Synergistic effect of mycotoxin contaminated feed

by Katia Pedrosa, Product Manager, Biomin Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria.

The contamination of raw materials and animal feeds with mycotoxin producing moulds responsible for a diversity of toxins such as aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids is a very frequent problem of the feed industry.

Often, individual levels of mycotoxins found in the commodities cannot explain the symptoms found in the field. One of the reasons which may explain this, is the co-occurrence of more than one mycotoxin in the finished feed.

There are several combinations of mycotoxins that frequently occur and their interaction may result in synergistic, additive and antagonistic effects which are up to now not given the right importance.

Diagnosis of mycotoxicoses in animals is difficult not only due to their occurrence at toxin concentrations below the detection limit but also due to the similarity to diseases originating from different etiologic agents. The information on combined toxic effects of mycotoxins is generally limited, particularly with respect to trichothecenes.

A theoretical consideration on the basis of cellular modes of action of mycotoxins is a starting point to screen certain combinations for their possible synergistic or additive effects.

Synergistic effects

Under commercial conditions, livestock are exposed to a complex mixture of mycotoxins derived mainly from Fusarium, Aspergillus, Penicilium, Alternaria and Claviceps spp. Poor livestock performance and/or disease symptoms, reported in commercial operations, may be due to synergistic interactions between multiple mycotoxins (Fig. 1).

Synergistic effects occur when the combined effects of two mycotoxins are much greater than the individual effects of each toxin alone. If the effect is additive then it might be possible to predict the outcome in terms of productivity.

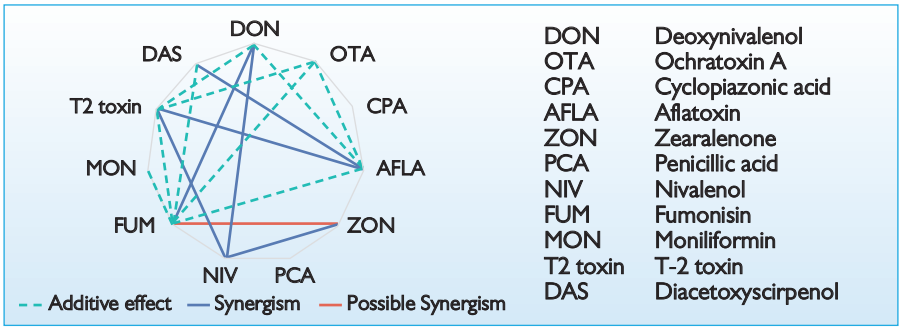

Fig. 1. Interactions between different mycotoxins (Cast report, 2003, Tajima et al. 2002).

Recent evidence indicates that most interactions involving Fusarium mycotoxins are ranging from additive to synergistic interactions varying from mortality to reduced feed intake and growth.

Regarding trichothecenes, additive effects seem to be the case for most combinations. There are some reports indicating synergistic effects of deoxynivalenol (DON) and fusaric acid; DON and fumonisin B1 (FB1); and diacetoxyscirpenol (DAS) and aflatoxins (Afla).

Cellular interference

According to Riley and Norred (1996) mycotoxins exhibit a diversity of biochemical and cellular mechanisms of toxicity; therefore it is thought that the best approach is trying to understand how they can interfere at cellular level and thus interact with the toxicity of other mycotoxins.

Riley (1998) considered that the study of the mechanism of action of toxic compounds is founded in the belief that cells are molecular machines. To understand how mycotoxins interfere with the cellular machinery, it is only necessary to understand how toxic compounds alter the behaviour of the molecules of life (Table. 1).

The toxicokinetic behaviour, metabolism and the toxicodynamic aspects influence the final outcome when human or experimental animals are exposed to a mixture of some mycotoxins.

Sows and gilts are highly susceptible to mycotoxins, which can greatly affect their health and productivity. Mycotoxins are commonly present in feed for farm animals and thus their consumption causes a variety of symptoms, depending on the type of mycotoxin, quantity, duration of exposure, as well as the health status and condition of the animal at the time of exposure.

In pigs, great concern is focused on the synergistic interactions between DON and fusaric acid (FA), DON and FUM, Afla and OTA and Afla in combination with T-2.

Additional adverse effects were identified by the combinations between T-2 toxin and OTA; DON and MON; T-2 toxin and FUM; FUM and moniliformin (MON); DAS and FUM, Afla and OTA, Afla and T-2 toxin.

With regard to the reproductive failure in pigs, ZON is the major responsible toxin, followed by ergot alkaloids and trichothecenes represented by T-2, which is aggravated by their combination.

Several studies have been carried out with combinations of mycotoxins and the amplification of toxicity in animals has been confirmed. For instance, a study was carried out in piglets with concentrations ranging from 10-40ppm of fumonisin B1 and 20-39ppm of OTA. Sudden death of piglets aged between 13 and 18 weeks was observed after several days of contamination. Piglets presented pathological signs of both toxins such as pulmonary oedema, kidney and liver lesions.

According to NOEL (No Observable Effect Limit) values (0.2-1.9µg/kg body weight per day of fumonisin B1 and 8-41µg/kg body weight of OTA), the mentioned concentrations should not show lethal effects. It was therefore supposed that this could be a result of a combination between OTA and fumonisin B1 that synergise each other.

Continued on page 9

Continued from page 7

Results of another experiment performed in weaned piglets with a combined administration of FUM, DON, T-2 and OTA in quantities normally present in feeds showed suppression of radical formation and antibody formation only after the combination between OTA and fumonisin B1 or DON, which did not occur when OTA was administered alone.

The mixture of Afla with cyclopiazonic acid was also studied in guinea pigs. Normally these two mycotoxins appear together in nature.

Synergistic interaction was seen on lethality, weight gain and histopathologic changes in the liver.

This study confirmed immune suppression of animals caused by mycotoxins and also that a mixture of mycotoxins can profoundly affect the animal. Additionally, a trial was conducted to determine the effect of feeding diets contaminated with DON and fusaric acid (FA) in swine.

Contamination with DON ranged from values between 0 to 7.5µg/g and for FA the values ranged from 12.2µg/g to 57.1µg/g.

In all diets containing DON, fast depressions (after one week) of growth and feed intake were verified.

With lower concentrations of DON and FA there was a significant reduction in feed intake and the reduction of the weight gains happened only after three weeks.

Mycotoxin	Initial lesion → Cascade of events
Aflatoxin	Metabolic activation → DNA modification → cell deregulation → cell death/transformation
Citrinin	Loss of selective membrane permeability → cell disruption → cell death (apoptosis)
Deoxynivalenol	Inhibition of protein synthesis → disruption of cytokine regulation → altered cell proliferation → cell death/apoptosis
Fumonisins	Sphinganine N-acety → disrupted lipid metabolism → cell deregulation → cell death/apoptosis
Moniliformin	Pyruvate and –ketoglutarate decarboxylase → loss of respiration control → cell death
Ochratoxin	Disruption of phenylalanine metabolism → reduced PEPCK → reduced glyconeogenesis → cell death (metabolic activation → inhibition of protein/DNA synthesis → apoptosis) (Altered membrane permeability → disruption calcium transport → cell deregulation → cell death)
Patulin	Nonprotein sulfhydryl depletion → altered ion permeability and/or altered communication → oxidative stress → cell death (inhibition of macromolecular biosynthesis → cell death)
T-2 toxin	Inhibition of protein synthesis → ? → cell death (apoptosis) (transient Ca^{2}+ elevation → endonuclease activation → apoptosis – cell death)
Zearalenone	Cytosolic oestrogen receptor → oestrogenic response → disruption of hormonal control → ?

Table 1. Probable primary biochemical lesions and the early cellular events in the cascade of cellular events leading to toxic cell injury or cellular deregulation of some selected mycotoxins (Adapted from Riley, 1998).

With increasing levels of FA combined with relatively constant amounts of DON caused a significant depression in weight gain after one week. Therefore, a toxicological synergism between DON and FA was confirmed. As pigs are particularly susceptible to FUM, a trial was carried out with diets contaminated with Afla and fumonisin B1.

The diets were fed singly and in combination to growing cross-bred barrows. Final body weight and body weight gain were significantly reduced in barrows that were fed Afla alone and Afla in combination with fumonisin B1 when compared to the control and fumonisin B1 diet groups.

This study indicated that giving a diet contaminated with the combination of both mycotoxins (Afla and fumonisin B1) to growing barrows, a more toxic response, than that seen in response to either toxin singly, is induced.

Another study was carried out to evaluate the effects of various concentrations of fumonisin B1 and Afla on swine alveolar macrophages. Results showed that incubation of alveolar macrophages between concentrations ranging 1.5-5µg/ml of fumonisin B1 led to a significant reduction in the number of viable cells when compared to the control levels.

Fumonisin B1, but not Afla, induced the apoptosis of swine alveolar macrophages with evidence of DNA laddering and nuclear fragmentation.

However, both fumonisin B1 and Afla exposure induced the expression of apoptosis-related heat shock protein 72 in alveolar macrophages. Swine alveolar macrophages treated with 50ng/ml of fumonisin B1 and 100ng/ml of Afla led to a reduction in phagocytic ability to approximately 55 and 36% of the control levels, respectively.

Moreover, incubation of alveolar macrophages with fumonisin B1 (2 and 10µg/ml) dramatically decreased the mRNA levels of interleukin-1 and tumour necrosis factor. It was therefore concluded that both fumonisin B1 and Afla are immunomodulatory agents to swine alveolar macrophages although they exert their effects via different biochemical mechanisms and that respiratory tract exposure to mycotoxins suppresses not only pulmonary but also systemic host defence systems.

Conclusion

Very often mycotoxicoses occur at very low toxin concentration levels. One reason for that may be the synergistic and additive effects between the mycotoxins that occur together, enhancing their toxicity. The presence of more than one mycotoxin in a high number of collected samples must raise the attention for these non-expected effects in animals.

Additional studies on the synergism among mycotoxins should predict possible in vivo effects of such multi-contaminations of mycotoxins and provide a better understanding of the importance of mycotoxins to the animal industry.

Preventing animals from getting contaminated and exposed to harmful effects of mycotoxin intoxication may often be the only option. Therefore, mycotoxin risk management strategies need to be taken into account as an option to find a solution for the current problem found in the field. ■