



Review article

Mycotoxins and the intestine

Leon Broom

Anpario PLC, Manton Wood Enterprise Park, Worksop, Nottinghamshire S80 2RS, United Kingdom

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ABSTRACT

Fungal biochemical pathways can yield various compounds that are not considered to be necessary for their growth and are thus referred to as secondary metabolites. These compounds have been found to have wide ranging biological effects and include potent poisons (mycotoxins). Mycotoxins invariably contaminate crops and (thus) animal feeds. The intestine is the key link between ingested mycotoxins and their detrimental effects on the animal. Effects on the intestine, or intestinal environment, and immune system have been reported with various mycotoxins. These effects are almost certainly occurring across species. Most, if not all, of the reported effects of mycotoxins are negative in terms of intestinal health, for example, decreased intestinal cell viability, reductions in short chain fatty acid (SCFA) concentrations and elimination of beneficial bacteria, increased expression of genes involved in promoting inflammation and counteracting oxidative stress. This challenge to intestinal health will predispose the animal to intestinal (and systemic) infections and impair efficient digestion and absorption of nutrients, with the associated effect on animal productivity.

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1. Introduction – mycotoxins and their diversity

Fungal biochemical pathways can yield various compounds that are not considered to be necessary for their growth and are thus referred to as secondary metabolites. These compounds have been found to have wide ranging biological effects and include potent poisons (mycotoxins). Fungi frequently infect crops and the subsequent contamination with mycotoxins poses a significant health risk to both humans and animals consuming the crop material. Currently, there are around 500 known mycotoxins. Recent work, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and analysing 83 different feed and feed raw materials, revealed that all of the samples contained a multitude of mycotoxin metabolites (Streit et al., 2013), with 26 to 30 different metabolites most frequently detected. Seven was the lowest number of metabolites detected in a sample, while 69 was the highest. The total number (range) of different metabolites that were detected was

139. These recent data, using the latest analytical techniques, are supporting the views expressed previously about the presence of mycotoxins. In 2005, Devegouda and Murthy suggested that “under practical conditions, no poultry feed is completely free of mycotoxins” and that “no feed can be expected to contain only one mycotoxin”. Similarly, Professor Tom Scott from the University of Saskatchewan, Canada, has been quoted as saying “mycotoxins, they are everywhere”. The reality is that nutritionists, producers, etc. are routinely dealing with crops/feeds with multiple mycotoxin contamination, even if their (perhaps older) analytical tests do not indicate/confirm this.

Silages (preserved forages or cereals) are a major component of ruminant feed rations worldwide. It is sometimes overlooked that silages can become contaminated with an array of mycotoxins from the field to feeding. It has become noteworthy that numerous ‘emerging’ mycotoxins (e.g., roquefortine C and mycophenolic acid) are being found. Given the prominence of silages in a ruminant’s diet and their normally significant levels of mycotoxin contamination, the very significant contribution of silages to the overall mycotoxin challenge of a ruminant is often overlooked.

2. Effects of mycotoxins

At the cellular level, toxins can catastrophically interfere with numerous pathways and processes. Some better understood

E-mail address: leon.broom@anpario.com.

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mycotoxins are known to inhibit protein synthesis (Creppy, 2002). Therefore, cells that are rapidly multiplying or synthesising/secretory protein(s) (e.g., intestinal, immune, etc.) would be particularly vulnerable to the effects of mycotoxins. There are excellent reviews available that consider the physiological (e.g., reproductive) effects of mycotoxins in animals (e.g., Cortinovis et al., 2013). Where data are perhaps lacking is in translating physiological effects into quantifiable performance effects. Recently, there have been two large studies that have employed meta-analysis techniques to assess the effects of mycotoxins on broilers (Andretta et al., 2011) and growing pigs (Andretta et al., 2012). The meta-analysis for broilers encompassed 98 published articles, consisting of over 1,400 diets and 37,000 birds. The analysis showed that, on average, mycotoxins reduced broiler feed intake by 12% and weight gain by 14%, with ochratoxins (OT) and aflatoxins (AF) having the greatest effect on these parameters. Effects were greatest on younger animals and, unsurprisingly, the type of mycotoxin and its concentration influenced the magnitude of the effect. For growing pigs, the meta-analysis encompassed 85 published articles, consisting of over 1,000 diets and 13,000 pigs. The effects of mycotoxins appear to be greater on growing pigs, with an 18 and 21% reduction in feed intake and weight gain, respectively. Aflatoxins were still mycotoxins having a greater impact but this time alongside deoxynivalenol (DON). Effects were, again, greater on younger animals, with mycotoxin type and concentration obviously key factors determining the size of the effect. For growing pigs, there seems to be a gender effect, with mycotoxins affecting males more than females. Both meta-analyses identified that nutritional factors (e.g., methionine concentration for growing pigs) affected the magnitude of the mycotoxin effect. Whilst this needs consideration, permitting the utilisation of expensive dietary components to minimise the effects of mycotoxins is unlikely to be cost effective. With ruminants, there is a general lack of data relating to effects on performance. In 1979, Noller et al. reported that DON and zearalenone (ZEA) reduced bodyweight gain of lactating cows by up to 44%, while DON at 6 mg/kg reduced milk fat by over 1 per cent from 3.92 to 2.77 (Trenholm et al., 1985). In other work, ZEA reduced the conception rate of heifers by 25% (Weaver et al., 1986). Typically, acute mycotoxin intoxications that cause death are uncommon. What is more common are the (chronic) negative effects of mycotoxin contamination on animal performance and health.

3. Mycotoxins and the gastrointestinal tract

When considering mycotoxins, the focus is normally only on the post absorptive effects of mycotoxins, whether they manifest in chronic or acute signs. This is a major oversight of the impact mycotoxins have in the intestinal environment. The gastrointestinal tract (GIT) is the initial site for interaction of ingested mycotoxins with the animal. Mycotoxins have varying bio-availabilities (Grenier and Applegate, 2013). Some will be more rapidly absorbed, whilst others will get further along the GIT. This is very important for a number of reasons. Firstly, whether absorbed into the systemic circulation or not, the cells of the GIT will potentially be exposed to the full range of ingested mycotoxins and in the highest concentrations. Secondly, toxins that get further along the GIT will have had more opportunity to interact with the microbial cells present in the intestine. These cells can also be vulnerable to the effects of mycotoxins. Recent work by Alassane-Kpembi et al. (2013) studied the effects of the Type B trichothecenes on intestinal epithelial cell viability. They demonstrated that these mycotoxins have a negative effect on the viability of the intestinal cells. When discussing feed mycotoxins, low concentrations are often dismissed as being of very little significance. The work of Alassane-

Kpembi et al. would suggest otherwise, as the effects on cell viability, per unit of mycotoxin, were much greater at the lower concentrations than at higher ones. They also reported that in almost all cases, the effects of combinations of the toxins were either additive or indeed synergistic, reinforcing the fact that it's inappropriate to consider any single mycotoxin in isolation. Obviously, the importance of intestinal cell viability in maintaining the performance and health of the animal cannot be overstated.

With techniques available to study gene expression, it is possible to evaluate the activity of cells in more detail than whether they are only viable or non-viable. Very recent work by Taranu et al. (2015) studied the effects of low concentrations (10 µM) of ZEA on gene expression of porcine intestinal cells (IPEC-1). Due to its oestrogenic activity, ZEA is typically associated with reproductive problems and is reported to have low toxicity when ingested. Although low concentrations of ZEA did not affect cell viability, Taranu et al. reported that 1,954 genes had an altered profile compared with the control. Of these, 190 genes were significantly differentially expressed, of which 70% were up-regulated. Genes coding for glutathione peroxidase enzymes (GPx6, GPx2, GPx1) were among those up-regulated, which provides further evidence for mycotoxins inducing oxidative damage. The real-time reverse transcription polymerase chain reaction revealed increased expression of cytokines involved in inflammation (e.g., tumor necrosis factor alpha, interleukin-6, and interleukin-8) and immune cell recruitment (e.g., interleukin-10). The increased expression of these molecules would demonstrate that ZEA modulates intestinal cell immune and/or cellular repair pathways. Inflammation has an energy and nutrient cost and can compromise the integrity of the intestine.

The intestinal microbiota also plays a crucial role in determining the health and performance of the animal. An optimal microbiota prevents colonisation of the intestinal epithelium by pathogens and penetration of the gut barrier, modulates the gut-associated lymphoid tissue (GALT) and systemic immunity, and influences gastrointestinal development. The combined effects are better digestive efficiency and utilisation of nutrients. It goes without saying that microbial cells can be susceptible to mycotoxins. Recent work by Ouethrani et al. (2013) demonstrated that ochratoxin A (OTA) significantly reduced acetic, butyric and total short chain fatty acid (SCFA) concentrations in a dynamic simulation model of the descending human colon. This would indicate that OTA is able to affect the composition and/or metabolism of the colonic microbiota. Moreover, and in support of this, the work of Ouethrani et al. revealed that OTA eliminated a strain of *Lactobacillus reuteri* from the descending colon microbiota, which was permanent. *L. reuteri*, which produces the bacteriocin, reuterin, can be a key resident of the GIT, having been shown to have positive effects on intestinal disorders, infection and immune responses. There are limited other data documenting the effects of mycotoxins on (intestinal) bacteria. Tenk et al. (1982) reported that T-2 caused an increase in the aerobic intestinal bacteria count, while chronic exposure to low doses of DON in pigs also increased aerobic intestinal bacteria (Waché et al., 2009). Recently, ZEA and DON administered individually, or in combination, negatively affected mesophilic aerobic bacteria (Piotrowska et al., 2014). Clearly, mycotoxins can influence the composition and/or fermentation products of the intestinal microbiota and, in doing so, affect the health and performance of the animal.

4. Effects on the immune system

There are various very good reviews outlining the effects of mycotoxins on the immune system, which the reader is referred to (e.g., Girish and Smith, 2008). It is, therefore, not necessary to say

too much further on this subject other than to remind ourselves of some of the principle effects. There is clear evidence that mycotoxins affect the immune system. Many of their effects on the immune system would be perceived as negative. Deoxynivalenol is particularly well studied here. Numerous immune cells (e.g., macrophages, B and T lymphocytes and natural killer cells) are very sensitive to DON, while DON has been demonstrated to alter cytokine secretion, increase cell apoptosis and suppress the antibody response to vaccination (Maresca, 2013).

Intestinal epithelial cells are a critical component of the innate immune system. In essence, these cells form a key barrier between the 'outside world' (arriving in the intestine as/with ingested feed) and the main internal (systemic) environment of the animal. Studies have shown that mycotoxins increase the permeability of the intestinal epithelial layer in numerous species (e.g., swine and poultry), which can result in excessive/uncontrolled leakage of foreign material into the animal. As well as affecting intestinal cell viability, we also know that mycotoxins can reduce cell proliferation, thus reducing the intestine's ability to repair and replenish itself. Mycotoxins have also been reported to affect the expression of cytokines by intestinal epithelial cells. For example, the previously mentioned work of Taranu et al. (2015) demonstrated increased expression of proinflammatory cytokines by intestinal cells exposed to ZEA.

5. Mycotoxins and infections

Clearly, negative effects of mycotoxins on the intestine and immune system mean that they can play a critical role in the initiation, progression and duration of intestinal (and also systemic) infections. With regards to coccidiosis, mycotoxins have been shown to negatively affect the cell-mediated response of broilers to infection, recovery of the intestine after infection and reduce the effectiveness of the anticoccidial treatment, lasalocid. Mycotoxins promote *Salmonella* invasion of, and passage across, intestinal epithelial cells, believed to be through effects on both host susceptibility and bacterial metabolism/the expression of virulence factors. In colibacillosis, the negative effects of mycotoxins are thought to be mediated through promotion of intestinal *Escherichia coli* colonisation and translocation, and detrimental effects on the immune response. Moreover, the detrimental effects of mycotoxins (e.g., DON) on the intestinal epithelium is a predisposing factor for necrotic enteritis. The mycotoxin(s) damages intestinal function/barrier, leading to impaired nutrient digestion and absorption, or even nutrient leakage, which provides substrates for *Clostridium perfringens* proliferation. These aspects have been covered in a nice review by Antonissen et al., 2014.

6. Conclusion

The intestine is undoubtedly the key link between ingested mycotoxins and detrimental effects on the animal. This concise review has attempted to draw together key works to highlight the very important interaction between mycotoxins, the intestine and animal health. Much of the information presented in this review primarily relates to the effects of low to moderate levels of mycotoxins rather than the artificially high concentrations used in some studies. Effects on the intestine or intestinal environment have been seen with various mycotoxins. These effects are almost certainly occurring across species. Most, if not all, of the reported effects of mycotoxins are negative in terms of intestinal health (e.g., decreased cell viability, reductions in short chain fatty acid concentrations and elimination of beneficial bacteria, increased expression of genes involved in promoting inflammation and counteracting oxidative stress). This 'challenge' to

intestinal health will predispose the animal to intestinal infections and impair efficient digestion and absorption of nutrients. Compromising the integrity of the intestine will also increase the likelihood of microbes or microbial products (or even the mycotoxins themselves) escaping from within the intestine to cause more widespread, systemic disease. This is why, in combination with the negative effects of mycotoxins on the immune system, we should always be considering the wider issues mycotoxins can cause, such as impaired vaccine responses, increased use of antimicrobials. All of these factors together affect animal productivity and producer profitability, and should always be taken into account when assessing the cost effectiveness of counteracting mycotoxins.

We now know that mycotoxin contamination of feed raw materials or finished feeds is widespread, that they do not typically occur alone and even low concentrations can have particularly damaging effects. For many of the mycotoxins, we have limited experience or information relating to their effects and, in time, as we better understand the array of mycotoxins that exist, our focus may need to shift to the 'new' or 'emerging' ones. Moreover, given much of the available information suggests synergistic, or at least additive, effects when multiple mycotoxins are present, we are still a long way from fully understanding/interpreting the implications of this mix of mycotoxins for animal (or human) health and performance and existing mycotoxin guidelines/permitted levels will need to change. However, not fully understanding the mycotoxin scenario does not mean that we can ignore this challenge to animal production. Animals will ingest mycotoxins. It would, therefore, be advisable to seek to deal effectively with these mycotoxins in the intestine (to minimise systemic effects) and to do so in a timely manner to limit intestinal damage. There are products available that help to alleviate the mycotoxin challenge to the animal. Products with efficacy against a broader range of mycotoxins would be preferable as they will remove more mycotoxins from the toxicological 'challenge' to the animal, whether they are the typically tested, or emerging mycotoxins, with similar physical or chemical characteristics.

Fungi are proposed to be the greatest threat to animal and plant health among all the taxonomic classes of pathogens (Fisher et al. 2012). We may only be just beginning to appreciate the importance of fungi and their metabolites (e.g., mycotoxins) to animal (and human) health and performance.

Conflict of interest

There are no conflicts of interest to report.

References

- Alassane-Kpembi I, Kolf-Clauw M, Gauthier T, Abrami R, Abiola FA, Oswald IP, et al. New insights into mycotoxin mixtures: the toxicity of low doses of Type B trichothecenes on intestinal epithelial cells is synergistic. *Toxicol Appl Pharma* 2013;272:191–8.
- Andretta I, Kipper M, Lehnen CR, Hauschild L, Vale MM, Lovatto PA. Meta-analytical study on productive and nutritional interactions of mycotoxins in broilers. *Poult Sci* 2011;90:1934–40.
- Andretta I, Kipper M, Lehnen CR, Lovatto PA. Meta-analysis of the relationship of mycotoxins with biochemical and hematological parameters in broilers. *Poult Sci* 2012;91:376–82.
- Antonissen G, Martel A, Pasmans F, Ducatelle R, Verbrugghe E, Vandenbroucke V, et al. The impact of Fusarium mycotoxins on human and animal host susceptibility to infectious diseases. *Toxins* 2014;6:430–52.
- Cortinovis C, Pizzo F, Spicer LJ, Caloni F. Fusarium mycotoxins: effects on reproductive function in domestic animals – a review. *Theriogenology* 2013;80:557–64.
- Creppy EE. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett* 2002;127:19–28.
- Devegowda G, Murthy TNK. In: Diaz DE, editor. *Mycotoxins: their effects in poultry and some practical solutions*. Nottingham: Nottingham University Press; 2005. p. 25–56.

- Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, et al. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 2012;484:186–94.
- Girish CK, Smith T. Impact of feed-borne mycotoxins on avian cell-mediated and humoral immune responses. *World Mycotoxin J* 2008;1:105–21.
- Grenier B, Applegate TJ. Modulation of intestinal functions upon mycotoxin ingestion: meta-analysis of published experiments in animals. *Toxins* 2013;5:396–430.
- Maresca M. From the gut to the brain: journey and pathophysiological effects of the food associated trichothecene mycotoxin deoxynivalenol. *Toxins* 2013;5:784–820.
- Noller CH, Stob M, Tutie J. Effect of feeding *Gibberella zeae* infected corn on feed intake, body weight gain, and milk production of dairy cows. *J Dairy Sci* 1979;62:1003–6.
- Ouethrani M, Van De Wiele T, Verbeke E, Bruneau A, Carvalho M, Rabot S, et al. Metabolic fate of ochratoxin A as a coffee contaminant in a dynamic simulator of the human colon. *Food Chem* 2013;141:3291–300.
- Piotrowska M, Śliżewska K, Nowak A, Zielonka L, Żakowska Z, Gajęcka M, et al. The effect of experimental *Fusarium* mycotoxicosis on microbiota diversity in porcine ascending colon contents. *Toxins* 2014;6:2064–81.
- Streit E, Schwab C, Sulyok M, Naehrer K, Krska R, Schatzmayr G. Multi-mycotoxin screening reveals the occurrence of 139 different secondary metabolites in feed and feed ingredients. *Toxins* 2013;5:504–23.
- Taranu I, Braicu C, Marin DE, Pistol GC, Motiu M, Balacescu L, et al. Exposure to zearalenone mycotoxin alters in vitro porcine intestinal epithelial cells by differential gene expression. *Toxicol Lett* 2015;232:310–25.
- Tenk I, Fodor E, Szathmáry C. The effect of pure *Fusarium* toxins (T-2, F-2, DAS) on the microflora of the gut and on plasma glucocorticoid levels in rat and swine. *Zentralbl Bakteriol Mikrobiol Hyg A* 1982;252:384–93.
- Trenholm HL, Thompson BK, Hartin KE, Breenhalgh R, McAllister AJ. Ingestion of vomitoxin (deoxynivalenol)-contaminated wheat by nonlactation dairy cows. *J Dairy Sci* 1985;68:1000–5.
- Waché YJ, Valat C, Postollec G, Bougeard S, Burel C, Oswald IP, et al. Impact of deoxynivalenol on the intestinal microflora of pigs. *Int J Mol Sci* 2009;10:1–17.
- Weaver GA, Kurtz HJ, Behrens JC, Robison TS, Seguin BE, Bates FY, et al. Effect of zearalenone on the fertility of virgin dairy heifers. *Am J Vet Res* 1986;47:1395–7.