers in the presence of FSH alone, and had no effect on progesterone and estradiol production (Pizzo et al., 2016). In granulosa cell cultures that were exposed to α-ZEL for 24 hours, 17β-estradiol levels were found increased is related to the inhibitory effects of the ZEN pathway of steroidogenesis (Minervini et al., 2001).

Conclusion

Understanding mycotoxins features and their effects on animal health, production, and reproduction parameters become crucial for the feed industry and livestock management. High-yielding dairy cows are more susceptible to NEB, leading to decreased lactational and reproductive performance. The feeding management that is aimed at alleviating these adverse effects of NEB may be disrupted by the mycotoxin presence in feedstuffs. Mycotoxins may be augmenting the adverse effects of NEB via hormonal and functional changes in the hypothalamic-ovarium axis in dairy cows. There are limited publications about the ZEN effects on dairy cows' reproduction. More research is needed to elucidate the ZEN effects on reproduction.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

References

Alassane-Kpembi, I., Puel, O., & Oswald, I. P. (2015). Toxicological interactions between the mycotoxins deoxynivalenol, nivalenol, and their acetylated derivatives in intestinal epithelial cells. *Archives of Toxicology*, *89,* 1337-1346. https://doi.org/10.1007/s00204-014-1309-4

Bauman, D. E., & Currie, W. B. (1980). Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science*, *63*(9), 1514-1529. https://doi.org/10.3168/jds.S0022-0302(80)83111-0

Bloomquist, C., Davidson, J, & Person, E. (1983). Toxicology, metabolism, and physiological effects of aflatoxin in the bovine. In: U. L. Diener, R. L. Asquith, & J. W. Dickens, (Eds.), *Aflatoxin and Aspergillus flavus in Corn.* Alabama Agric. Exp. Sta. Auburn University.

Castro, N., Kawashima, C., Van Dorland, H. A., Morel, I., Miyamoto, A., & Bruckmaier, R. M. (2012). Metabolic and energy status during the dry period is crucial for the resumption of ovarian activity postpartum in dairy cows. *Journal of Dairy Science*, *95*(10), 5804-5812. https://doi.org/10.3168/jds.2012-5666

Civelek, T., Aydin, I., Cingi, C. C., Yilmaz, O., & Kabu, M. (2011). Serum non-esterified fatty acids and beta-hydroxybutyrate in dairy cows with retained placenta. *Pakistan Veterinary Journal*, *31*(4), 341-344.

Coppock, R. W., Mostrom, M. S., Sparling, M. S., Jacobsen, B., & Ross, S. C. (1990). Apparent zearalenone intoxication in a dairy herd from feeding spoiled acid-treated corn. *Veterinary and Human Toxicology*, *32*(3), 246-248.

Danicke, S., & Winkler J. (2015). Invited review: Diagnosis of zearalenone (ZEN) exposure of farm animals and transfer of its residues into edible tissues (carry over). *Food and Chemical Toxicology*, *84*, 225-249. https://doi.org/10.1016/j.fct.2015.08.009

Danicke, S., Matthaus, K., Lebzien, P., Valenta, H., Stemm, K., Ueberschar, K. H., Razzazi-Fazeli, E., Böhm, J., & Flachowsky, G. (2005). Effects of Fusarium toxin-contaminated wheat grain on nutrient turnover, microbial protein synthesis, and metabolism of deoxynivalenol and zearalenone in the rumen of dairy cows. *Journal of Animal Physiology and Animal Nutrition*, *89*(9-10), 303-315. https://doi.org/10.1111/j.1439-0396.2005.00513.x

Desjardins, A. E, & Proctor, R. H. (2007). Molecular biology of Fusarium mycotoxins. *International Journal of Food Microbiology*, *119*(1-2), 47-50.

Edite Bezerra da Rocha, M., da Freire, F. C. O., Erlan Feitosa Maia, F., Guedes, M. I. F., & Rondina, D. 2014. Mycotoxins and their effect on human and animal health. *Food Control*, *36*, 159-165. https://doi.org/10.1016/j.foodcont.2013.08.021

EFSA CONTAM. (2017). Panel Scientific opinion on risk for animal health related to the presence of zearalenone and its modified forms in feed. *EFSA Journal*, *15*, 4851.

EFSA. (2016). Panel on Contaminants in the Food Chain. Scientific opinion on the appropriateness to set a group helath-based guidance value for zearalenone and its modified forms. *EFSA Journal*, *14*, 4425.

Eskola, M., Kos, G., Elliott, C. T., Hajslova, J., Maya, S., & Krska, R. 2020. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25. *Critical Reviews in Food Science and Nutrition*, *60*(16), 2773-2786. https://doi.org/10.1080/10408398.2019.1658570

Fenwick, M. A., Llewellyn, S., Fitzpatrick, R., Kenny, D. A., Murphy, J. J., Patton, J., & Wathes, D. C. (2008). Negative energy balance in dairy cows is associated with specific changes in IGF-binding protein expression in oviduct. *Reproduction*, *135*(1), 63-75. https://doi.org/10.1530/REP-07-0243

Fiedler, K., Schutz, E., & Geh, S. (2001). Detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials. *International Hygiene and Environmental Health*, *204*(2-3), 111-121. https://doi.org/10.1078/1438-4639-00094

Fink-Gremmels, J. (2008). The role of mycotoxins in the health and performance of dairy cows. *The Veterinary Journal*, *176*, 84-92. https://doi.org/10.1016/j.tvjl.2007.12.034

Fushimi, Y., Takagi, M., Monniaux, D., Uno, S., Kokushi, E., Shinya, U., Kawashima, C., Otoi, T., Deguchi, E., & Fink-Gremmels, J. (2015). Effects of dietary contamination by zearalenone and its metabolites on serum anti-müllerian hormone: Impact on the reproductive performance of breeding cows. *Reproduction in Domestic Animals*, *50*(5), 834-839. https://doi.org/10.1111/rda.12599

Gromadzka, K., Waskiewicz, A., Chelkowski, J., & Golinski, P. (2008). Zearalenone and its metabolites: Ocuurance, detection, toxicity and guidelines. *World Mycotoxin Journal*, *1*(2), 209-220. https://doi.org/10.3920/WMJ2008.x015

Grummer, R. R., Mashek, D. G., & Hayirli, A. (2004). Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice*, *20*(3), 447-470. https://doi.org/10.1016/j.cvfa.2004.06.013

Harcarova, M., Conkova, E., Proskovcova, M., & Falis, M. (2020). In vivo assessment of zearalenone toxicity. *Folia Veterinaria*, *64*(2), 60-65. https://doi.org/10.2478/fv-2020-0018

Hayirli, A., Grummer, R. R., Nordehim, E. V., & Crump, P. M. (2002). Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. *Journal of Dairy Science*, *85*(12), 3430-3443. https://doi.org/10.3168/jds.S0022-0302(02)74431-7

Huzzey, J. M., Mann, S., Nydam, D. V., Grant, R. J., & Overton, T. R. (2015). Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. *Preventive Veterinary Medicine*, *120*(3-4), 291-2917. https://doi.org/10.1016/j.prevetmed.2015.04.011

Kallela, K., & Ettala, E. (1984). The oestrogenic Fusarium toxin (zearalenone) in hay as a cause of early abortions in the cow. *Nordisk Veterinaer Medicine*, *36*(9-10), 305-309.

Kiessling, K. H., Pettersson, H., Sandholm, K., & Olsen, M. (1984). Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. *Applied and Environmental Microbiology*, *47*(5), 1070-1073. https://doi.org/10.1128/aem.47.5.1070-1073.1984

King, R. R., McQueen, R. E., Levesque, D., & Greenhalgh, R. (1984). Transformation of deoxynivalenol (vomitoxin) by rumen microorganisms. *Journal of Agricultural and Food Chemistry*, *32*(5), 1181-1183. https://doi.org/10.1021/jf00125a061

Kinoshita, A., Keese, C., Meyer, U., Starke, A., Wrenzycki, C., Danicke, S., & Rehage, J. (2018). Chronic effects of *Fusarium* mycotoxins in rations with or without increased concentrate proportion on the insulin sensitivity in lactating dairy cows. *Toxins*, *10*(5), 188. https://doi.org/10.3390/toxins10050188

Kramer, R. 1997. *Zearalenone in Pasture and its Effects on Reproduction in Ewes.* MApplSc thesis, Masset University, Parmerston North, New Zealand.

Kuiper-Goodman, T., Scott, P. M., & Watanabe, H. (1987). Risk assessment of the mycotoxin zearalenone. *Regulatory Toxicology and Pharmacology*, *7*(3), 253-306. https://doi.org/10.1016/0273-2300(87)90037-7

Kuiper-Goodman, T., Scott, P. M., & Watanabe, H. (1987). Risk assessment of the mycotoxin zearalenone. *Regulatory Toxicology and Pharmacology*, *7*(3), 253-306. https://doi.org/10.1016/0273-2300(87)90037-7

Kumari, S., Prasad, S., Patbandha, T. K., Pathak, R., Kumerasan, A., Boro, P., Maniamaran, A., & Mohanty, T. K. (2016). Metabolic indicators for retention of fetal membranes in Zebu and crossbred dairy cattle. *Animal Production Science*, *56*(7), 1113-1120. https://doi.org/10.1071/AN14941

Li, Y., Zhang, B., He, X., Cheng, W. H., Xu, W., Luo, Y., Liang, R., Luo, H., &Huang, K. (2014). Analysis of individual and combined effects of ochratoxin A and zearalenone on HepG2 and KK-1 cells with methematical models. *Toxins*, *6*(4), 1177-1192. https://doi.org/10.3390/toxins6041177

Li. L., Zhang, T., Ren, X., Li, B., & Wang, S. (2021). Male reproductive toxicity of zearalenone-meta-analysis with mechanism review. *Ecotoxicolgy and Environmental Safety, 221*, 112457. https://doi.org/10.1016/j.ecoenv.2021.112457

Malekinejad, H., Colenbrander, B., & Fink-Gremmels, J. (2006). Hydroxstreoid dehydrogenases in bovine and porcine granulosa cells convert zearalenone into its hydroxylated metabolites α-zearalenol and β-zearalenol. *Veterinary Research Communications*, *30*, 445-453. https://doi.org/10.1007/s11259-006-3325-1

Marquardt, J. P., Horst, R. L., & Jorgensen, N. A. (1977). Effect of parity on dry matter intake at parturition in dairy cattle. *Journal of Dairy Science, 60*(6), 929-934. https://doi.org/10.3168/jds.S0022-0302(77)83965-9

Martins, C., Torres, D., Lopes, C., Correia, D., Goios, A., Assunçao, R., Alvito, P., Vidal, A., De Boevre, M., De Saeger, S., & Nunes, C. (2020). Food consumption data as a tool to estimate exposure to mycoestrogens. *Toxins, 12*, 118. https://doi.org/10.3390/toxins12020118

Metzler, M., Pfeiffer, E., & Hildebrand, A. (2010). Zearalenone and its metabolites as endocrine disrupting chemicals. *World Mycotoxin Journal*, *3*(4), 385-401. https://doi.org/10.3920/WMJ2010.1244

Minervini, F., Dell'Aquilla, M. E., Maritato, F., Minoia, P., & Visconti, A. (2001). Toxic effect of the mycotoxin zearalenone and its derivatives on in vitro maturation of bovine oocytes and 17β-estradiol levels in mural granulosa cell cultures. *Toxicology in Vitro, 15*(4-5), 489-495. https://doi.org/10.1016/S0887-2333(01)00068-6

Mirocha, C. J., Harrison, J., Nichols, A. A., & McClintock, M. (1968). Detection of a fungal estrogen (F-2) in hay associated with infertility in dairy cattle. *Applied Microbiology*, *16*(5), 797. https://doi.org/10.1128/am.16.5.797-798.1968

Mirocha, C. J., Weaver, G., Gustafsson, B., Chi, M., Pathre, S. V., Robinson, T. S., & Bates, F. (1978a). *Pharmacological and Toxicological Studies on Zearalenone in Food Producing Animals.* Food and Drug Administration. Washington, DC.

Mirocha, C. J., Pathre, S. V., Behrens, J., & Schaueramer, B. (1978b). Uterotropic activity of cis and trans isomers of zearalenone an zearalenol. *Applied and Environmental Microbiology*, *35*(5), 986-987. https://doi.org/10.1128/aem.35.5.986-987.1978

Monniaux, D., Clement, F., Dalbies-Tran, R., Estienne, A., Fabre, S., Mansanet, C., & Monget, P. (2014). The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? *Biology of Reproduction*, *90*(4), 1-11. https://doi.org/10.1095/biolreprod.113.117077

Monniaux, D., Drouilhet L., Rico, C., Estienne, A., Jarrier, P., Touze, J. L., Sapa, J., Phocas, F., Dupont, J., Dalbies-Tran, R., & Fabre, S. (2013). Regulation of anti-Müllerian hormone production in domestic animals. *Reproduction, Fertility and Development*, *25*(1), 1-16. https://doi.org/10.1071/RD12270

Moretti, P., Probo, M., Cantoni, A., Paltrinieri, S., & Giordano, A. (2016). Fluctation of neutrophil counts around parturition in Holstein dairy cows with and without retained placenta. *Research in Veterinary Science*, *107*, 207-212. https://doi.org/10.1016/j.rvsc.2016.06.015

Mostrom., M. S. (2012). Zearalenone. In: R. C. Gupta (Ed.), *Veterinary Toxicology*. London, Elsevier.

Nakamura, U., Rudolf, F. O., Pandey, K., & Kadokawa, H. (2015). The non-streoidal mycoestrogen zeranol suppresses luteinizing hormone secretion from the anterior pituitary of cattle via the estradiol receptor GPR30 in a rapid, non-genomic manner. *Animal Reproduction Science*, *156*, 118-127. https://doi.org/10.1016/j.anireprosci.2015.03.009

Overton, T. R., & Waldron, M. R. (2004). Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science*, *87*, 105-119. https://doi.org/10.3168/jds.S0022-0302(04)70066-1

Pizzo, F., Caloni, F., Schreiber, N. B., Cortinovis, C., & Spicer, L. J. (2016). *In vitro* effects of deoxynivalenol and zearalenone major metabolites alone and combined, on cell proliferation, steroid production and gene expression in bovine small-follicle granulosa cells. *Toxicon*, *109*, 70-83. https://doi.org/10.1016/j.toxicon.2015.11.018

Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F., Marasso, S. L., Ricci, A., Canavese, G., Lore, A., Prelle, A., Gullino, M. L., & Spadora, D. (2013). Development of a microcantilever-based immunosensing method for mycotoxin detection. *Biosensor & Bioelectronics*, *40*(1), 233-239. https://doi.org/10.1016/j.bios.2012.07.029

Rico, C., Fabre, S., Medigue, C., di Clemente, N., Clement, F., Bontoux, M., Touze, J. L., Dupont, M., Briant, E., Reny, B., Beckers, J. F., & Monniaux, D. (2009). Anti-Müllerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. *Biology of Reproduction*, *80*(1), 50-59. https://doi.org/10.1095/biolreprod.108.072157

Roine, K., Korpinen, E. L., & Kallela, L. (1971). Mycotoxicosis as the probable cause of infertility in dairy cows. *Nordisk Veterinaer Medicin*, *23*(12): 628-633

Ropejko, K., & Twaruzek, M. (2021). Zearalenone and its metabolites- General overview, occurrence, and toxicity. *Toxins, 13*, 35. https://doi.org/10.3390/toxins13010035

Rudolf, F. O., & Kadokawa, H. (2014). Expression of estradiol receptor, GPR30, in bovine anterior pituitary and effects of GPR30 agonist on GnRH-induced LH secretion. *Animal Reproduction Science*, *139*(1-4), 9-17. https://doi.org/10.1016/j.anireprosci.2013.04.003

Schatzmayr, G., Zehner, F., Taubel, M. Schatzmayr, D., Klimitsch, A., Loibner, A. P., & Binder, E. M. (2006). Microbiologicals for deactivating mycotoxins. *Molecular Nutrition & Food Research*, *50*(6), 543-551. https://doi.org/10.1002/mnfr.200500181

Seeling, K., Boguhn, J., Strobel, E., Danicke, S., Valenta, H., Ueberschar, K. H., & Rodehutscord, M. (2006). On the effects of Fusarium toxin contaminated wheat and wheat chaff on nutrient utilization and turnover of deoxynivalenol and zearalenone in vitro. *Toxicology in Vitro*, *20*, 703-711. https://doi.org/10.1016/j.tiv.2005.10.006

Shin, E. K., Jeong, J. K., Choi, I. S., Kang, H. G., Hur, T. Y., Jung, Y. H., & Kim, I. H. (2015). Relationship among ketosis, serum metabolites, body condition, and reproductive outcomes in dairy cows. *Theriogenology*, *84*(2), 252-260. https://doi.org/10.1016/j.theriogenology.2015.03.014

Silva, L. A., Mello, M. R. B., Piao, D. O., Silenciato, L. N., Quadros, T. C. O., Souza, A. H., & Barbero, R. P. (2021). Effects of experimental exposure to zearalenone on reproductive system morphometry, plasma oestrogen levels, and oocyte quality of beef heifer. *Reproduction in Domestic Animals*, *56*(5), 775-782. https://doi.org/10.1111/rda.13917

Takagi, M., Mukai, S., Kuriyagawa, T., Takagaki, K., Uno, S., Kokushi, E., Otoi, T., Budiyanto, A., Shirasuna, K., Miyamoto, A., Kawamura, O., Okamoto, K., & Deguchi, E. (2008). Detection of zearalenone and its metabolites in naturally contaminated follicular fluids by using LC/MS/MS and in vitro effects of zearalenone on oocyte maturation in cattle. *Reproductive Toxicology*, *26*(2), 164-169. https://doi.org/10.1016/j.reprotox.2008.08.006

Taylor, V. J., Beever, D. E., & Wathes, D. C. (2003). Physiological adaptations to milk production that affect fertility in high-yielding dairy cows. *British Society of Animal Science Occasional Publication*, vol 29, (pp. 37-71). Nottingham, UK: Nottingham University Press.

Tola, M., & Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food & Agriculture*, *2*(1), 1191103. https://doi.org/10.1080/23311932.2016.1191103

Towers, N. R., & Sprosen, J.M. (1993). Zearalenone-induced fertility in sheep and cattle in New Zealend. *New Zealend Veterinary Journal*, *41*, 223-224.

Valenta, H., & Vemmer, H. (1996). *In vitro*-Unterschungen zum Metabolismus von Zearalenon bei Inkubation mit Pansensaft; *Proceedings of the 18 Mykotoxin-Workshop*; Kulmbach, Germany. 10-12 June 1996 [(accessed on 14 May 2022)]. (pp, 10-12).

Visser, J.A., & Themmen, A. P. (2014). Role of anti-Müllerian hormone and bone morphogenetic proteins in the regulation of FSH sensitivity. *Molecular and Cellular Endocrinology*, *382*(1), 460-465. https://doi.org/10.1016/j.mce.2013.08.012

Wankhade, P. R., Manimaran, A., Kumerasan, A., Jeyakumar, S., Ramesha, K. P., Sejian, V., Rajendran, D., & Varghese, M. R. (2017). Metabolic and immunological changes in transition dairy cows: A review. *Veterinary World*, *10*, 1367-1377. https://doi. org/10.14202/vetworld.2017.1367-1377

Weaver, G. A., Kurtz, H. J., Behrens, J. C., Robinson, T. C., Sequin, B.E., Bates, F. Y., & Mirocha, C. J. (1986). Effect of zearalenone on fertility of virgin dairy heifers. *American Journal of Veterinary Research*, *47*(6):1395-1397.

Winkler, J., Kersten, S., Meyer, U., Stinshoff, H., Locher, L., Rehage, J., Wrenzycki, C., Engelhardt, U.H., & Danicke, S. (2015). Diagnostic opportunities for evaluation of the exposure of dairy cows to the mycotoxins deoxynivalenol (DON) and zearalenone (ZEN): reliability of blood plasma, bile and follicular fluid as indicators. *Journal of Animal Physiology and Animal Nutrition*, *99*(5), 847-855. https://doi.org/10.1111/jpn.12285

Yang, F., Li, L., Chen, K., Li, C., Wang, Y., & Wang, G. (2019). Melatonin alleviates β-zearalenol and HT-2 toxin-induced apoptosis and oxidative stress in bovine ovarian granulosa cells. *Environmental Toxicology and Pharmacology*, *68*, 52-60. https://doi.org/10.1016/j.etap.2019.03.005

Zhang, G. L., Feng, Y. L., Song. J. L., & Zhou, X. S. (2018). Zearalenone: A mycotoxin with different toxic effect in domestic and laboratory animlas' granulosa cells. *Frontier in Genetics*, *9*, 667. https://doi.org/10.3389/fgene.2018.00667

Zheng, N., Gao, Y. N., Liu, J., Wang, H. W., &Wang, Q. J. (2018). Individual and combined cytotoxicity assessment of zearalenone with ochratoxin A or α-zearalenol by full factorial design. *Food Science Biotechnology*, *27*(1), 251-259. https://doi.org/10.1007/s10068-017-0197-9

Zhu, L., Yuan, H., Guo, C., Lu, Y., Deng, S., Yang, Y., Wei, Q., & Wen, H. (2012). Zearalenone induces apoptosis and necrosis in porcine granulosa cells via a caspase-3-

and caspase-9-dependent mitochondrial signaling pathway. *Journal of Cellular Physiology*, *227*, 1814-1820. https://doi.org/10.1002/jcp.22906