

Negative effects of zearalenone on reproductive productivity in dairy cattle

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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 olumsuz 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 mikotoksinle 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 sığırları

Introduction

Zearalenone (ZEN) is one of the most common mycotoxins that is produced by *Fusarium* spp. mainly *F. graminearum*, *F. culmorum*, and *F. verticillioides* (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-Kpembé 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.

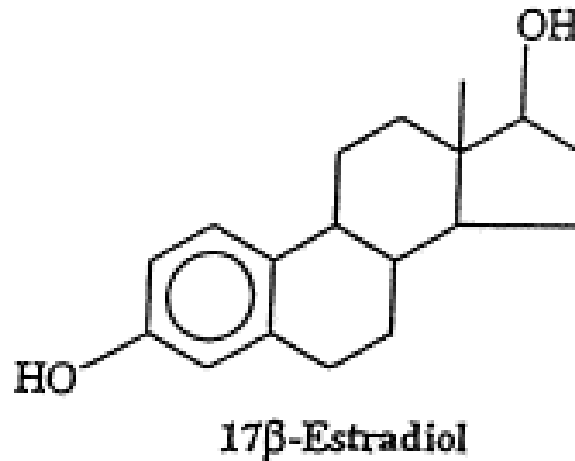


Figure 1: Chemical structure of 17β-estradiol.

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 bond 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.

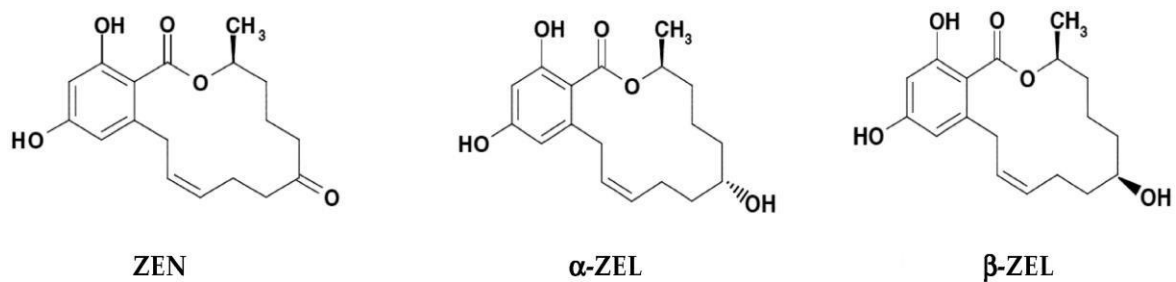


Figure 2: The chemical structure of zearalenone, α -zearalenol, and β -zearalenol (Ropejko and Twaruzek, 2021)

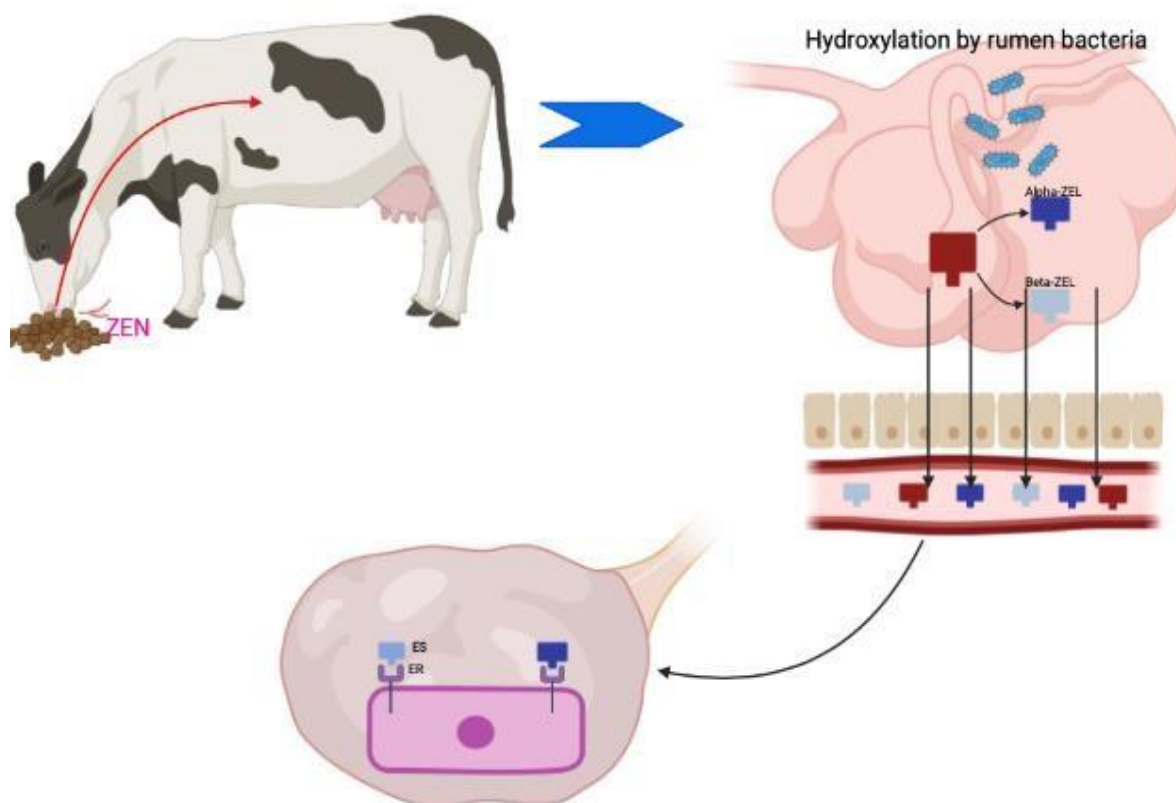


Figure 3: An illustration of ZEN metabolism in the rumen. ZEN and *its* metabolites bind to estrogen receptors and exert estrogenic effects on animals. 17 beta-estradiol and ZEN-and *its* metabolites bind to estrogen receptors

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 ($\sim 8.82 \mu\text{M}$).

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 (94 μ M) 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 μ M. 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 μ M 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.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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