Zearalenone Toxicity Research Postprint

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Abstract

Zearalenone (ZEA) is a common feed mycotoxin primarily produced by various *Fusarium* species. It has been detected in grains such as corn, wheat, rice, and barley, and exhibits estrogenic effects. ZEA causes reproductive dysfunction in animals, accompanied by reduced growth performance. Globally, the annual economic losses to the livestock industry due to ZEA contamination are extremely severe, making it an urgent and critical issue. This review summarizes research progress on the reproductive toxicity, immunotoxicity, hepatotoxicity and nephrotoxicity, genotoxicity, and tumor induction of ZEA, and provides an overview of current challenges.

Full Text

Study on the Toxicity of Zearalenone

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Abstract

Zearalenone (ZEA) is a common mycotoxin produced by various *Fusarium* species that is frequently detected in cereals such as corn, wheat, rice, and barley. Due to its estrogen-like effects, ZEA causes reproductive dysfunction and reduced growth performance in animals. The economic losses to global livestock production from ZEA contamination are substantial and represent an urgent challenge. This review summarizes research progress on ZEA' s reproductive toxicity, immunotoxicity, hepatotoxicity and nephrotoxicity, genotoxicity, and tumor induction, while also addressing current challenges in the field.

Keywords: zearalenone; toxicity; interaction; mycotoxin

Globally, approximately 25% of grains suffer from mycotoxin contamination, which reduces their feed value, while over 2% of crops are completely lost due to severe toxin contamination. Zearalenone (ZEA) contamination is particularly serious. Mold contamination occurs both in the field and during storage; poor storage conditions promote rapid fungal growth and mycotoxin production, reducing the nutritional value of feed ingredients and damaging various organs and systems in animals. ZEA primarily contaminates corn, wheat, rice, barley, millet, and oats, and was first isolated from corn infected with *Gibberella zeae* in 1962 [1]. In addition to *Fusarium graminearum* and *Fusarium oxysporum*, other species such as *Fusarium tricinctum* and *Fusarium equiseti* also produce ZEA. Currently, ZEA has been detected in grains and agricultural products worldwide. Liu et al. [2] analyzed mycotoxin contamination in feed and feed ingredients in several regions of China during the first half of 2017, finding that among 356 samples, ZEA was detected in 95% with an average concentration of 689.0 g/kg, primarily contaminating corn and its by-products and oilseed meals. Placinta et al. [3] conducted a worldwide survey of mycotoxin contamination in cereals and by-products, demonstrating the ubiquitous presence of ZEA residues in selected grain materials and animal feed samples across countries, confirming the severity of ZEA contamination and the urgent need for effective control measures.

Currently, numerous studies have shown that consumption of ZEA-contaminated feed causes various negative effects in animals. This review systematically introduces the hazards of ZEA to animals, including damage to the reproductive system, immune function, liver and kidney organs, genetic material, and tumor induction. It also elaborates on the interaction effects between different toxins and provides suggestions and prospects for current problems in mycotoxin research, aiming to help livestock practitioners better understand this global issue of mycotoxin contamination.

1 Physicochemical Properties of ZEA

ZEA, also known as F-2 toxin, is a white crystalline substance with the molecular formula C H O . It is a resorcylic acid lactone, chemically named 6-(10-hydroxy-6-oxo-undec-1-enyl) -resorcylic acid lactone, primarily produced by *Fusarium* species, with a relative molecular mass of 318 and a melting point of 161–163°C [4]. ZEA is polar, though less so than aflatoxin, insoluble in water, carbon disulfide, and carbon tetrachloride, but soluble in alkaline aqueous solutions. Hidy et al. [5] investigated ZEA solubility in various solvents, finding that solubility decreases in the following order: acetone, ethanol, methanol, dichloromethane, acetonitrile, benzene, and n-hexane.

2 Toxicity Research on ZEA

ZEA and its secondary metabolite zearalenol (ZAL) share structural similarities with endogenous estrogen, enabling specific, competitive binding to estrogen receptors in animals. This activates estrogen response elements and triggers dimerization [6-7], producing a range of estrogen-mimicking effects that cause nervous system excitation, endocrine disruption, impaired secondary sexual characteristics, and often death due to excessive estrogen levels. The binding capacity to estrogen receptors varies among toxins: -zearalanol (-ZAL) > -zearalenol (-ZOL) > -zearalanol (-ZAL) > ZEA > -zearalenol (-ZOL) [8]. ZEA primarily damages the reproductive systems of female animals, reducing production performance while also compromising immune function and exhibiting hepatotoxicity and cytotoxicity, with potential to induce tumors [9]. ZEA contamination poses significant threats to human and animal health, causing substantial economic losses to livestock production.

2.1 Reproductive and Developmental Toxicity

ZEA' s reproductive toxicity primarily manifests through competitive binding with estrogen receptors, producing estrogen-like effects that alter receptor structure and facilitate translocation to the nucleus. This modifies chromatin structure, affecting DNA transcription and RNA translation, thereby disrupting normal protein synthesis [7]. ZEA impairs cellular physiological functions and reproductive system performance, causing reproductive failure and reduced animal productivity [10]. Female animals typically exhibit genital lesions, ovarian atrophy, pseudopregnancy, abortion, and stillbirth; prepubertal females often experience infertile first estrus. Males show decreased libido and reduced sperm quality, while offspring may suffer congenital malformations [11]. Species susceptibility varies, with sensitivity ranking as pigs > rats > cattle and poultry, with young sows being most vulnerable [12]. Research indicates that approximately 0.2 mg/kg ZEA in feed can cause vulvar swelling, increased uterine volume and weight, and ovarian degeneration with internal cavitation in young sows [13].

Xiao [10] found that ZEA-poisoned pigs showed no mortality or significant changes in body condition or temperature. Symptoms varied by growth stage, primarily manifesting as estrous-like genital conditions, with irregular estrus and infertility after multiple matings in replacement gilts. Obremski et al. [14] reported that sexually mature sows fed ZEA-contaminated corn experienced follicular atresia and apoptosis-like changes in uterine granulosa cells, impairing reproductive performance. Wang et al. [15] found that adding 1.5 mg/kg ZEA to gestating sows' diets significantly reduced litter size. Since fetuses obtain nutrients and excrete waste through the placenta, ZEA' s inhibition of placental innate immunity compromises fetal development, increasing stillbirth and weak piglet rates. Gajęcka et al. [16] fed two groups of 2-month-old gilts diets containing 20 or 40 g/kg ZEA for 48 days, observing uterine wall hyperplasia with congestion and swelling in the 20 g/kg group, and endometrial connective tissue

fibrosis with uterine wall cell necrosis in the 40 g/kg group. Shan et al. [17] demonstrated that boars fed ZEA-contaminated diets exhibited 'feminization' symptoms including testicular atrophy and vas deferens degeneration, severely reducing semen quality.

Beyond swine, ZEA poses significant reproductive risks to other animals. Dong et al. [18] showed that increasing ZEA doses significantly reduced reproductive organ weights in male mice, indicating testicular and epididymal damage that decreased sperm count and motility. Dailey et al. [19] reported that feeding laying hens ^{1} C-labeled ZEA resulted in detectable radioactivity in egg yolks, posing human health risks through consumption of such eggs and potentially causing estrogen hyperfunction.

2.2 Immunotoxicity

The immune system comprises immune organs, cells, and molecules, with immune function intimately linked to these three components. Damage to immune organ structure, alterations in immune cell characteristics and numbers, or abnormal immune molecule secretion all compromise normal immune function and threaten animal health [20]. Lymphocyte proliferation capacity serves as an important immune function indicator. Yang et al. [21] demonstrated that 1.0 mg/kg ZEA significantly reduced peripheral blood lymphocyte proliferation rates in weaned sows, with virus antibody levels decreasing linearly as dietary ZEA concentration increased. Liang et al. [22] reported that mice injected with 25 mg/kg ZEA for 6 consecutive days developed pathological changes in both thymus and spleen, with the thymus being more sensitive. Vlata et al. [23] found that 30 g/mL ZEA inhibited immune expression in human peripheral blood mononuclear cells, specifically suppressing T and B lymphocyte proliferation. Obremski et al. [24] fed prepubertal gilts diets containing 0.1 mg/kg ZEA for 42 days, observing altered T and B lymphocyte subpopulations in ileal lymphoid tissue and changes in gastrointestinal neurochemical coding, likely attributable to ZEA' s effects on estrogen receptors or its pro-inflammatory properties, reflecting metabolic and transformational changes in the nervous system.

2.3 Hepatic and Renal Toxicity

As the primary metabolic organ with detoxification functions and the largest digestive gland, the liver is also the main site of ZEA metabolism, making it vulnerable to ZEA-induced physiological impairment [4]. Zhu et al. [25] found that feeding broilers diets containing 2 mg/kg ZEA caused oxidative liver damage and toxin residues. Aspartate aminotransferase and alanine aminotransferase are important diagnostic indicators for liver disease; elevated levels indicate oxidative liver cell damage [26]. Han et al. [27] reported that ZEA exposure significantly increased these enzyme activities in mice, indicating liver pathology. Wang [28] fed sows low-concentration ZEA diets (0.5, 2.0 mg/kg), finding liver ZEA residues of 291.14 and 1,352.05 ng/kg respectively, demonstrating a linear

relationship between toxin intake and residue levels. Mirocha et al. [29] fed broilers diets containing 100 mg/kg ZEA for 8 days, detecting ZEA accumulation of 59–103 g/kg in muscle and 681 g/kg in liver.

The kidneys, as primary excretory organs, eliminate ZEA through enterohepatic circulation and represent a target organ for ZEA toxicity [22]. ZEA accumulation induces glomerular atrophy, renal tubular epithelial cell degeneration, and proteinuria, causing organic kidney lesions [30-31]. Liang et al. [32] intraperitoneally injected female mice with ZEA, deoxynivalenol (DON), ZEA+DON, or control solvent, finding that ZEA accelerated renal apoptosis, increased serum creatinine and urea nitrogen, and induced oxidative stress. Furthermore, the ZEA+DON combination exhibited sub-additive toxic effects on kidney function.

2.4 Genotoxicity

DNA, the primary genetic material and main component of chromosomes, is damaged by ZEA' s genotoxic effects, which inhibit DNA and protein synthesis and interfere with cell division, blocking genetic expression. Zhen et al. [33] reported that ZEA damages genetic material in porcine Leydig cells in a dose-dependent manner until cell death. Zheng [34] found that ZEA causes cytoskeletal protein and nuclear damage in rat Sertoli cells, resulting in nuclear structural indentation and, in severe cases, nuclear rupture, while inhibiting protein synthesis and significantly reducing expression of multiple proteins. Muthulakshmi et al. [35] demonstrated that ZEA causes heart damage and spinal curvature in zebrafish, along with DNA damage, apoptosis, and histological changes in fertilized embryos, with damage severity positively correlating with ZEA concentration and exposure duration.

2.5 Tumor Induction

Numerous studies demonstrate ZEA' s tumor-inducing potential and carcinogenicity. Yu et al. [36] investigated ZEA' s effects on tumor-related gene expression in estrogen-dependent breast cancer cells (MCF-7), finding that ZEA promotes tumor development and exhibits carcinogenic properties. Schoental et al. [37] reported that rats fed diets contaminated with ZEA and its metabolites developed mammary fibroadenomas, pituitary adenomas, testicular interstitial cell tumors, and uterine fibroids, with similar studies consistently inducing these conditions. While some research suggests ZEA is carcinogenic even at low concentrations, other studies indicate carcinogenicity only at high doses. These discrepancies may relate to species, sex, age, and physiological status, warranting further investigation into ZEA' s carcinogenic mechanisms to reach more definitive conclusions.

2.6 Interaction of ZEA with Other Mycotoxins

Mycotoxin interactions occur when feed is contaminated with two or more mycotoxins simultaneously, producing combined toxic effects that can be additive,

synergistic, or antagonistic. While most research focuses on single mycotoxin effects on animal performance, reproduction, and immunity, studies on interactions between different mycotoxins remain limited. However, since single fungal species may produce multiple toxins, feed ingredients are often co-contaminated, and even singly-contaminated ingredients can result in multiple toxins in complete feed. Synergistic effects of multiple mycotoxins pose more serious health threats.

Lei [38] demonstrated that different mycotoxins exhibit varying toxic effects on target cells and organs, with dose-dependent relationships between ZEA and aflatoxin B1 (AFB1). At low AFB1 concentrations, ZEA reduces AFB1 nephrotoxicity, but high AFB1 concentrations create synergistic effects with ZEA. Both ZEA+AFB1 and ZEA+DON combinations affect pro-inflammatory cytokine expression in mouse spleen, impairing immune function. Levkut et al. [39] showed that ZEA+DON mixtures severely inhibited phagocytic capacity in broilers without affecting leukocyte counts, whereas DON alone did not suppress phagocytosis, suggesting synergistic effects between ZEA and DON. Williams et al. [40] reported that growing pigs fed ZEA+DON-contaminated corn exhibited reduced average daily feed intake and weight gain, impairing growth performance. Li et al. [41] co-exposed HepG2 and KK-1 cells to ZEA and ochratoxin A (OTA), demonstrating combined effects that increased damage to target cells and organs.

3 ZEA Limit Standards

ZEA contamination has attracted worldwide attention, as it is present in virtually all feed ingredients and complete feeds. Contamination during cultivation, harvest, or storage causes numerous adverse consequences, including reduced animal performance, disease, and mortality, inflicting substantial economic losses on producers and serious threats to human health.

However, ZEA limit standards vary globally, lacking uniformity. Australia limits ZEA in grains to 50 g/kg; France permits 200 g/kg in grains and vegetable oils; Russia allows 1,000 g/kg in durum wheat, flour, and wheat germ; Uruguay limits corn and barley to 200 g/kg; and Italy restricts ZEN (zearalenone) to 100 g/kg in cereals and cereal products. Chinese standards specify: ZEA in corn and complete feed must not exceed 500 g/kg; in piglet complete feed, 150 g/kg; and in gilt complete feed, 100 g/kg [42]. These regional differences likely reflect variations in livestock breeds, environmental factors, and animal tolerance to ZEA, necessitating continued research for more scientifically grounded standard development and implementation.

4 Summary

ZEA toxicity primarily affects reproduction, immunity, liver and kidney function, genetic material, and tumor induction. Since its discovery, researchers worldwide have extensively investigated ZEA' s physicochemical properties,

metabolic mechanisms, prevention, degradation, and control measures. However, challenges remain: ZEA' s interactions with biological macromolecules in vivo involve complex metabolic processes, and its interactions and synergistic effects with other mycotoxins require further clarification. Additional research is needed to differentiate the effects of mycotoxin combinations from single-toxin toxicity. Ensuring safety of livestock products and effectively controlling mycotoxin contamination requires coordinated global efforts to refine standards and promote their implementation across relevant industries.

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