

Mycotoxins



How to combat multiple mycotoxins in feed

The last two years, we have encountered much larger amounts of aflatoxins in corn than usually found in Europe, due to drought and high temperatures occurring during the harvest of 2012.

Mycotoxins are commonly found in feed. However, the toxicity of combinations of mycotoxins cannot always be predicted. Using a wide adsorption spectrum of a toxin binding product is therefore key.

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Based on their common occurrence and known and suspected effects on human and animal health, aflatoxins (AF), fumonisins (FB), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEA) are recognized as the five most important agricultural mycotoxins. Recent surveys have demonstrated the regular occurrence of low levels of multiple mycotoxins in cereals. However, the toxicity of combinations of mycotoxins

cannot always be predicted based upon their individual toxicities. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive or synergistic. Data on the combined toxic effects of mycotoxins are limited and, therefore, the actual health risk from exposure to mycotoxins is unknown. Worldwide surveys on the occurrence and contamination levels of mycotoxins in raw materials also indicate that DON and FB are the most frequently detected mycotoxins, so it is of interest to determine their toxic effect when present simultaneously in feedstuffs.

Effects on intestinal wall integrity

The intestinal tract is the first barrier against ingested antigens, including mycotoxins, endotoxins and pathogenic bacteria. Following ingestion of mycotoxin-contaminated feed, enterocytes may be exposed to high concentrations of toxins. Both DON and FB have a direct effect on gut health and

integrity. DON damages the epithelial cells, decreasing the villi length and thus their surface, resulting in poor nutrient absorption and an increased risk of negative energetic balance. DON impairs the barrier function of the intestine by two mechanisms. It decreases the intestinal expression of claudin proteins and activates MAP-Kinases that regulate tight junction proteins on one side, and decreases trans-epithelial electrical resistance (TEER) on the other side. This results in an increased risk of trans-epithelial passage of both bacteria and endotoxins into the systemic system. DON also suppresses the normal immune response to pathogens and simultaneously induces autoimmune like effects and upregulates or downregulates critical functions associated with activated macrophages. It is interesting that experimental dysregulation of Immunoglobulin A (IgA) persisted up to four months after a discrete period of dietary exposure to DON.

Fumonisin is also very immunosuppressive and impair both specific (B and T lymphocytes) and nonspecific (T lymphocytes, macrophages) immune functions. They cause both stimulation and suppression of responses to foreign antigens, decrease total immunoglobulins, IgG and macrophages phagocytic activity and also decrease the intestinal expression of interleukin 8 (IL-8). As IL-8 is implicated in the recruitment of neutrophils during inflammatory response, the decreased IL-8 production could lead to an impaired recruitment of neutrophils and thus be associated with an increased susceptibility to enteric infection. Moreover, decreased epithelial cell replication has been reported in the presence of fumonisins. Consequently, the renewal of damaged intestinal cells does not work properly. This partly explains the synergistic action between FB and DON. In the presence of FB, the damage caused by DON on villi or intestinal integrity is not properly repaired, and lower levels of DON (and other trichothecenes) cause more severe troubles.

Transfer rate of aflatoxins

The joint effect of FB and DON on gut integrity increases the absorption of other mycotoxins and toxins. At the end of 2012 and throughout 2013, we have encountered much larger amounts of aflatoxins in corn than usually found in Europe. Problems first appeared in Italy and the Balkan countries with surroundings. The reasons were the drought and high temperatures occurring during the harvest of 2012. The amounts observed are not affecting the health of animals but are dangerous from the point of view of milk quality and public health. Indeed aflatoxin B1 (AFB1) passes into milk in the form of aflatoxin M1 (AFM1) which is a potent carcinogen. The problem has spread all around Europe and we now have to face a mycotoxin which was not familiar to us before. Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, fungi being more characteristic of tropical regions. In the USA and in most Latin American countries the maximum authorised level of AFM1 in milk is 0.5 ppb, while the European

Union limit is 0.05 ppb (or 50 ppt, which is how it is usually expressed when analysed). If we rely on the average transfer rate of AFB1 to AFM1 of 1.7% in milk, the limit of 0.5 ppb in milk would be reached when AFB1 level in feed exceeds 30 ppb of AFB1 expressed on dry matter.

However in Europe, the limit of 0.05 ppb in milk is expected to be reached when feed exceeds 3 ppb of AFB1 on dry matter, making it difficult to enforce legislation in regions where raw materials are naturally contaminated with AFB1, like Italy. This is what happened this year with corn from Eastern Europe and Italy.

Many authors believe that the metabolism rate of AFB1 into AFM1 ranks between 1 and 3%, with an average of 1.7%, but this rate can vary greatly among animals, from one day to another and between milkings, due to nutritional or physiological factors such as intake level, digestion rate, health status, milk production or individual sensitiveness to mycotoxins or other concomitant stresses. Transformation rates as high as 6.2% have been measured on high production cows during the peak of lactation.

Clay structures and polysaccharides

With such variability in metabolism rate and the low limit for AFM1 in milk in the EU, it is essential to use a good toxin binder to protect cows from absorbing these low quantities of AFB1. Aflatoxins are lipophilic and of low molecular weight, so they are assumed to be properly adsorbed by several aluminosilicates, especially of the bentonite-montmorillonite type. This type of materials shows adsorption rates of more than 90% when measured *in vitro*. However, it is acknowledged that *in vivo* adsorption is lower, and with low legislated level, any quantity of free AFB1 can be absorbed and metabolised to AFM1. In this situation, gut integrity is a key factor to warrant a good protection. Using a broad spectrum toxin binder, able to properly protect against the deleterious effects of DON and FB on the intestine will allow an efficient protection against AFB1. This effect can easily be demonstrated by analysing the AFM1 in milk. DON and FB are mycotoxins with

higher molecular weights and more difficult to adsorb by conventional detoxifying agents. Specific technologies can modify clay structure at the nano scale, increasing the interlayer space of the material and thus improving its adsorption capacity for larger molecules. This modification can be done by using natural agents such as algal polysaccharides. Ulvans, polyanionic polysaccharides present in green algae, are sulphated xylorhamno-glucuronans. They are formed by a succession of disaccharides composed of an uronic acid and a sulphated rhamnose. They interact with montmorillonite via silanol groups on the edges of the layers and compensation cations in the interlayer space of montmorillonite. The presence of ulvans in the interlayer space of the montmorillonite increases the accessible adsorptive surface and the number and types of adsorption sites, resulting in a matrix similar to the structure of activated charcoal. The adsorption of mycotoxins in this innovative material is a complex mechanism involving CEC (cation exchange capacity) and surface area of montmorillonite, the polyanionic structure of ulvans and the "microtubular" structure formed in the interlayer space, allowing ionic and hydrophobic interactions with mycotoxins. When testing this new material using the TIM-1 system in TNO, results were even better than those obtained with activated charcoal for big mycotoxins such as DON and FB. In addition, the use of this product did not impair the bio-accessibility of nutrients.

Conclusion

Changing weather conditions and increasing international trading lead to different contamination situations, with different effects on animal health and quality of animal products, to which we need to adapt from one year to another. Protection is key, and becoming a more important factor in order to obtain profitable performance and good quality products from our animals. In this sense, the wider the adsorption spectrum of the toxin binding products used, the more protected we are and the less worried we need to be toward changing, unfamiliar situations. Improve protection, improve performance. **AAF**