Physiological disturbance caused by mycotoxins

Low levels of mycotoxins have a significant negative metabolic and physiological impact on livestock animals. Therefore a multifunctional and preventive approach is crucial to maintain a high level of performance in today's livestock production.

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Mould can infect almost every agricultural commodity all over the world, during plant growth and/or after harvest. A great variety of these fungi can produce mycotoxins, which can accumulate in raw materials and feed. More than 100 countries nowadays have regulations for maximum levels of mycotoxins, but these regulations do not seem to assure complete safety.

Masked mycotoxins

Plants protect themselves from xenobiotic compounds like mycotoxins, by converting them into more polar metabolites. These metabolites are stored in plant vacuoles or conjugated to structures such as cell wall components. Typical examples of such called "masked mycotoxins" are Zen4G as derivative from Zearalenone, DON 3G and DON 4G as masked forms of Deoxynivalenol, Ochratoxin α as conjugate of Ochratoxin A and many more derivatives have been identified over the last few years. Unfortunately, these molecules escape from regular analytical techniques because there is a need for specific procedures for sample preparation. Also standards of the hidden toxins are commercially not yet available. Recent findings by "Laboratory of Food Analysis" of the University in Ghent indicated a concentration of masked DON varying from 30-98% of the DON level in corn. Very little is known about the availability of the masked mycotoxins in the animals' metabolic system. In 2011, Berthiller was able to demonstrate that 62% of the DON 3G in a diet is transformed back into DON by the microbial population in the large intestine. It would be unwise to ignore this additional and large range of toxins.

Oxidative stress and organ damage

Mycotoxins are incorporated in the cell membrane, affecting its poly-unsaturated fatty acids (PUFA) and leading to detrimental changes in its structure. It is not clear at present if mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or the increased tissue susceptibility to lipid peroxidation is a result of compromised antioxidant system. It seems likely that both processes are involved in this stimulation. In that respect, Abado-Becognee (1998) demonstrated a level of malonaldehyde (MDA), an indicator of tissue oxidation, being more than four times higher in kidney cells in the presence of Fumonisin B1. Considering that mycotoxins are among the stress factors that might create increased oxidation rates in tissues and cell structures, it has to be considered that important organs such as blood, liver, kidneys, etc. might get affected by the aggressiveness of these molecules. The functionality of the liver, being the largest solid and multitasking organ in the body, is challenged in high performing animal production. It can be easily understood that this threat might lead to metabolic disturbance and loss on performance.

Intestinal integrity

The intestinal tract is the first barrier against ingested antigens, including mycotoxins and pathogenic bacteria. Following ingestion of mycotoxin contaminated feed, enterocytes may be exposed to high concentrations of toxins. Tight junctions play a crucial role in the good functioning of the intestinal barrier and are key when it comes to intestinal integrity. Ana-Paula et al. observed an increased level of inflammatory cytokines (TNF-α and IL-1β) in

the ileum of pigs after ingestion of DON, Fumonisin or a combination of both. This increased level of cytokines could be linked to tight junction ileal barrier defects and decreased expression of tight junction proteins such as occludin and E-cadherin (*Figure 1*). The concentration of the mycotoxins was low and did not affect the technical performance of the animals but did show physiological impact on the intestine. This research confirmed earlier investigations by Pinton et al. (*Figure 2*) which observed in *vitro* a significant reduction of Trans Epithelial Electrical Resistance (TEER) in intestinal epithelial cell monolayers treated with different concentrations of DON. Finally this research concluded that the reduction in TEER is related to a reduced expression of tight junction proteins caused by DON exposure. Bouhet et al. (2003) investigated and concluded similar effects on TEER of epithelium cells when exposed to non-cytotoxic levels of Fumonisin B1. The above summary of data clearly indicates that current legal limits of mycotoxins do not exclude physiological and metabolic changes which might affect general health and performance.

Double trouble: Mycotoxins and pathogens

Vandenbroucke et al. observed the intestinal and systemic infection phase of *Salmonella Typhimurium* in pigs (*Figure 3*). Intestinal cell lines, pretreated with non-toxic levels of DON, showed an increased invasiveness of *S. Typhimurium* and an increased translocation through the cell layer. Within an intestinal loop model, it has been demonstrated that simultaneous exposure of the intestinal tract by non-cytotoxic concentrations of DON and *S. Typhimurium* resulted in an increased inflammation, which was not observed when exposed to only DON or *S. Typhimurium*. An in *vitro* approach as model for the systemic phase of the infection revealed, at low concentrations of DON, an enhanced uptake of *S. Typhimurium* by the macrophages. It is well known that Salmonella can shelter and multiply in macrophages while being spread throughout the body.

Antonissen et al. (2012) challenged broiler chickens with *Clostridium perfringens*, being fed a control diet or a diet contaminated with DON (< 5000 ppb). Chickens that received DON had significantly more lesions (46,6%) compared to the group challenged by *C. perfringens* without DON-exposure (19.5%). Within the current philosophy of disease prevention, mycotoxin control should become part of the basic strategy.

Better prepare than repair

Part of the answer is to build dietary strategies to enhance liver function (as well as kidney function) and to aid in reducing intestinal absorption of toxins and their related oxidative stress. Recent data from the Polish University of Warmińsko-Mazurski in Olsztyn (Prof Andrzej Gugołek) demonstrated the impact of Zearalenone (with or without treatment of Escent®) on the performance of rabbits. Feed, contaminated with 116 ppb of Zearalenone, was used as control diet. Escent was included in the same feed as treatment group. The trial started at day 35 of age and from day 39, body weight was significantly different between both groups ($p<0,05$), while from day 56 onwards significance was even more pronounced ($p<0,01$). At the end of the trial, 12% better growth with the Escent group. Dressing % was increased by 4.6% as well as liver colouration.

In Eastern Europe, a trial has recently been carried out where the feed was naturally contaminated with a low, but multiple levels of mycotoxin (Aflatoxin 3 ppb - Fumonisin <100 ppb - T2 28 ppb – DON 72 ppb and Zearalenone 55 ppb). Escent has been dosed at 1 kg/tonne. The treatment with Escent resulted in a live weight which was 6.2% higher, a FCR which was 5.2% better and a mortality which was 3.75% compared to 5.8% in the control diet.

From mycotoxins, it is know that they might cause sub-clinical and clinical signs of intoxication. Based on the safety assessments, legal limits have been set forward for different toxins. Today's research clearly indicates we understand only a tiny part of the complex mycotoxin puzzle. New challenges of low and legally allowed levels of mycotoxins do have a significant metabolic and physiological impact on the animals. Therefore a multifunctional and preventive approach is indispensable to maintain the high levels of performance in today's competitive animal rearing. AAF

Figure 1. The effect of DON, Fumonisin B1 and a combination of both on the expression of the tight junction proteins occludin and E-cadherin. (Source: Ana-Paula et al., 2011)

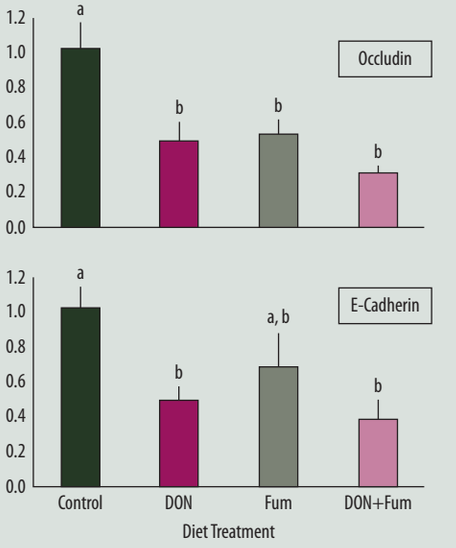

Figure 2. Non-cytotoxic doses of DON (on cellular level) decrease the TEER of porcine intestinal cells in a dose and time dependant manner. (Source: Pinton et al., 2009)

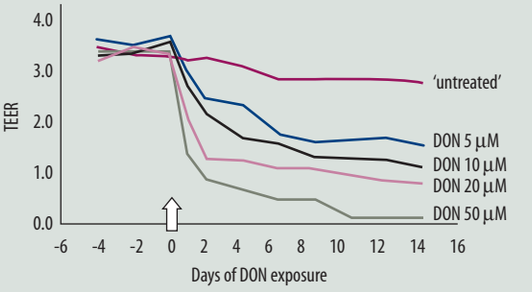

Figure 3. Impact of DON on transepithelial passage of Salmonella Typhimurium refers to a significantly higher translocation of the bacteria compared to the unexposed control cells ($p<0.05$). (Source: Vandenbroucke et al., 2011)