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Review

Adverse Effects of *Fusarium* Toxins in Ruminants: A Review of *In Vivo* and *In Vitro* Studies

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Abstract: With an increased knowledge of the mechanism of action of *Fusarium* mycotoxins, the concept that these substances are deleterious only for monogastric species is obsolete. Indeed, most mycotoxins can be converted into less toxic compounds by the rumen microflora from healthy animals. However, mycotoxin absorption and its conversion to more toxic metabolites, as well as their impact on the immune response and subsequently animal welfare, reproductive function, and milk quality during chronic exposure should not be neglected. Among the *Fusarium* mycotoxins, the most studied are deoxynivalenol (DON), zearalenone (ZEN), and fumonisins from the B class (FBs). It is remarkable that there is a paucity of *in vivo* research, with a low number of studies on nutrient digestibility and rumen function. Most of the *in vitro* studies are related to the reproductive function or are restricted to rumen incubation. When evaluating the production performance, milk yield is used as an evaluated parameter, but its quality for cheese production is often overlooked. In the present review, we summarize the most recent findings regarding the adverse effects of these mycotoxins with special attention to dairy cattle.

Keywords: deoxynivalenol; zearalenone; fumonisins; immune system; reproduction; gastrointestinal function; milk

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1. Introduction

Mycotoxins are secondary metabolites produced by many filamentous fungi belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium* spp. that can cause toxic responses when ingested by humans and other vertebrates [1–3]. Mycotoxins are generally very stable and can be detected in animal feeds and homegrown forage [4–6]. The *Fusarium* spp. produce mycotoxins that are usually detected in several feeds because the *Fusarium* molds are widespread and able to contaminate field crops in the temperate and warm climate zones [7]. There are also other studies that have described the toxicological effects of *Fusarium* toxins in farm animals in the last decade [8–10]. The mycotoxin deoxynivalenol (DON) can cause numerous problems in animals, including gastrointestinal disorders, soft stools, immunosuppression, and a general decrease in performance due to feed refusal [9–11], and because the immune system requires energy to work properly, e.g., because of immune deficiency or inflammation [2]. The biological mechanisms underlying these responses are not well understood, but dysbiosis in the rumen and/or gut milieu, increased permeability of the rumen and/or gut epithelia, and damage of gut epithelium are common signs [12]. The fumonisins (FBs) are mycotoxins that are cytotoxic, hepatotoxic, and nephrotoxic and can be absorbed in the gut [13]. This results in an immunosuppressive condition in the intestine involving molecular systems including the downregulation of the innate immunity marker myeloid differentiation primary response 88 (MyD88). The MyD88 plays a role in the immune cell activation through toll-like receptors (TLRs), acting in the first phase of the pathogens' recognition and starting the inflammatory cascade [14,15]. Zearalenone (ZEN)

has a resorcyclic acid lactone structure, and its conformation is similar to that of estradiol, allowing this mycotoxin to bind to estrogenic receptors, leading to an estrogenic action [13]. Therefore, this toxin is mostly known by its adverse reproductive effects.

Although the mechanism of action of *Fusarium* mycotoxins are well described in the intestines of monogastrics, it is important to understand if these mycotoxins will be able to reach the intestine of ruminants. As reviewed by Fink-Gremmels [16], several mycotoxins can be inactivated by the rumen flora. For instance, DON is converted into the less toxic de-epoxidized metabolite (de-DON or DOM-1) given physiological rumen conditions, whereas others pass the rumen–reticulum compartment unchanged (e.g., FBs) or are converted into metabolites characterized by a biological activity greater than the mother molecule (e.g., ZEN converted into α-zearalenol (α-ZEL)). Hence, various mycotoxins can modify the rumen flora due to their antimicrobial activity and exert a modulating effect on the immune system of the host animal, even at low doses. There is limited conclusive scientific evidence regarding the real effect of mycotoxins on the digestive, physiological, and pathophysiological parameters of dairy cows [2–6]. For instance, in the opinion of the European Food Safety Authority (EFSA) concerning DON as an undesirable substance in the agro-food chain [11], effects ascribable to this mycotoxin ingestion in dairy cows are described as a loss of appetite, a reduced feed intake and rumination activity, immuno-suppression, and an up-regulation of pro-inflammatory cytokines, but it was also stated that only a few feeding studies with dairy cows were available. Similarly, a recent scientific opinion of EFSA [17] on FBs stated that there is scarce information concerning the adverse effects of these mycotoxins in ruminants, particularly dairy cows. Furthermore, the effects of mycotoxins on cheese-making characteristics have been poorly investigated [18]. Consequently, an evaluation of the economic impact of mycotoxins on ruminant livestock production is an area of research that is still poorly explored.

In the present review, we provide an overview of the most recent (2015–2022) *in vivo* and *in vitro* studies related to the adverse effects of *Fusarium* mycotoxins in dairy cattle. Most of the information is restricted to DON, ZEN, and FBs, but information from other *Fusarium* mycotoxins, e.g., T-2/HT-2 and beauvericin (BEA), is given when available.

2. General Toxicology of *Fusarium* Toxins

2.1. Deoxynivalenol

2.1.1. Toxicity

The mycotoxin DON is a type B trichothecene with a carbonyl group at the C-8 position, and it is produced by *F. graminearum* (Gibberellazeae), *F. culmorum*, *F. nivale*, *F. poae*, *F. roseum*, and *F. tricinctum*. There are two less toxic forms of DON, which are 3-acetyl-deoxynivalenol (3-Ac-DON) and 15-acetyl-deoxynivalenol (15-Ac-DON). They can be present at the same time with DON and are detected at lower concentrations with respect to the parental molecule [19]. DON and 3-Ac-DON can be produced by chemotype Ia of *F. graminearum*, whereas DON and 15-Ac-DON can be produced by the chemotype IIa of *F. graminearum*. The LD_{50} of DON for mice varies from 49 to 70 mg kg^{-1} with intraperitoneal DON injection, 46 to 78 mg kg^{-1} for oral DON administration, and 140 mg kg^{-1} for 1-day old broiler chicks with DON oral administration [20]. No information of LD_{50} is available for cows, but the no observed adverse effect level (NOAEL) is 5 mg/kg feed [21]. DON is often called "vomitoxin" because it can cause vomiting in swine depending on the dose [21]. In addition, de Bovver [22] reported that pigs and other sensitive animals with acute exposure to DON have gastrointestinal problems such as diarrhoea and melena. The chronic exposure to DON can also cause different effects such as anorexia, reduced weight gain, retarded growth, and nausea and negatively impacts the immune and nervous systems [23–25].

The major effect of DON at the cellular level is the inhibition of protein and nucleic acid synthesis via binding to the ribosome and by activating cellular kinases, mitogen-activated protein kinases (MAPKs), and the dysregulation of steroidogenesis [11,26–29]. In brief, DON can bind to the 60S ribosomal subunit and consequently inhibits protein synthesis [29].

The binding of DON to the ribosomes stops the translation and induces the activation of several ribosome-associated MAPKs; this is called the ribotoxic stress response [29–31]. Upon exposure to DON at low/moderate concentrations, MAPK activation drives a surge in the expression of proinflammatory cytokines [31,32].

2.1.2. Absorption, Distribution, Metabolism, and Excretion

The absorption of DON in ruminants is approximately 7–10% [33,34]. Besides the fact that the intact ruminal epithelium is a barrier against DON [35], most of the ingested DON is de-acetylated and de-epoxyded by ruminal protozoans and some bacteria before absorption [33,36]. In pigs and chickens, the distribution of DON occurs quickly and transiently within all tissues, such as serum, muscle, abdominal fat, stomach, intestine, liver, kidneys, heart, brain, lung, skin, spleen, testicles, ovaries, and adrenals [37,38], but accumulation does not occur in several animal species [39]. The serum half-life of DON was found to be 4 h (3.5–4.7 h), and this mycotoxin almost disappears 24 h after ingestion by dairy cows [40]. In the rumen, the DON degradation results in the formation of its derivative, the de-epoxy-DON (also called DOM-1 or de-DON) [41]. After exposure to DON for several weeks, DOM-1 represents the major portion of 81–93% [35] and 94–99% [42] of the total flow of DON plus DOM-1. However, the total flow of DON and DOM-1 accounted only for 4–28% and 12–77% of DON-intake, possibly because there is an absorption of DON and/or DOM-1 across the rumen epithelia or a complete degradation by rumen microbes. Another important step in the metabolism of DON is the conjugation with glucuronic acid and the sulfonation of xenobiotics, which are considered detoxification pathways that increase the water solubility and therefore increase their excretion via urine and bile [43,44]. In dairy cows, approximately 100% of DOM-1 was found to be conjugated in serum, while DON was not detectable at all. In milk, the conjugation degree varied from 33 to 80% and 73 to 92% for DON and DOM-1, respectively, while the ranges in urine were from 21 to 92% and 86 to 100%, respectively [42]. Sulfonation is mediated by sulfotransferases, which catalyse the transfer of a sulfonyl group to a hydroxyl group on an acceptor molecule [43]. The quantitative importance *in vivo* has only been poorly investigated so far. DON is efficiently absorbed in the gastrointestinal tract and then excreted in the urine as a glucuronidated form [45]. Larsen et al. [46] observed that DON and its metabolites are usually excreted via feces, but a little part is present also in the urine. In cows, most of the ingested DON is excreted in the urine and feces, and DOM-1, of which 80% is glucuronidated in the urine and feces, is excreted in the unconjugated form even three days after replacing the contaminated diet by a non-contaminated one [47]. These authors suggested measuring unconjugated and conjugated DOM-1 in urine and feces to determine the exposure via feed.

2.2. Zearalenone

2.2.1. Toxicity

ZEN is a mycotoxin produced by *F. graminearum*, *F. roseum*, *F. culmorum*, and *F. crookwellense*. This mycotoxin is a 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone ($C_{18}H_{22}O_5$) presenting a keto group and an olefinic double bond [48]. ZEN is a lipophilic substance with a high melting point (164–165 °C) [48] and has a lower polarity. Furthermore, this mycotoxin has an estrogenic effect because it acts like the sex hormone 17 β-estradiol (E_2), binding to estrogen receptors and consequently inducing an estrogenic-like response that interferes with the reproductive function of animals [49]. These effects include the disturbance of the estrous cycle, pathological changes of the reproductive tract of males and females, decreased fertility, decreased neonatal survival in females and their offspring, and impaired spermatogenesis [50–52]. Females are more sensitive to ZEN than males, and among the species, pigs are the most sensitive ones [50]. The LD_{50} values are <20 g/kg body weight in mice, >10 g/kg body weight in rats, and >5 g/kg body weight in guinea pigs [51]. No information on LD_{50} and the NOAEL is available for cows [48]. The immunomodulatory effects of ZEN were demonstrated in mice [53]. Oxidative stress was

observed in bovine neutrophils exposed *in vitro* to ZEN, which is a risk for animal defense against pathogens [54].

2.2.2. Absorption, Distribution, Metabolism, and Excretion

ZEN is a lipophilic substance rapidly absorbed upon oral exposure in some animal species such as pigs, poultry, and fish [55–57]. The ZEN oral bioavailability is different between the species, and it was suggested that the oral bioavailability changes with age, with a lower bioavailability in younger animals [58]. Information on ZEN absorption in ruminants is scarce, as rumen microbiota efficiently degrade ZEN usually into α-zearalenol (α-ZEL) and β-ZEL, which is mostly produced via hepatic biotransformation [48]. After absorption, ZEN and its metabolites, e.g., α-ZEL and β-ZEL, are rapidly distributed to the liver, kidneys, and other organs, explaining the low concentration of ZEN and ZEN metabolites detected in edible tissues upon oral administration. Danicke et al. [59] and Winkler et al. [60] demonstrated that the enterohepatic recirculation of ZEN occurs in cattle. *In vitro* studies using rumen fluid demonstrated a higher conversion of ZEN into α-ZEL than into β-ZEL [61]. The metabolite α-ZEL is 60 times more potent than ZEN, whereas the potency of β-ZEL is 0.2× of that of ZEN. However, under *in vivo* conditions, β-ZEL is the predominantly produced metabolite [48]. It was demonstrated that the ratio of ZEN, α-ZEL, and β-ZEL in the bile of cows that were fed a ZEN contaminated diet ranged from 12 to 20%, 6 to 13%, and 68 to 76%, respectively [59]. This may explain why cattle are less sensitive to ZEN than pigs. It is important to bear in mind, however, that this proportion may be affected by feed intake and rumen pH [62,63]. Urine and fecal excreta contain β-ZEL (51–58%), followed by ZEN (25–29%) and α-ZEL (12–20%) [64]. Urine excretion of ZEN, α-ZEL, and β-ZEL mostly occurs as the glucuronide conjugate (74, 53, and 70% of total ZEN, respectively), whereas free and sulphate conjugates are excreted at a percentage of total ZEN of 11% and 16% for ZEN, 36% and 11% for α-ZEL, and 11% and 19% for β-ZEL, respectively [64].

2.3. Fumonisins

2.3.1. Toxicity

FBs are mycotoxins produced by *F. verticillioides*, *F. proliferatum*, *F. anthophilum,* and *F. nygamai*. This substance is a C-20 or C-19 long chain amino-polyol backbone with two methyl groups on the backbone and two propane-1,2,3-tricarboxylic acids (called tricarballylic acid, TCA), and the side chains are esterified to hydroxy groups at positions C14 and C15. FBs are polar compounds that are soluble in water and in polar solvents. There are more than 28 different forms of FBs that are classified as A-, B-, C-, and P-series in relation to their different substituent groups. The substances of group B are often present in the feed commodities, for example, fumonisins B1 (FB1), fumonisins B2 (FB2), fumonisins B3 (FB3), fumonisins B4 (FB4), and the other FBs of group B or the other groups are less than 5% of the total FBs [17]. The backbone of the B-type FBs is similar to the sphinganine (Sa) and sphingosine (So), especially for the amino and hydroxy functions in positions C2 and C3. Hence, the FBs toxicity is characterized by the disruption of the sphingolipid metabolism [65]. FBs are responsible for stopping the sphingolipid biosynthesis, causing leucoencephalomalacia in horses, pulmonary edema in swine, and hepatotoxicity in rats, mice, and rabbits [17]. Hepatic toxicity, an increase in serum enzymes and bilirubin, and biliary duct hyperplasia were observed in neonatal calves that were intravenously treated with FB1 at a dosage of 1 mg/kg for 7 d [66]. Dietary exposure of calves to 148 mg/kg FBs for up to 31 d resulted in changes in liver function without indications of severe liver disease, feed intake, and weight gain [67]. The sphingolipid metabolism of steers fed contaminated diets with FBs at levels up to 90 mg/kg for 110 d was affected, but these animals did not present any adverse health effects or impaired performances [68]. Ruminants are considered less sensitive to FBs than other livestock species [10,69,70] because FBs are poorly absorbed by ruminants [70]. There is a lower milk yield in Holstein and Jersey cows

fed diets containing 100 mg/kg of FBs from 7 d before calving to 70 d after parturition [71]. However, such tested contamination levels are not found under field conditions.

2.3.2. Absorption, Distribution, Metabolism, and Excretion

The bioavailability of the toxin FB is poor (1–6%), and the absorbed fraction is rapidly distributed in the liver and kidneys and excreted via the feces. Fumonisins are characterised by rapid elimination through the systemic circulation with half-lives of a few hours. Intravenous administration (50 or 200 µg/kg body weight) or oral gavage (1 or 5 mg/kg body weight) in dairy cows resulted in a very rapid distribution phase ($t\frac{1}{2}$ α ~ 2 min) and a slower but still rapid elimination phase ($t\frac{1}{2}$ β ~ 15–18 min) [17]. They are no longer detectable in plasma 120 min after administration [72]. These authors also observed that the distribution volumes (Vd ~ 0.25 L/kg) indicate that before being excreted, the mycotoxin concentrates mainly in the extracellular compartments and is poorly absorbed by the tissue. Furthermore, plasma from cows given FB1 orally had no detectable FB1 concentrations [72]. After hydrolysis, the FB1 is converted into partially hydrolysed FB1 (pHFB1) and HFB1, which is less toxic than FB1 [10], and can be detected in tissues and excreta. After oral exposure to 400 mg/kg FB1, FB1 and its metabolite HFB1 are mainly excreted via feces (6 mg/kg FB1 and 14 mg/kg HFB1) and, to a lesser extent, via urine (0.1–0.7 mg/kg FB1) in cattle [73]. FB2 has a poor bioavailability with a lower urinary and fecal excretion when compared with FB1 [17].

3. Carry-Over of *Fusarium* Mycotoxins

In ruminants, the carry-over of DON and DOM-1 to animal products was investigated for transmission in the milk, but there is no information on residues in edible tissues. Prelusky et al. [40] observed that following a single oral dose of 1.7 mg/kg body weight (BW) to lactating cows, the concentrations of free and conjugated DON in the milk were 1–3 ng/mL at 8 h and <1–2 ng/mL at 20 h after dosing, respectively. In a study by Charmley et al. [74], no DON and DOM-1 residues were measured in milk from cows when up to 100 mg/d was consumed for 70 d. This carry-over of DON and DOM-1 is negligible (0.0001 and 0.0011%, respectively) [75]. Residues of ZEN and its metabolites have been found in the edible tissues of dairy cows [76,77]. The enterohepatic recirculation of ZEN is responsible for a high accumulation in the liver compared to other tissues [76]. In cattle, there is a carry-over to the liver and the milk [64,78,79]. Danicke et al. [76] calculated the carry-over factor for ZEN and its metabolites from animal feed to edible animal products and demonstrated a carry-over factor in dairy milk of 0.008. Milk contained ZEN, α-ZEL, and β-ZEL, but the consumption of this product does not lead to a significant exposure of ZEN to humans. The major exposure of ZEN to humans is mainly driven by cereal consumption [52,57,76]. In cattle, most of the ZEN is metabolized into β-ZEL [59], but sometimes, it is also possible to detect similar levels of free α-ZEL and β-ZEL and conjugated α-ZEL almost five times higher than conjugated β-ZEL in dairy milk [64]. However, these authors did not comment on these differences. Increased feed intake may influence ZEN metabolism, especially in high milk yielding cows [76], but more information is needed to understand this mechanism. The carry-over of FBs in the milk is negligible [80]. Spotti et al. [81] concluded that FB1 can cross the mammary barrier but did not provide evidence of the mycotoxin fate in the udder tissue.

4. Legislation for *Fusarium* Mycotoxins in Cattle Feed

The maximum regulated/recommended maximum levels of *Fusarium* mycotoxin in the final diet for cattle in different countries are given in Table 1. DON levels in feed are regulated according to animal age and class (calves younger than 4 months and dairy) only in Canada and South Africa [82,83]. Recommended maximum levels for DON in the final feed for cattle range from 1 to 10 mg/kg feed depending on cattle age and class. For instance, in the US, the maximum recommended level is similar to that in Europe for adult cattle (5 mg/kg feed) [84,85], but this maximum level is increased to 10 mg/kg feed when considering ruminating beef and feedlot cattle older than 4 months [84]. In China, a

maximum of 1 mg/kg DON in the feed of calves and lactating animals is recommended [86]. Maximum DON levels in South Korea and Japan range from 2 and 3 mg/kg [87,88]. In South Africa, both ZEN and FB maximum levels are also regulated in feed [83], whereas maximum levels of ZEN are recommended in Canada [82]. The maximum recommended ZEN levels in the feeds from ruminants are generally 0.5 mg/kg in Europe, China, South Korea, and Japan [85–89]. In Canada, the maximum recommended ZEN level is much higher (10 mg/kg feed), but it decreases to 1.5 mg/kg feed if other mycotoxins are present in the diet [82]. In the US, there are no recommended maximum levels for ZEN in dairy feed. In the US FDA guideline, a maximum of 10 mg/kg of feed for ruminants younger than 3 months, 30 mg/kg of feed for lactating dairy cows and breeding ruminants, and 60 mg/kg of the diet for calves over 3 months that are intended for slaughter are recommended [90]. The maximum recommended levels of FB1+FB2 are very low (4 mg/kg feed) in Japan when compared to the other regions [88]. Furthermore, in Japan, the sum FB1+FB2+FB3 is considered, whereas in most of the other countries, the recommendation covers the sum FB1+FB2. In both Europe and South Korea, a maximum of 20 FB1+FB2 mg/kg feed designated for calves and 50 mg/kg feed for adult ruminants are recommended [85,91].

Table 1. Regulated and recommended levels of *Fusarium* toxins in the final diet of livestock.

Maximum Level (mg/kg)	DON	ZEN	FB	Reference
Regulated				
Canada	1 [1] 5 [2]	-	-	[82] [82]
South Africa	2 [3] 3 [4] 5 [4]	0.5 [5]	50 [6]	[83] [83] [83]
Recommended				
Canada	-	10 [7]	-	[82]
US	5 [8] 10 [9]	-	10 [10] 30 [11] 60 [12]	[84,90] [84,90] [90]
Europe	2 [13] 5 [2]	0.5	20 [13,14] 50 [2,14]	[85] [85]
China	1 [15] 3 [16]	0.5	20 [14,17] 50 [2,14]	[86] [86]
South Korea	2	0.5	20 [14,18] 50 [2,14]	[87,89,91] [91]
Japan	3 [19]	0.5 [19]	4 [19,20]	[88]

[1] young calves and lactating dairy animals; [2] cattle; [3] calves up to 4 months (moisture of 12%); [4] dairy cattle (moisture of 12%); [5] calves and dairy cattle (moisture of 12%); [6] beef (FB1) (moisture of 12%); [7] when other mycotoxins are present, it is recommended a maximum level of 1.5 mg/kg feed; [8] dairy cattle older than 4 months; [9] ruminating beef and feedlot cattle older than 4 months; [10] breeding younger than 3 months; [11] ruminants including lactating cows; [12] breeding ruminants ≥3 months old raised for slaughter; [13] calves <4 months; [14] FB1+FB2; [15] calves and lactating animals; [16] other ruminants; [17] calves; [18] calves <3 months; [23] cattle; [19] Composite feeds (include mixed feeds); [20] FB1+FB2+FB3.

5. Occurrence of *Fusarium* Mycotoxins and Their Sources of Exposure

The synthesis of DON is favourable in cold and wet periods that are followed by a short dry period, and when there is a wet condition with a warm day and cool night [92]. The fungi responsible for producing DON are important plant pathogens and are responsible for causing the *Fusarium* head blight (FHB) in wheat and in barley. DON is present around the world, and it frequently contaminates cereal grains such as wheat (57% positive), maize (41% positive), oats (68% positive), barley (59% positive), rye (49% positive), and rice (27% positive) [93]. A survey on ensiled feed revealed that corn and wheat silage

were found to be the major source of DON in the cattle diet, the average concentrations were 859 and 621 µg/kg, and the maximum concentrations were 3142 and 1165 µg/kg, respectively [94]. In Argentina, in farms with a mycotoxin problem due to the incorrect technique of ensiling, a survey revealed that 28.2% of contaminated corn silage and 50.0% of contaminated alfalfa silage were contaminated with more than 2 mg/kg of DON [95]. The analyses of 74,000 samples from 100 countries in 10 years revealed a prevalence of ZEN in 45% of samples of complete animal feed and feedstuffs [96]. Reisinger et al. [97] found a high percentage of the samples (67.7%) contaminated with ZEN in maize silage. These authors showed that in 5.1% of the analysed samples, the ZEN levels exceeded the European Union (EU) guidance levels for cereals, cereal products, and forages. A survey in the US evaluating 711 corn grains and 1117 corn silages in the period of 2013–2019 detected the presence of ZEN in 21.2% of the corn grain samples with a maximum level of 2.9 mg/kg and only 0.42% exceeding the recommended maximum limits and in 17.8% of the corn silages with a maximum level of 4.0 mg/kg and only 1.25% exceeding the recommended maximum limits [98]. The *F. verticillioides* and *F. proliferatum*, responsible for FB production, are also causative agents of *Fusarium* ear rot, which is a disease of the corn plant [99]. The FB levels in the corn silage varied from 340 to 2490 µg/kg of DM, with a higher concentration in the sample from the top layer and the sidewalls of the silo where there is usually air infiltration and less fermentation [94]. An emerging issue related to mycotoxin contamination of forages and the factors affecting their occurrence at pre-harvest in the field, or during ensiling and the storage of forage crops, has progressed and was the topic of different reviews [5,6,94,97]. Gallo et al. [6] summarized the mycotoxin occurrence data in animal feeds, with a special emphasis on their presence in different kinds of forages, as shown in Figure 1. In summary, DON was detected at a level higher than 1 mg/kg in 33% of the samples, whereas most of the ZEN and FB were detected at medium and low levels, which are much lower than the maximum recommended levels in the complete feed. Therefore, it remains necessary to understand the effects of the co-occurrence of these mycotoxins [100] and their interactions with other factors that are averse to animal health and production.

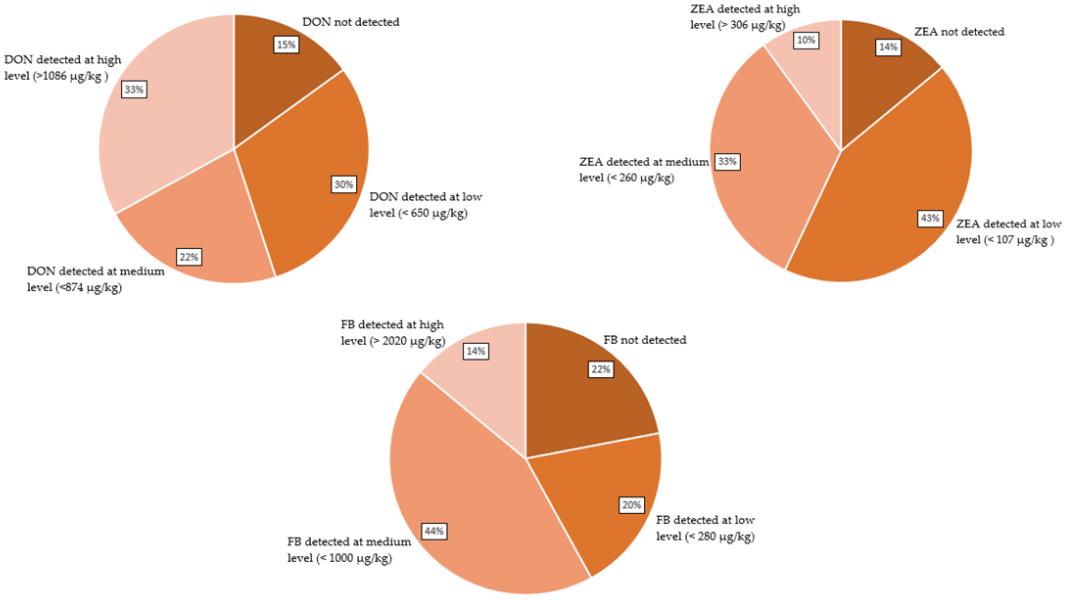

Figure 1. Distribution of *Fusarium* mycotoxins in silage. Adapted from tables in Gallo et al. [6].

Silage, which is the main constituent of cattle ration in several parts of the world, can present high levels of contamination if it is not produced according to good silage practices. This represents a novel area of research about mycotoxicosis in livestock. Gallo et al. [101] characterized sixty-four corn silages for chemicals, bacterial communities,

and concentrations of several fungal metabolites. Silages were grouped in five clusters based on the detected mycotoxins, and they were characterized for their contamination by (1) low levels of *Aspergillus* and *Penicillium* mycotoxins; (2) low levels of *Fusarium* mycotoxins; (3) high levels of *Aspergillus* mycotoxins; (4) high levels of non-regulated *Fusarium* mycotoxins; and (5) high levels of FBs and their metabolites. High occurrences were observed for FBs, but the levels were still close to the recommended levels.

6. Preventing Field Contamination with *Fusarium* Mycotoxins

To minimize mycotoxin contamination in animal diets, it is important to adopt the good agriculture practise followed by the good manufacturing practises during the handling, storage, processing, and distribution of the raw material. Following the HACCP system (hazard analysis critical control point) will be useful to identify and control hazards in the production and processing system. It is necessary to consider the climate conditions of the specific years and the environment where the specific culture is grown. Wegulo [102] reported that it is useful to cultivate crops resistant to the infection of this fungi to inhibit the growth of the fungi and to use specific agro-technical methods such as sowing at the correct time (which influences the harvesting time and the presence of the fungi in the environment), adopting the rotation of the culture, ploughing the residue of the culture in the soil, not exceeding with nitrogen fertilization, using fungicides on the crop if necessary, and avoiding damage to the kernel. Zorn et al. [103] compared the effectiveness and economic impact of ploughing, crop rotation, and choice of variety in decreasing DON contamination in the field. These authors observed that ploughing decreased DON contamination in the feed by circa 32%, crop rotation (maize–no maize) decreased the contamination by 7%, and the choice of wheat variety decreased contamination by 36%, but ploughing was preferred because when selecting a variety, its nutrient composition should also be considered.

Excess precipitation during anthesis (flowering) makes conditions favourable for dissemination and infection by *Fusarium* spp.; thus, irrigation during anthesis and during the ripening of the crops, specifically wheat, barley, and rye, should be avoided [104]. The mycotoxin contamination depends on moisture control. In fact, the grain should be dried to 15% humidity or lower before storage because if the product is dry and kept dry, there is no deterioration. Adequate storage requires no delay, good clean storage facilities, insect control, moisture of grains and humidity control, temperature control, rodent control, chemical preservatives, and antifungal agents (such as acids, mould inhibitors), and the hygiene in the silos may decrease the growth of toxicogenic moulds [105]. However, as *Fusarium* toxins are already produced before storage, such controls will decrease the risks of co-contamination with other relevant mycotoxins [100]. Regarding ZEN, the larvae of the European corn borer (*Ostrinia nubilalis*) and Western corn rootworm (*Diabrotica virgifera*) are harmful for the plant and facilitate the infection by *Fusarium* [1]. The synthesis of FBs is favourable in the hot and dry period followed by humid conditions and insect damage [106].

7. Detoxification of *Fusarium* Mycotoxins in Feedstuffs and Diets

Mycotoxin detoxification can be performed before/during processing or via dietary supplementation with mycotoxin decontaminants. *Fusarium* mycotoxins are resistant to processing. For instance, DON remains stable at 120 °C, moderately stable at 180 °C, and partially stable at 210 °C [107]. ZEN can be deactivated after 60 min at 175 °C [108], and FBs remain stable even after exposure to 250 °C [109]. DON is also stable under weakly acidic conditions, but at the same time, it is unstable in alkali conditions [110].

Before dietary preparation, grains can be subjected to physical decontamination. Dehulling and milling may decrease contamination in the flour, but at the same time, the mycotoxin concentration will be increased in by-products that are often used in animal feed, such as hulls and bran [111,112]. Cleaning and sorting based on kernel uniformity, weight, size, and shape appear as an option to remove *Fusarium* mycotoxins from wheat and corn, but for feed production, usually air blowing and sieving are applied [113,114]. However,

the efficiency of the method must be combined with minimal mass loss. Furthermore, removal must result in an economic advantage, covering the costs with the discharge of the residuals. Removal of DON reached 38% by cleaning and sorting, but mass loss ranged from 28 to 32% [113]. With physical removal, it was possible to reduce DON and ZEN contamination in maize by 73% and 79%, respectively, but with a mass loss of 69% [115]. The contamination of maize with FBs is mostly observed in the broken kernels (10× more than in the intact ones) and in the fines (<3 mm), which represent 5–20% of the total mass [114]. By removing the fines, the FB levels can decrease from 26 to 69% [116]. The rate of removal will depend on the level of contamination, and physical cleaning does not interfere with the nutrient composition of the grains. Ozone (O_3) gas has been indicated to degrade *Fusarium* mycotoxins such as DON, but moisture must be considered [117,118]. The ozonation of maize contaminated with 1000 ppm DON will reach a 90% reduction if moist ozone is used, but dry ozone will result in a decrease of 70% [117]. Moreover, DON elimination via ozonation will be more efficient in ground maize than in whole wheat kernels [119]. Ozone can also degrade both ZEN and FBs in aqueous solutions, but FBs can be converted to another toxic metabolite [120]. Therefore, when deciding on ozonation, it is crucial to determine if degradation of the parental mycotoxins will result in metabolites with a similar or even a higher toxicity.

During the grain processing, there is a variable reduction in the DON content. When whole corn grains contaminated with 7.5–14.8 mg/kg DON were brushed in a laboratory, the decontamination rate ranged from 75.3 to 83.6% [121]. Under the laboratory scale, the highest detoxification (95%) was obtained with extrusion cooking of corn flour [122], a process indicated for human foods. Brushing of the whole corn grain is performed to remove dust from the surface of the kernels. All these decontamination rates will also depend on the physicochemical parameters used during the extrusion process, such as temperature, compression, moisture, and additives [123]. Extrusion cooking resulted in a decrease in the ZEN in corn grits at a range of 65–83% [124], and the levels of FBs in extruded corn grits or alkaline cooked whole kernel corn decreased by 64–72% and >90%, respectively [125]. Specifically, rinsing grain with water or a sodium carbonate water solution could lower the concentration of mycotoxin DON in wheat and maize [123]. Autoclave treatment of DON-contaminated maize and wheat with sodium bisulphite is useful in reducing the DON concentration but cannot be easily performed in practice [117, 118]. Danicke et al. [126] found that the hydrothermal treatment of DON-contaminated wheat in the presence of sodium metabisulphite ($Na_2S_2O_5$) can reduce the toxic effects of DON. In wheat, γ-radiation can reduce the concentration of DON [127], but the radiation produces metabolites or results in even more toxic by-products.

The detoxification of mycotoxins can also be performed by either adsorption (binding) or biotransformation of these contaminants before they are absorbed in the gut of the host. These feed additives must be able to inactivate or remove the mycotoxins without producing more toxic or carcinogenic products, without altering the nutrient value of the diet, and be stable during feed processing, e.g., pelleting. The compounds able to bind mycotoxins are classified as adsorbents, and those enzyme-based additives are classified as biotransforming agents. Adsorbent materials are added to the feed to bind the toxin during the digestive process in the gastrointestinal tract, thus reducing the toxin bioavailability. Their binding capacity will depend on the structure of the target mycotoxin. Most of the commercially available adsorbents contain organoclays in their composition. Organoclays are obtained by modifying bentonites of zeolites with quaternary amines (organophilization). Quaternary amines are surfactants that have a hydrophilic and a lipophilic end. The adsorption of different mycotoxins by organoclays is strongly reduced when complex media are used instead of a buffer solution because the binding of mycotoxins by organoclays is non-specific and, therefore, the other components of the diet are bound together. Although bentonites can sequester aflatoxins, these clay minerals are not as efficient in binding DON, ZEN, or FBs [128]. On the other hand, a combination of a clay with the cell wall of green seaweed (algoclay-based decontaminant) was able to decrease the ZEN exposure [129].

Biotransformation enables an irreversible conversion of mycotoxins into a less toxic or a non-toxic molecule by enzymes or microorganisms. This degradation occurs in the gastrointestinal tract of animals. The advantage of biotransformation is that it is highly specific, defined, and irreversible. Enzymes can act rapidly and independently of the environmental condition, but sufficient moisture (>20%) is needed and the enzymes must be stable during feed preparation and storage.

Besides decontamination, diets are also supplemented with antioxidants to minimize the negative impact of mycotoxins. Specifically for FBs, antioxidants may be used to reduce the toxicity of FB1 [130]. The use of these decontaminants as additives is indicated for diets with mycotoxin levels below the maximum recommended levels, and they are not intended for use in highly contaminated diets.

Regarding silages, Gallo et al. [131] examined the effectiveness of a combined inoculant of hetero-fermentative *Lactobacillus buchneri* LB1819 and homo-fermentative *Lactococcus lactis* O224 on the quality of maize silage at two different densities and observed that inoculation reduced FB2 and roquerfortine C in the diets but increased the fusaric acid. As *Fusarium* mycotoxins are mostly produced in the field, silage inoculation or treatment will have a major effect on *Aspergillus* and *Penicillium* mycotoxins.

8. Effects of *Fusarium*-Toxins on Livestock Production Performance

The effects of *Fusarium* toxins on livestock found in trials published from 2015 to 2021 are shown in Table 2.

In Table 2, most of the trials were performed with a combination of *Fusarium* toxins. However, studies on the interaction of mycotoxins with other toxins, other chemical compounds, or living organisms have been mostly focused on monogastrics [132]. Such studies in ruminants are lacking, but some field trials and *in vitro* experiments indicate the importance of determining the effects of feed contamination with different *Fusarium* toxins, especially because DON and ZEN may co-exist in the final diet [133]. There is also the possibility of the co-contamination with FBs. Beef cattle fed a diet contaminated with 1.7 mg/kg DON combined with 3.5 mg/kg FBs presented a decreased rumen pH, a decreased body weight, a decreased crude protein digestibility, an altered immune cell chemotaxis, and an altered immunoglobulin production and cell membrane structure [134,135]. Dairy cows fed diets contaminated with DON (up to 0.9 mg/kg) and FB (up to 1.3 mg/kg) presented a decrease in dry matter and neutral detergent fibre (NDF) digestibility and an affected immune response together with decreased milk production and a lowered quality for cheese production [136]. However, FBs have a very low oral availability in ruminants. Therefore, this mycotoxin should not be of the highest concern regarding productivity in this species. In a recent study, it was shown that dry cows fed an artificially contaminated diet with 22 mg/kg FB presented an increased activity of liver enzymes, indicating hepatoxicity and altered ruminal microbiota [137]. These authors also observed a compensatory mechanism for the affected rumen function by an increase in feed intake and improved fiber breakdown due to an increase in the rumination time. In *in vitro* studies, a decrease in the ruminal fermentation during exposure to DON and FB was observed [138]. An increase in the Sa:So ratio was observed in steers fed a diet contaminated with up to 90 mg/kg FB, but the performance was not affected [68].

The combination of DON and ZEN in the diet of dry cows affected leucocyte counts only [139], whereas in lactating cows, an impaired immunological status [140,141], decreased milk yield [142], and decreased fat content in milk [140,142] were observed. Dairy cows fed with a diet contaminated with 8.21 mg/kg of DM of DON and 0.09 mg/kg of ZEA for 4 weeks revealed altered rumen fermentation and reduced protein flow to the duodenum, possibly because of the synergistic toxic effects of ZEN and DON [143]. Dietary exposure to 5.9 mg/kg ZEN altered the ruminal microbiota and decreased rumen pH, but a compensatory increased feed intake was observed in dry cows [137]. When dairy cows were exposed to ZEN at a level below 1 mg/kg, only an altered protein metabolism was observed, but the immune status was not impaired [144]. In Europe, the recommended

maximum level of ZEN in the diet is 0.5 mg/kg, and rarely, a total mixed ration (TMR) will achieve more than 5 mg/kg ZEN.

Table 2. Effects of Fusarium toxins on livestock revealed from trials published from 2015 to 2022.

Mycotoxins	Animal	Type of Study	Diet Digestible	Physiological Immune State	Body Weight	Milk	Reference
DON (0.2, 1.7 mg/kg TMR) FB (0.2, 3.5 mg/kg TMR)	Beef cattle	ET	↓↓ pH Rumen	↓	↓↓		[134]
DON (1.7 mg/kg diet) FB (3.5 mg/kg diet)	Beef cattle	ET	↓↓ CP	↓↓			[135]
DON (340.5, 733, 897.3 µg/kg DM) FB (127.9, 994.4, 1247.1 µg/kg DM)	Dairy cows	ET	↓ DM e NDF digestibility	↑ plasma liver transaminase ↓ expression genes for immune and inflammatory		↓↓↓ production and curd firmness	[136]
FBs (22 mg/kg TMR)	Dry cow	ET	Altered rumen microflora ↑ fiber breakdown	Hepatotoxicity (↑ liver enzymes)	↑FI		[137]
FB (≤5, 15, 30, 60, 90 mg/kg)	Steers	FT		↑ SA:SO ratio			[68]
DON (40 to 274 µg/kg DM) ZEN (0 to 274 µg/kg DM)	Dry cow	ET		↓↓↓ total leucocyte count			[140]
DON (455 µg/kg DM) * ZEN (550 µg/kg DM) *AFB1 (10.0 µg/kg DM) *	Red dairy cows	ET		↓		↓↓Fat ↑↑ SCC in CTR	[141]
DON (0.06, 2.31, 4.61 mg/kg diet) ZEN (0.02, 0.29, 0.58 mg/kg DM)	Holstein cows	ET		↓↓			[142]
DON (163 µg, 1966 µg/kg TMR DM) ZEN (19 µg, 366 µg/kg TMR DM	Holstein Friesian cows	ET	↓DM			↓ milk yield, milk fat and protein kg	[143]
ZEN (5.9 mg/kg TMR)	Dry cow	ET	Altered rumen microflora ↓ rumen pH ↑ fiber breakdown		↑FI		[137]
ZEN (<1 ppm into diet)	Cow	ET	Altered protein metabolism				[145]
DON (340.5, 733, 897.3 µg/kg DM) FB (127.9, 994.4, 1247.1 µg/kg DM)	Rumen donor animal	*In vitro*	↓↓ gas production ↓ acetic acid				[138]
DON, NIV, ENN, MPA, ROQ-C, ZEN (for corn silage: 12 mg/kg DON, 60 mg/kg NIV, 1 mg/kg ENN B, 6 mg/kg MPA, 2 mg/kg ROQ-C, 3 mg/kg ZEN)	Ruminal liquid fermentation	*In vitro*	No effect on AGV				[61]

DM: Dry matter; ET: Experimental trial; FI: Feed intake; FT: Field Trial; ↑: increase; ↓ decrease * Recalculated from data presented in the article.

9. Effects of *Fusarium*-Toxins on Livestock Health Status

9.1. Oxidative Stress and Immunity

Oxidative stress is a condition of exaggerated production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from cells combined with the inability to counteract promptly their negative effects with appropriate antioxidant systems or radical scavengers that should be able to maintain the redox homeostasis [145]. It is well known that severe oxidative stress can trigger the immune response by activating the nuclear factor-kB (NF-kB) pathway, which promotes the upregulation of proinflammatory cytokines [146] and then activates the innate immune response. The site of the activation of oxidative stress and the consequent immune response as well as circumstances that can affect these cellular responses remain unclear. Indeed, other mechanisms might stimulate the immune response when mycotoxins are present in the diet. In addition, many factors can modulate this response, such as the dosage and duration of the mycotoxin administration, the diet composition, the physiological state, and the clinical status of the animals. Overall, mycotoxins can trigger the immune and oxidative responses of animals at least at three different levels:

- Interacting with the microbiota composition in the gastrointestinal tracts (GIT), which in turn might challenge the epithelial cells of the GIT or the immune cells located at the gut level;
- Interacting directly with the epithelium or immune cells resident in the GIT;
- Interacting with organs and tissues in case mycotoxins or modified forms are absorbed or transferred into the bloodstream.

DON causes ribotoxic stress favoring apoptosis, altering the inflammatory response and reducing the expression of cell adhesion proteins [9]. Both *in vitro* [147–150] and *in vivo* studies [151,152] reported that DON causes oxidative stress. In target tissue such as liver, kidney, lymphoid organs, intestine, and blood/serum, DON is responsible for the modification of the intracellular antioxidant defense system. Bovine mammary alveolar cells (MAC-T) were cultured in the presence of DON, resulting in a significant decrease in cell proliferation, increased apoptosis, and altered cell morphology [153]. Such effects were previously reported, where DON decreased the proliferation and induced apoptosis in bovine mammary epithelial cells due to oxidative stress and inflammation [154]. Although the effect of DON on milk nutrients has not yet been elucidated, mammary oxidative stress and inflammation contribute to a decrease in milk yield, casein synthesis, and milk fat content when cows are fed DON-contaminated diet [74,153]. Feeding mid-lactation cows with TMR naturally contaminated with 3.5 mg/kg DON and other *Fusarium* mycotoxins at negligible levels resulted in a stimulated primary humoral response [155]. Beef cattle fed a diet contaminated with 1.7 mg/kg DON combined with 3.5 mg/kg FBs presented altered immune cell chemotaxis, immunoglobulin production, and cell membrane structure [134]. Because FBs are poorly absorbed by ruminants, it is probable that these effects were mostly caused by dietary DON exposure. Besides its xenoestrogenic role, ZEN is also known for its genotoxicity and immunotoxicity [156,157]. *In vivo* studies with piglets showed that ZEN leads to oxidative stress, as this molecule acts on the integrity of DNA and mitochondria, decreasing cell proliferation and, at the same time, modulating the inflammatory response [158]. It was observed *in vitro* that ZEN induces ROS and lipid peroxidation, leading to oxidative damage in DNA and mitochondria, apoptosis, and the modulation of pro-inflammatory cytokines [159,160]. Oxidative stress in MAC-T cells was also observed after exposure to ZEN, which was characterized by a decrease in the mitochondrial membrane potential, increased endoplasmic reticulum stress, and subsequent apoptosis [161]. The ability of DON and ZEN to target mammary cells is not surprising because these cells are one of the most metabolically active cells, especially during lactation, being more susceptible to oxidative stress and apoptosis [162].

9.2. Gut Function

The forestomach and intestine are very sensitive to the presence of toxic molecules [163,164]. In particular, microbes can either promote disorders or diseases as a consequence of the changes occurring in the gut milieu or as a reaction of the host mechanisms of defense caused by the presence of contaminants or immunostimulants at the epithelium level. The ability of *Fusarium* mycotoxins to interact with epithelial cells of the GIT and to alter intestinal defense mechanisms has been previously demonstrated [12,164,165]. These mycotoxins decrease the surface area available for nutrient absorption, modulate the expression of nutrient transporters, and above all, cause a loss of barrier function, increasing the permeability of the GIT barrier, as well as modify the mucus secretion and the immunoglobulin and cytokine production [164,166]. Consequently, diets contaminated with mycotoxins worsen the animal health status [167], mainly when the hygienic conditions are unsatisfactory, or the host immune defenses are ineffective or deficient in some component. Dietary contamination with mycotoxins can strengthen stressful conditions and make the recovery of the physiological status in the GIT more difficult. This hypothesis is partly supported by our recent research [136], where feeding mid lactating dairy cows with *Fusarium* mycotoxin contaminated feed caused an immunosuppressive effect on circulating leukocytes and a weakening of the systemic inflammatory response. Although it is known in monogastrics that DON induces gut damage and enhances bacterial translocation, specific studies related to the effects of *Fusarium* mycotoxins on the gut integrity of cattle are scarce. Hence, it is speculated that similar interactions can occur in the GIT of cattle, favoring the growth and/or pathogenicity degree of other pathogens (bacteria, virus, and parasites) when DON is not properly degraded in the rumen, reaching the small intestine. Jovaisiene et al. [138] suggested that dietary exposure to *Fusarium* mycotoxins, specifically DON, affects the intestinal immunoglobulin synthesis in cows, as observed in rabbits [168].

10. Effect of *Fusarium* Toxins on Reproductive System

10.1. In Vivo Experimental Trials

Most reports describing the negative impact of mycotoxins on the reproductive performance of livestock are focused on ZEN. This is expected because this toxin can bind estrogen receptors and cause clear clinical/reproductive effects. However, other *Fusarium* mycotoxins such as DON, DOM-1, FBs, T-2/HT-2, and beauvericin (BEA) should not be neglected because these toxins may impair the function of reproductive cells and further embryo development [169–172]. Reproductive studies in dairy cattle are scarce, especially those performed in the field or in *in vivo* controlled experimental trials with realistic contamination levels of mycotoxins (Table 3). When cows were exposed to DOM-1 via intrafollicular injection (100 ng/mL), a decrease in the follicular development was observed [173]. These authors also exposed cows to DON via feed (3 or 6 ppm) and detected serum levels of DOM-1 10 to 20 times higher than those of DON, reaching 10 to 20 ng/mL. Although no effects on embryo production were observed in the feeding trial, attention should be given to the long-term exposure to DOM-1. In contrast with monogastric species, clinical signs of impaired reproduction caused by ZEN are not always visible in ruminants, probably because of the low rate of α-ZEL absorption [2]. In heifers, abortion was observed only when the diet was contaminated with 10 mg/kg ZEN [174]. Dietary contamination with 0.75 mg/kg ZEN tended to decrease the testicular weight of bulls [175]. Although no other parameter related to sperm function or reproductive endocrinology was evaluated by these authors, it was shown that dietary T-2 (another *Fusarium* mycotoxin) decreases the sperm of bulls [176]. Cows exposed to ZEN at a dose close to 1 mg/kg diet presented an increase in the population of ovarian antral follicles and increased the synthesis of the anti-Müllerian hormone by follicular granulosa cells [144], a characteristic of polycystic ovaries [177]. This hormone is produced by granulosa cells from growing follicles and regulates the recruitment of the quiescent ovarian follicles. In large ovarian follicles, there is a decrease in the secretion of anti-Müllerian Hormone (AMH) [178]. The findings from [144] suggest that by increasing AMH synthesis, ZEN may accelerate the

depletion of the ovarian reserve of gametes. At a lower level in the diet (0.3 mg/kg), ZEN decreases the oocyte quality but does not interfere in the endocrine profiles or further embryo development [179].

Table 3. Effects of *Fusarium* toxins on livestock reproductive function (*in vivo* studies in cattle).

Mycotoxins	Animal	Type of Study	Exposure	Dose	Findings	Reference
ZEN	Bulls	ET	Dietary ZEN *	0.32 mg/kg	Tendency of testicular weight decrease	[175]
	Heifers	ET	Dietary ZEN	10 mg/kg	Abortion	[174]
	Heifer	ET	Dietary ZEN	0.3 mg/kg	↓ oocyte quality No effect on estradiol level No effect on further embryo development	[179]
	Cows	ET	Dietary ZEN	<1 mg/kg	↑ ovarian antral follicle population ↑ synthesis of AMH by granulosa cells No effect on fertility	[144]
DOM-1	Cows	ET	Injection of DOM-1 in ovarian dominant follicle	100 ng/mL	↓ follicular size	[173]
		ET	Dietary DON	6 mg/kg	No effect on the number of viable embryos Serum level of DON: 1.37 ng/mL Serum level of DOM-1: 20.4 ng/mL	[173]
T-2/HT-2	Bulls	ET	Dietary T-2	0.22–0.60 mg/kg	↓ sperm quality (low motility; poor morphology)	[176]

AMH: Anti-Müllerian hormone; ET: Experimental trial; ↑: increase; ↓ decrease; DOM-1: de-epoxidized DON
* Diet also contained 7.8 mg/kg DON.

10.2. In Vitro Experimental Trials

In vitro studies are commonly performed using somatic cell lines to evaluate the steroidogenic activity of some mycotoxins instead of using spermatozoa or those cells obtained from ovarian follicles. Endocrine disruption and stress can be measured *in vitro* with specific cell lines. However, reproductive cells are complex structures and are more indicated for *in vitro* studies to evaluate each developmental stage of the cell, fertilization capacity, and further embryo development [180]. Sperm cells are male reproductive cells, and their major role is the fertilization of an oocyte (female reproductive cell) and the delivery of epigenetic elements to the formed embryo [181]. Depending on the developmental stage, the oocyte will be surrounded by a few layers of somatic cells (granulosa cells), forming the so-called preantral follicles. In later developmental stages, the oocyte starts growing and is surrounded by granulosa and theca cells, the latter being able to secrete androgens that will be converted into estradiol by granulosa cells (for a review, see [182]). Besides, a cavity of fluid is formed within the ovarian follicle (follicular fluid), resulting in an antral follicle. This fluid contains the metabolic products from the granulosa cells, e.g., hormones, as well as nutrients and antioxidants [183]. To be fertilized, the oocyte must reach maturation and be ovulated by leaving the follicular structure. Hence, it is not always possible to compare studies performed with cell lines or somatic cells to those where the sperm or oocyte is exposed to mycotoxins [184]. Granulosa cells are used as species-specific models to evaluate the effects of toxins on steroidogenesis, oxidative stress, and apoptosis. In cattle, most

cells are collected from small ovarian follicles (1–7 mm) [169,185,186], whereas one study was performed with granulosa cells harvested from large follicles (8–22 mm) [187]. It was noteworthy that granulosa cells from large follicles are less sensitive to mycotoxin exposure than those from small follicles. Due to the richness in proteins [183] and antioxidants in the follicular fluid, perhaps there are differences in the effect of mycotoxin exposure on oocytes enclosed in an antral follicle or not. For instance, in pigs, the follicular fluid protects granulosa cells when they are exposed to alternariol, but such an effect is not observed after exposure to beauvericin [188]. Table 4 summarizes the main *in vitro* trials performed with bovine somatic cells, sperm, and oocytes.

DON is not an estrogenic compound, but it can induce ribotoxic stress in cells, including the reproductive ones. The estradiol and progesterone synthesis decreased in granulosa cells from small follicles after exposure to 0.1 and 0.33 μM DON, respectively [169]. However, when granulosa cells from large follicles were exposed, a decrease in estradiol and progesterone synthesis was observed at much higher DON levels (3× and 10×, respectively) [187]. This indicates that the sensitivity of granulosa cells to mycotoxins will depend on the follicular developmental stage. Moreover, depending on the developmental stage, oocytes can be more sensitive to mycotoxins. Oocytes from gilts are more susceptible to DON and BEA than those from sows, probably because of a difference in the redox balance [189]. In pigs, the negative impact of DON was previously demonstrated by the inhibition of oocyte maturation [190]. Bovine theca cells were cultured in the presence of DON or DOM-1 to determine the effect of these mycotoxins on steroidogenesis [171]. These authors observed that 0.5 ng/mL DON decreased progesterone synthesis, and at a double dose it did not affect cell viability. In healthy ruminants, the rumen-associated bacteria can convert more than 90% of DON into its less toxic metabolite DOM-1 [60]. However, DOM-1 reduces steroid production and induces the death of ovarian cells in cattle [171]. These authors exposed bovine theca cells to DON and DOM-1, demonstrating that 0.5 ng/mL DOM-1 decreased progesterone and testosterone synthesis, and at 1 ng/mL, it caused endoplasmic reticulum stress and cell death. These findings are important because in healthy cows, the DON converted into DOM-1 [191] will reach the blood stream and the ovarian follicular fluid [60]. Dairy cows fed a diet contaminated with approximately 8 mg/kg DON may present serum levels of DOM-1 ranging from 4 to 28 ng/mL, whereas the DOM-1 serum levels in those fed diets with much lower DON levels (0.25 ppm) can reach 5 ng/mL DOM-1 [42]. The negative impact of 100 ng/mL DON on bovine granulosa cells was also previously demonstrated [26]. However, as aforementioned, DON is barely recovered in the serum from dairy cows [75,192]. Furthermore, such a serum level requires an intake of feed with extremely high levels of this mycotoxin. To reach a peak of 20 ng/mL DON in serum, a cow should be fed a dose of 1.84 mg/kg BW DON, e.g., giving a bolus with 920 mg DON [40]. However, chronic exposure may interfere with oocyte development and sperm quality.

The development of antral follicles in gilts born from sows exposed to 0.2 or 0.5 mg/kg ZEN via the diet was not affected, whereas apoptosis and cytoplasm vacuolization was observed in the oocytes enclosed in preantral follicles [193]. Similar signs of degeneration were observed in oocytes from sheep preantral follicles exposed to ZEN *in vitro*, where ZEN impaired the proliferation of granulosa cells from primary and secondary preantral follicles and caused DNA double-strain breaks in the oocytes from primordial follicles [194,195]. The presence of mycotoxins in the follicular fluid cannot be excluded. Takagi et al. [196] detected ZEN, α-ZEL, and β-ZEL in normal (18.8%, 6.3%, and 6.3%, respectively) and cystic (35.0%, 10.0%, and 15.0%, respectively) bovine follicles.

Zearalenone not only binds estrogen receptors but also promotes inflammatory processes in cells and reproductive organs. Bovine oviductal fluid plays an important role in sperm capacitation via interaction with glycosaminoglycan, which is secreted by oviductal epithelial cells [181]. When exposed *in vitro* to 100 ng/mL ZEN, bovine oviductal epithelial cells (BOEC) experienced an inflammatory process, impairing an interaction with sperm and subsequent fertilization [197]. Dietary ZEN is rapidly converted to α-ZEL, which is

a major concern for reproduction. Estradiol synthesis is increased in bovine granulosa cells exposed *in vitro* to 30 µg/mL α-ZEL but not when exposed to ZEN at the same concentration [198]. These authors also showed that both mycotoxins were able to impair oocyte maturation, indicating that granulosa cells cannot always predict the action of a toxin in oocytes. Bovine granulosa cells were exposed *in vitro* to 0.09 and 3.1 µM α-ZEL and presented a decrease in the synthesis of estradiol and progesterone, respectively, whereas exposure to β-ZEL at the same concentrations did not affect cell function [169]. At a higher concentration of 25 µM, β-ZEL was able to induce endoplasmic stress in granulosa cells with a subsequent decrease in the estradiol secretion [185]. These authors observed a similar behavior when bovine granulosa cells were exposed to 50 nM HT-2 toxin, the metabolite from the T-2 toxin. Although the T-2 toxin does not disturb specifically the hormonal homeostasis, this mycotoxin and its metabolite HT-2 cause apoptosis and induce oxidative stress in different cell types, including those involved in the reproductive function [185,186].

FBs at a dose of 5 µg/mL disturb the normal function of bovine granulosa cells by stimulating aromatase activity and the subsequent estradiol synthesis [172] and can activate androgen receptors in the human mammary gland and Caco-2 cells [199].

Table 4. Effects of Fusarium toxins on livestock reproductive function (*in vitro* studies with bovine cells).

Mycotoxins	Cell Type	Dose	Findings	Reference
DON	Granulosa cells [1]	0.1 µM	↓ estradiol secretion	[169]
	Granulosa cells [1]	0.33 µM	↓ progesterone secretion	[169]
	Granulosa cells [2]	0.33 µM	↓ estradiol secretion	[187]
	Granulosa cells [2]	3.3 µM	↓ progesterone secretion	[187]
	Granulosa cells [3]	100 ng/mL	↓ estradiol secretion ↓ progesterone secretion	[26]
	Theca cells	0.5 ng/mL	↓ progesterone secretion No effect on testosterone secretion	[171]
	Theca cells	1 ng/mL	No effect on cell viability	[171]
DOM-1	Theca cells	0.5 ng/mL	↓ progesterone secretion ↓ testosterone secretion	[171]
	Theca cells	1 ng/mL	↑ cell death; endoplasmic reticulum stress	[171]
	COCs	100 ng/mL	No effect on oocyte maturation No effect on embryo cleavage ↓ blastocyst formation and expansion	[173]
	Sperm	10 ng/mL	↓ motility and strength	[173]
HT-2	Granulosa cells [3]	50 nM	↓ estradiol secretion ↑ oxidative stress; endoplasm reticulum stress	[185]
	Granulosa cells [3]	12.5 nM	↓ cell viability ↑ oxidative stress	[186]
ZEN	Bovine oviductal epithelial cells (BOEC)	100 ng/mL	Inflammation Disrupt interaction between sperm and BOEC	[197]
	Granulosa cells [4]	30 µg/mL	No effect on estradiol synthesis	[198]
	COCs	1000 ng/mL	↓ oocyte maturation	[196]
	COCs	30 µg/mL	↓ oocyte maturation	[198]
α-ZEL	Granulosa cells [1]	0.09 µM	↑ estradiol secretion	[169]
	Granulosa cells [1]	3.10 µM	↓ progesterone secretion	[169]
	Granulosa cells [2]	3.10 µM	No effect on estradiol and progesterone secretion	[187]

Table 4. *Cont.*

Mycotoxins	Cell Type	Dose	Findings	Reference
	COCs	30 µg/mL	↓ oocyte maturation	[198]
	Granulosa cells [4]	30 µg/mL	↑ estradiol	[198]
β-ZEL	Granulosa cells [1]	3.10 µM	No effect	[169]
	Granulosa cells [3]	25 µM	↓ estradiol secretion ↑ oxidative stress; endoplasm reticulum stress	[185]
	Granulosa cells [3]	10 µM	↓ cell viability ↑ oxidative stress	[186]
FB1	Granulosa cells [1]	5 µg/mL	↑ estradiol No effect on progesterone secretion	[172]
	Granulosa cells [1]	10 µM	↓ estradiol secretion No effect on estradiol or progesterone secretion No effect on cell proliferation	[170]
BEA	Granulosa cells [1]	3 µM	↓ estradiol secretion ↓ progesterone secretion ↓ cell proliferation	[170]

COCs: Cumulus–oocyte complexes; [1]: granulosa cells from small follicles (1–5 mm); [2]: granulosa cells from large follicles (8–22 mm); [3]: granulosa cells from 2–5 mm follicles; [4]: granulosa cells from 2–7 mm follicles; ↑: increase; ↓ decrease.

11. Conclusions

The effects of the *Fusarium* mycotoxins DON, ZEN, and FBs are well documented in monogastrics, whereas information related to ruminants remains needed. It is difficult to compare the results of the available data because in addition to being scarce, the *in vivo* studies in beef and dairy cattle are usually performed with co-contaminated feeds, e.g., DON and ZEN of DON and FBs at variable dietary contamination levels. However, with the knowledge of oral availability, rumen degradation, and toxicity, it is possible to determine which mycotoxins are probably of concern for ruminants.

With an absorption of approximately 10%, most of the ingested DON is converted into DOM-1 by rumen microorganisms. Such a degradation will depend on the function of the rumen, and chronic exposure should not be neglected. It is known that DON may induce inflammatory processes even if present in the diet at levels below the maximum recommended levels. This mycotoxin may also interfere with milk composition, and a study indicates that DON impairs the milk quality. Milk quality and cheese processing are two of the neglected parameters when evaluating the effects of mycotoxins. Most probably, there is an underestimation of the economic impact caused by this mycotoxin in dairy farms. The DON biomonitoring together with the compilation of data on milk production, milk quality, and cheesability could assist in the evaluation of experimental trials. Although DOM-1 is much less toxic than DON, there is a concern about its effect on female reproductive cells. This metabolite can be found in the ovarian follicular fluid at similar levels to those measured in serum and should be considered in further *in vivo* and *in vitro* reproductive studies.

In ruminants, most of the ingested ZEN is converted into β-ZEL, a metabolite with a much lower estrogenicity (0.2× of ZEN). Such a conversion will also depend on the animal health status, productivity, and feed intake. A higher feed intake may lead to a decreased conversion into β-ZEL. Nevertheless, it seems that cattle are very resistant to this mycotoxin and a reproductive adverse effect will occur when the mycotoxin is present at a high concentration or when the feed contains not only this mycotoxin as a xenoestrogen source but also phytoestrogens. Although *in vitro* studies indicate that ZEN decreased rumen pH, this effect is not commonly observed *in vivo.* When performing *in vitro* studies, it is crucial to consider the exposure cell type (somatic or reproductive cells), as well as if

the tested cells have α- and/or β-estrogen receptors, because these factors may influence the results.

The negative impact of FBs in cattle is observed only when these animals are exposed to extremely high levels of the mycotoxin, e.g., 90 mg/kg, which are far higher than the maximum recommended levels and usually are not realistic contamination levels. Furthermore, the oral availability of this mycotoxin is very low, ranging from 1 to 6%. Other mycotoxins produced by *Fusarium* spp., such as BEA and T-2, are also a source of oxidative stress, but their impact on ruminant health and production is not yet well elucidated.

Finally, from the three summarized mycotoxins, DON poses more of a concern than the others regarding animal production, followed by ZEN. Based on the collected data, FBs under the current occurrence in commodities cause no harm to ruminants. The effect of the interaction among a mixture of mycotoxins (including the non-*Fusarium*), between mycotoxins and veterinary drugs or other chemical compounds, or microorganisms (gastrointestinal flora or pathogenic organisms) should be considered. For instance, corn silages could be contaminated with a large number of mycotoxins produced by *Fusarium* spp. and other mycotoxigenic fungi able to grow in silage conditions (i.e., a high level of carbon dioxide and a low level of oxygen and pH). Screening studies with *in vitro* rumen incubation followed by *in vitro* intestinal exposure together with a proper surveillance of in-farm exposure may produce crucial data to develop intervention strategies of prevention and remediation. The application of non-invasive methods of monitoring, such as the analysis of urine or feces, could also support the knowledge of mycotoxin contamination in cattle.

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