Mysore J. Agric. Sci., 57 (3) : 15-23 (2023)

Why Despite Rumen Degradation of Mycotoxins Ruminants are Still at Potential Risk for Mycotoxin Toxicity

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Received : June 2023
Accepted : July 2023

Abstract

Despite widespread occurrence of mycotoxin contamination of ruminant diets is evident globally; ruminants were traditionally considered less susceptible to mycotoxins due to rumen microbial degradation. But, growing body of evidence is highlighting that tolerance of ruminants to mycotoxins is a 'consideration' but not a fool-proof process of complete mycotoxin-inactivation. Yet, remarkably there is a paucity of research studies of mycotoxins in ruminants than in monogastric farm animals (pigs and poultry). Available data show a low number of studies that are mainly related to feed intake, nutrient utilization/digestibility or on production performance (milk yield and growth rate). *In-vitro* studies mostly focus on rumen incubation and immune cells proliferation; with very few articles focusing on reproductive function. However, none of the articles have explicitly explained the diverse set of mechanistic link for the occurrence of deaths and many adverse effects being reported in ruminants following mycotoxin exposure. Thus in the present review, we summarizes the factual evidence explaining how despite rumen microbial action the mycotoxins are potential risk factors in ruminants diets. The current article further enlists and explains various factors that are contributing to the adverse effects of mycotoxins in ruminants despite mycotoxin level being below permissible regulatory limits.

Keywords : Mycotoxins, Ruminants, Animal feed, Adverse effects, Rumen microbes

Mycotoxins are a global animal public health concern including ruminants. Mycotoxins are secondary fungal metabolites with a worldwide spectrum of occurrence and consequently their exposure both in humans as well as animals is high globally. Alarmingly, the global occurrence of mycotoxins in animal feed is not new and has been a persistent scenario since several decades (Rodrigues, 2014; Rodrigues & Naehrer, 2012 and Biomin, 2020). A study performed in 2013 on feed samples from across the world demonstrated that approximately 70 per cent of samples tested was mycotoxin contaminated (Streit *et al.*, 2013). It is no surprise that co-occurrence of multiple mycotoxins is quite common (Biomin, 2020). The situation is further complicated by increasing prevalence of mycotoxin due to climate change (Battilani *et al.,* 2016), putting the animals including ruminants at higher risk for mycotoxin exposure and related toxicity.

Mycotoxins in animal feed occur when fungi infects the food-crops during growth and harvesting (field mycotoxins) as well as when it infects the food-stuff during drying, transport and storage (storage mycotoxins). While corn, wheat, rice, barley, oats and rye are the common cereals affected by mycotoxins, nevertheless straw and silages are also contaminated. The most commonly occurring mycotoxins are produced mainly by the fungi of the genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and *Stachybotrys* (Robbins *et al.,* 2000). Although several hundred mycotoxins have been identified, the most

common mycotoxins found to be implicated in their toxicities include aflatoxins, trichothecenes (T-2, deoxynivalenol - DON), fumonisins (FUM), zearalenone, ochratoxin and ergot alkaloids (Grenier and Oswald, 2011). Among them, fusarium mycotoxins (FUM, DON) were the most prevalent mycotoxins in animal feed in Europe, Asia and worldwide as well indicated by global survey reports (Rodrigues & Naehrer, 2012 and Biomin, 2020). In addition, more recently, there are emerging mycotoxins that are more-and-more detected in animal feed that includes enniatin, phomopsin A, alternariol, etc., suggesting that mycotoxins in ruminants is a critical issue due to their constant presence.

The problem with mycotoxins is their toxicity (mycotoxicosis) and associated disease. Deaths due to mycotoxin contamination are frequently reported both in humans as well as animals, such as death of 125 people in Kenya in 2004 and death of dogs in USA in 2006, following ingestion of aflatoxin-contaminated food and feed, respectively (Lewis *et al.,* 2005 and Susan S. Lang, 2006). More recently, over 110 dogs died following exposure to aflatoxins in pet food (FDA, 2021). However, it was previously considered that ruminants were less susceptible to mycotoxins due to degradation of mycotoxin by rumen microbes. For example, mycotoxin DON was almost completely bio-transformed to de-epoxy DON (94-99%) an non-toxic metabolite by rumen microbes (Seeling *et al.,* 2006). But despite the noted rumen mycotoxin degradation, it was noted that ruminants are not completely exempt from toxic effects of mycotoxins as evidenced by deaths regularly reported in ruminants caused by mycotoxin contamination (Marczuk *et al.,* 2012). This raises specific concerns for the presence of noticeable toxic effects in ruminants despite primary considerations that ruminants are less susceptible to mycotoxins due to degradation of mycotoxin by rumen microbes. The current article thus focuses on providing the plausible mechanistic explanation for the occurrence of toxic effects of mycotoxins in ruminants. The article initially lists the mycotoxin toxic effects despite lack of evident clinical manifestation. Then the article explains how the mycotoxin occurrence factors and rumen microbial factors are responsible for the reported mycotoxin toxic effects in ruminants.

Adverse Effect of Mycotoxins in Ruminants Despite Degradation by Rumen Bacteria

The toxic effects of mycotoxins in ruminants are noticed as deleterious effects on animal production as well as on health. The detailed toxic effects of mycotoxins in ruminants have been saliently described elsewhere by other authors (Gallo *et al.,* 2015; Iheshiulor *et al.*, 2011 and Ogunade *et al.,* 2018). The current article rather focuses on the question that 'why is the presence of toxic effects of mycotoxins in ruminants despite microbial degradation and more importantly despite the mycotoxin level in feed being within permissible regulatory limits?'. Indeed, various factors influence the outcome of the toxic effects of mycotoxins that involves a complex interaction of animal-related factors with dietary changes based on the specific age-needs, toxin-related factors and the envirnmental /manage-mental factors as summarised by Gallo *et al.* (2015). Briefly, the presence of mycotoxins could a) affect the production and performance of animals, despite lack of toxic effect; b) exert sub-clinical toxic effect despite absence of clinical toxicity; or c) cause clear toxic effect. These three different outcomes are explained below.

While mortality is the easiest identifiable toxic effect, despite reports of deaths from mycotoxins mortality is not always the manifested clinical sign of mycotoxicosis. More frequently mycotoxicosis in ruminants occurs as a range of non-specific symptoms negative impact on animal health, productivity and performance (Kiyothong *et al.*, 2012). Reduced rumen motility (AFB1) (Cook *et al.*, 1986), reduced microbial protein synthesis (DON) (Danicke, 2002), switch in the rumen microbial community composition (DON) (Seeling *et al.*, 2006) to inhibition the multiplication of cellulolytic rumen microorganisms (fusaric acid) (May *et al.*, 2000) are some of the general effects of presence of mycotoxins in ruminant feed, noticed even in absence of evident toxicity. This further explains the negative effect of mycotoxins on

feed intake and feed conversion efficacy, that has been thoroughly discussed by Rodrigues (2014), in-turn affecting animal performance. Further, in situation where the mycotoxins exert sub-clinical toxic effect without apparent the clinical symptomatic disease the negative impact on animal health, productivity and performance is more pronounced, compared to their effect on animal performance without any toxicity. This is more so, in ruminants. Due to the rumen degradation of mycotoxins, the level of exposure is considered generally low, however chronic low dose exposure leads to sub-clinical effects that are evidenced to a higher degree in terms of reduction in performance trait (Whitlow and Hagler, 2005). Reduced milk yield, reproductive performance, higher disease incidence (Whitlow and Hagler, 2005) are all some of the common effects of mycotoxicosis in ruminants. Increased somatic cell count (SSC), loss of body condition, lameness with no apparent disease such as reported cases of increase in mastitis and laminitis, correlated to DON exposure in Nothern-Europe and increase in lameness (subclinical laminitis) and impaired fertility (cystic ovaries) in dairy herds following AFB exposure have been reviewed by Rodrigues (2014). While effects such as increased SCC reduce farm profitability, other immune effects such as increased incidence of disease and the resultant increased animal-to-human transmission of pathogens are of public health concern (Oswald *et al.,* 2005). Indeed studying immune effects is crucial, but of all the parameters assessed, immune-toxicity of mycotoxins in ruminants has been given less priority as compared with other farm animals and rodents. It is imperative to focus on immune effect, as impaired immune response to mycotoxin exposure has been demonstrated to occur at levels that had no effect on growth rate in other species (Smith and Hamilton, 1970). Furthermore, ruminants were demonstrated to be more susceptible to the immunotoxic effects of mycotoxins than pig and poultry immune cells, which occur at low doses corresponding to the low dose long term exposure. However, many immune effects including evaluation of vaccine response, outcome of a disease due to interaction of mycotoxin in the pathogenicity of pathogens requires to be elucidated for many mycotoxins in ruminants. Additionally, many deleterious effects were seen in cattle even when feed mycotoxin levels were within regulatory limits. Such as reduced IgA concentrations (Korosteleva *et al.,* 2007), depressed neutrophil phagocytic activity in cows fed DON contaminated diet 3.5mg/kg dry matter (Korosteleva *et al.,* 2009) and low dose DON (0.6mg/kg of dry matter) long term (63 days) exposure in dairy cattle showed negative influence on somatic cell count, blood parameters and immunity (Jovaisiene *et al.,* 2016). Most of these toxic effects were associated with immuno-toxicity. More importantly, recent research results show shocking results that mycotoxin metabolites of microbial degradation that were previously considered non-toxic (Example: de-epoxy DON metabolite of microbial degradation of DON) is found to be toxic to bovine ovarian cells (Guerrero-Netro *et al.,* 2017 and Pizzo *et al.,* 2016). These data suggest that ruminants are more-or-less at the same level of risks from mycotoxins as monogastric animals such as pigs, dogs, etc. This makes studies on mycotoxin-toxicity in ruminants a necessity than an option, for mitigating the mycotoxin problem and ensuring global public health.

Factors that Complicate the Exposure of Ruminants to Mycotoxins

Indeed the different sources of ruminant diet complicate the actual exposure of ruminants to mycotoxins. Ruminants diets are composed of forages (that includes fresh grass, hay, straw and/or silages) and concentrates (that includes cereals and/or protein feed - such as oil seed cakes) (Gallo *et al.,* 2015). Due to this considerable diversity of the feeding systems adopted and the diverse feeds used in their diets, ruminants in particular are exposed to higher variety of mycotoxins coming both from field mycotoxins (occurs before the harvest - forage, cereals, silage) and storage mycotoxins (occurs after the harvest - cereals, concentrates, silage making) (Gallo *et al.,* 2015). Forages make up a major portion of the ruminant diet and could be contaminated by the mycotoxins in the field as well during storage, resulting from poor storage management practices.

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Presence of mycotoxins in straw and silages is also fairly common as evident by surveys (Biomin, 2016). Trichothecenes (DON, T-2), zearalenone, fumonisins, aflatoxins and ergot alkaloid are some of the field-derived mycotoxins, in forages and cereals used for ruminant diets (Driehuis, 2013). Silage in particular, could have mycotoxins from pre-harvest, while ensiling and after ensiling. Roquefortine C, mycophenolic acid, gliotoxin, fumigaclavines, monacolin K and citrinin are the mycotoxins that occur during ensiling process (Driehuis, 2013). The presence of mycotoxins in silage is extensively reviewed in other articles (Ogunade *et al.,* 2018; Wambacq *et al.,* 2016) and could be referred for more detailed description. Further, cereals that make up the major portion of concentrates added to enhance the energy density in ruminant feed are known to be commonly contaminated by mycotoxins. In a 3 year survey Rodrigues and Naehrer (2012), presented evidence that 81 per cent of livestock feed (cereals, distillery grains, finished feed) samples collected worldwide were found to contain at least one mycotoxin. Moreover, a study reported two or more mycotoxins in 48 per cent of the samples. Similarly, contamination of cereals, silage and complete feed were reported in a 4 year survey in Poland (Kosicki *et al.,* 2016).

Additionally, various factors further influence the risk of higher mycotoxin 'exposure and load' in ruminants compared to monogastric animal. As an example based on report by Mostrom and Jacobsen (2011), in the United States during 2008, approximately 25 to 30 per cent of the agricultural commodities were retained for on-farm use and farms where the forage is farm-grown and silage is farm-made, the chances of both field and storage mycotoxin production is higher in ruminant feed (Mostrom and Jacobsen, 2011). Further, the increased need to feed nutrient dense diets to dairy cattle to meet their greater nutrition demand required to accommodate for the higher milk production, increases their chances to mycotoxin exposure from concentrates. Adding to this, ruminants are at higher risk for mycotoxin exposure also due to common consideration that ruminants are less susceptible to mycotoxin in feed on-account of mycotoxin detoxification by rumen microbes. This conception leads to common practice of diverting the spoiled feed of more susceptible species such as pigs to cattle feeding (Mostrom, 2022).

Rumen Microbiota Plays a Key Role in Toxicity of Mycotoxin in Ruminants

Indeed, it has been demonstrated that some of the common mycotoxins found in animal feed (including DON and OTA) are degraded in the rumen, thereby enhancing the resistance of ruminants to feed borne mycotoxins. However, tolerance of ruminants to mycotoxins is a 'consideration' and not a clear-cut conclusive reality (Fink-Gremmels, 2008). For example, 'high-yielding cows fed energy rich diets', as well as 'young ruminants' and 'animals in the transition period' are more susceptible to the negative effects of these mycotoxin toxic metabolites (Rodrigues, 2014). In addition, species difference also plays an intricate role in symptoms of mycotoxicosis. For example, sheep are more tolerant to roquefortine C (Ogunade *et al.,* 2018), while more susceptible to zearalenone (Knutsen *et al.,* 2017), than cattle. Thus, as stated by Rodrigues (2014), ruminal metabolization of mycotoxins is not equivalent to complete toxin-inactivation. Evidently, rumen microbial community carries out this ruminal mycotoxin degradation. Thus the actual extent of ruminal mycotoxin degradation and absorption is dependent on at-least three factors: a) rumen microbial population, b) feeding regime and c) the anti-microbial properties of the mycotoxins themselves. The details of this multi-factor dependence are explained below.

Rumen Microbial Population

While rumen detoxification is true for certain mycotoxins (such as DON), but it is not a fool-proof process that ensures complete detoxification. As the ruminal degradation of mycotoxins is rumen microbiota dependent, factors that influence the stability/activity/diversity of rumen microbiota such as dietary composition and the resulting alterations in pH, could impact the mycotoxin degradation potential. It has been shown that depending on

various factors such as dietary starch intake, rumen pH, microbial activity, level of mycotoxin, etc. The degradation and in-turn bioavailability of mycotoxins varies substantially. Bioavailability of AFB1 and OTA were increased in starch-induced low ruminal pH in non-lactating dairy cows (Pantaya *et al.,* 2014, 2016). Further, not all mycotoxins are degraded in rumen (fuminosins, aflatoxins) and on the other-hand certain mycotoxins are transformed into more toxic metabolite (zearalenone to α-zearalenol) (Danicke *et al.,* 2005). Globally, dairy cattle under the conditions of modern feeding strategies fed diets containing high-energy low fibre are more prone to sub-acute ruminal acidosis (SARA). SARA combined with increased stress of high performance and compromised microbial activity could lead to more mycotoxins escape rumen detoxification and in-turn more mycotoxins absorbed in intestines.

More importantly, differences in mycotoxin degradation between young and adult ruminants enhance the susceptibility of young ruminants to mycotoxicosis. Although calves carry the same diversity of microbiota in their rumen as adult cattle, the proportions are not the same (Jami *et al.,* 2013). The condition could be explained by the fact that although, weaned calves are technically capable of surviving on a complete solid feed based diet, including forages, their diet is mostly concentrate based. Further, they are not completely capable of deriving their nutritional requirement completely from forages until around 4-5 months. Yet, it takes almost 6 months for their rumen to resemble the adult microbial ratios (Jami *et al.,* 2013) and 2 years to be completely similar (Dill-Mcfarland *et al.,* 2017). Thus, emphasizing that ruminants are not always resistant to mycotoxin toxicity.

Feeding Regime

Feeding regime has a profound influence on the rumen pH and microbial diversity. It is well known that providing higher concentrates (such as in modern intensive farming system) the rumen pH decreases and the cattle are more prone to sub-acute ruminal acidosis (SARA). However, during ruminal acidosis (excess energy feed) transformation of mycotoxins in a compromised. *In-vitro* studies on rumen fluid had shown that starch based diet, which is primary concern for SARA reduces the degradation rate of DON than cellulose diet (Jeong *et al.,* 2010). Continuing with the example of DON, *in-vitro* studies suggests that its degradation by primary rumen culture was only 35 per cent as opposed to 100 per cent by chicken large intestinal contents (He *et al.,* 1992). Moreover, the biotransformation of DON was inhibited at pH 5.2, driving the authors to suggest that the detoxification of DON is pH dependent. Moreover, despite a faster transformation of DON into DOM-1 by the original rumen culture, the proportion of detoxification was reduced for subsequent subcultures (He *et al.,* 1992).

Incidentally *in-vivo* experiments show that bioavailability of certain mycotoxins (AFB1, OTA) increases in starch-induced low ruminal pH (Pantaya *et al.,* 2016). Adding to this, under conditions of high concentrate diet that favour ruminal acidosis or SARA, there is alteration in the microbial activity or diversity in rumen (AlZahal *et al.,* 2017) as well as an evident increased compromise of rumen mucosal barrier function (Owens *et al.,* 1998). Such altered microbial activity (with or without altered rumen mucosal barrier function) could be the possible cause for the compromised detoxification and increased bioavailability of mycotoxins as demonstrated by Pantaya *et al.* (2016). Particularly rumen pH of calves is mostly acidic around weaning up-to 12 weeks of age (Quigley *et al.,* 1992 and Suarez-Mena *et al.,* 2015), thus calves are more prone to SARA. This raises the risk of calves to exposed mycotoxins, due to increased absorption.

The Anti-Microbial Properties of the Mycotoxins

Mycotoxins are known for their antibacterial properties. Most of the mycotoxins common in animal feed including DON, FB1, T-2, AFB1 have antibacterial effect (Ali-Vehmas *et al.,* 1998 and Bisht *et al.,* 2011). Subsequently, mycotoxins have been demonstrated to negatively impact certain aspects of rumen fermentative capacity both *in-vitro* and *in-vivo*.

As an example, DON was shown to reduce ammonia-N and total gas production, *in-vitro* (Jeong *et al.,* 2010), whereas in adult cattle, the reduced efficiency of microbial protein synthesis (24%) in rumen and the flow of microbial protein into the duodenum (21%) (Danicke *et al.*, 2005). Such effects on nutrient utilization could be more pronounced in calves and other young ruminants due to still developing rumen and rumen microflora.

Overall, three different types of factors that include a) mycotoxin factor (mycotoxin dose and the anti-microbial activity of mycotoxins); b) animal factor (age group of animal, the rumen microbial population and the rumen digestion kinetics), as well as c) feed related factor (feed composition, more specifically relating to starch and the fibre content in the diet) are the key factors that decide the actual rate of degradation/absorption of mycotoxins in ruminants. This in-turn decides the resultant toxic effects noticed in ruminants, despite the initial consideration of less susceptibility of ruminants to mycotoxins due to rumen microbial mycotoxin-metabolization. While it seems that generally young ruminants are more susceptible to mycotoxicosis due to lack of ability to efficiently degrade mycotoxins than adults. Yet, susceptibility of adult ruminants to mycotoxins could not be neglected.

Studies of mycotoxins in ruminants have received less attention than in monogastric farm animals (pigs and poultry). But, despite previous consideration that ruminants are less susceptible to mycotoxins due to rumen microbial degradation, the extent of mycotoxin-detoxification depends on the mycotoxin-dose, animal age group, feeding regime, rumen digestion dynamics and the microbial community, thus increasing susceptibility of ruminants of different age groups to mycotoxins. Further mycotoxins-metabolites of microbial degradation (de-epoxy-DON), previously considered non-toxic was shown to be toxic by recent research in cattle. Additionally, some of the mycotoxin-metabolites produced by rumen microbial degradation are more toxic than parent compound (Zearalenone to α-Zearalenol). As evident, for most of the known mycotoxins either their complete toxic profile/mechanism of action/toxic dose are not completely available, in ruminants. In this regard, with view of growing risk of mycotoxin contamination of ruminant feed, it is imperative to screen the mycotoxins for their toxicity in ruminants relying on more and more sensitive markers.

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