

Evaluating mycotoxin binders with ducklings

Contrary to common belief, aflatoxins are still present in many feed components and by-products worldwide. A number of companies claim that their products efficiently minimise this animal and public health risk by mycotoxin binding. A new tool using ducklings as testing animals allows evaluation of mycotoxin binders in 'real-life' conditions. This highly reproducible model allows the comparison of toxin binders for their efficacy in 'real life' conditions, highlighting wide differences of performances in this model.



Ducklings are one of the most sensitive species to aflatoxin contamination.

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In the USA, mycotoxigenic fungi in barley silage and hay from beef feedlots, as a source of mycotoxins, have just been proved to be a significant component in the aetiology of the Jejunal Haemorrhage Syndrome in beef cattle. Barley kernel samples collected in Spanish grain stores from 2008 to 2010 were found to contain aflatoxin contamination (aflatoxin B1, AfB1) in up to 12% of the samples. Also in Spain, AfM1 has been identified in the Manchego cheese supply chain. The process led to an average 4-fold increase of the AfM1 median concentration in the final curd, in relation to the corresponding tank milk. Another example was in brewer's grain

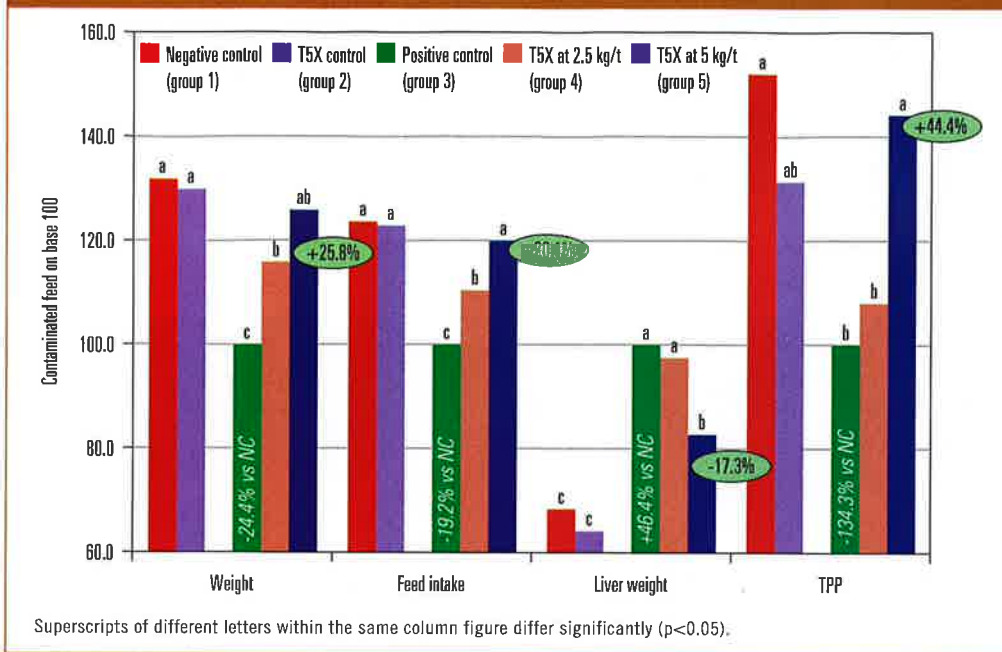
samples used as a by-product in the feed of swine in Argentina, where over 50% of the samples were naturally contaminated with AfB1; samples collected at day 7 of storage showed AfB1 levels exceeding the maximum allowed for pig feedstuffs.

It should be remembered that aflatoxins have been found in airborne dust from feed factories, and that naturally occurring aflatoxins (as a group) were evaluated as carcinogenic to humans by the International Agency for the Research on Cancer. Furthermore, it is possible that climate change might have an effect on the content and the geographical presence of AfB1 in cereals.

Ducklings: an aflatoxin 'sentinel' model

An impressive number of publications have presented approaches to minimise mycotoxin contamination of feed, and nearly as many products are on the market with a claim of toxin-binding capacities. However, only a limited number of methods are available to compare these strategies in a real-life situation. Such a method, that uses live ducklings, has recently been developed and validated. It now provides results on the efficacy of aflatoxin binding products in 10 days. For this method Pekin ducklings were chosen because they are considered the most sensitive avian species to mycotoxins, even

Figure 1 - Evaluation of the efficacy of T5X as an aflatoxin binder in a broiler chick model (5 replicates) exposed to a 2,800 ppb aflatoxin spiked diet for 21 days.



though aflatoxins are proven to also be highly toxic to broilers and turkeys. A preliminary validation study has been performed, during which day-old ducklings received either a non-contaminated control feed, or the same feed spiked with known concentrations of AFB1 (50, 125, 250 and 500 ppb) for 21 days. Each cage (24 birds) received the defined diet and two replicates were performed per diet (240 ducklings in total). The birds were observed daily and weighed weekly. After 21 days, the birds were sampled and necropsied.

Plasma protein as biomarker

A wide range of blood parameters were chosen, as potential biomarkers: the plasma parameters (total blood proteins, albumin and cholesterol concentrations) were found to be significantly correlated to the level of in-feed aflatoxin. Total plasma proteins presented less individual variation within a batch, and were retained as a reliable biomarker. This was verified in another validation study where ducklings were exposed, for 10 days, to a control diet, a diet containing 100 ppb of AFB1 or the latter diet supplemented with either 5 or 7.5 kilogramme/tonne of an aflatoxin binder.

Again, the blood parameters proved to be significantly impaired in the tainted diet group, as compared to the negative control group. Furthermore, the impact of aflatoxin on these parameters was alleviated in the groups of ducklings exposed to the diet contaminated and supplemented by the toxin binder. This allowed the calculation of the level of protection for each dose of binding product and for each parameter.

In a nutshell, these trials proved the duckling model is validated for a 100 ppb AFB1 diet exposure over a 10-day period, an exposure level compatible with both the field situation and ethical grounds.

A validated screening tool

The model was then used to screen different components for their toxin-binding capacities, in order to generate new products with validated efficacy in 'real-life' conditions. Over time, more than 50 products with mycotoxin binding claims or potential ones have been challenged in this model, with highly variable results, allowing comparison of the level of protection they provide (after 1 to 19 tests). The compilation of these trials shows that some

products provide extremely poor protection, below 10%, in this challenge model. On the other hand, Neovia's T5X consistently provided the highest level of protection of all the compared products, with an average level of 87.6% over 20 tests and a minimal protection of 75%. Hence, the original 'in vivo duckling model', based on the reproducible evaluation of biochemical parameters, involves a limited number of animals and enables a rapid, repeatable and economic evaluation of the real efficacy of mycotoxin binders. It is a powerful tool that has led to the development of the aflatoxin binder T5X.

Independent broiler trial

A recent statement from the European Food Safety Authority (EFSA) on the establishment of guidelines for the assessment of additives intended as 'substances for reduction of the contamination of feed by mycotoxins' specifies that 'efficacy shall be demonstrated in in vivo studies (normally short-term) and should be performed in the relevant target species for which the additive is intended'. This did not come as a surprise, although the statement does not stress whether such trials should be performed by a third party. Neovia chose Lamic* in Brazil to trial the in vivo efficacy of T5X in broilers. That trial was performed in 2010, in an experimental setting of the university, in five replicates.

Feed intake, weight and feed conversion rate (FCR) were measured on a weekly basis. Total plasma protein, which is a reliable biomarker of aflatoxin exposure, was measured on 12 birds per group at the end of the trial (day 21), and birds were then necropsied; the relative weight of the liver was calculated (g/100 g of liveweight).

Highly significant impact of aflatoxins

FCR was not significantly modified among groups over the 3-week period of the study. However, the impact of aflatoxins on the physiology of the birds was significant on all other

measured parameters: the birds in the positive control had both a decreased liveweight at 21 days (-24.4%) and a decreased feed consumption over the same period (-19.2%) as compared to the negative control group. As a consequence of the toxicity of the aflatoxins, the relative weight of the liver of the birds increased by 46.4% in 21 days, and the total plasma protein concentration decreased by 34.3%. Hence, as in the duckling model, the chick model confirms that the measurement of total plasma proteins is a reliable indicator of in-feed exposure to mycotoxins.

Dose-dependent effect

The incorporation of T5X in the basal diet did not cause any significant modification in any of the measured parameters. When incorporated in the contaminated diet at either 2.5 or 5 kilogramme per tonne (kg/t), T5X had a significant and dose-dependent effect, its protective effect being higher at the 5 kg/t concentration (*Figure 1*). At that concentration, the impact of the high mycotoxin challenge is minimised as compared to the positive control: -25.8% for the final liveweight, +20.1% for the feed intake without any impact on the FCR, -17.3% on the relative liver weight and +44.4% on the total plasma protein concentration. The conclusion of this trial was that T5X "meets the minimum approval requirements" for an aflatoxin-binding additive in Brazil.

Effect on nutrients

The EFSA guidelines for the assessment of additives claiming to reduce the contamination of feed by mycotoxins warns that 'there is the possibility that the availability of crucial nutrients could also be affected' by this binding capacity. 'Consideration should thus be given to the extent to which the supply of nutrients, micronutrients and other additives to the animals could be reduced'. It recommends 'that apparent digestibility of crude protein, zinc, retinyl or tocopheryl esters, thiamine or pyridoxine and a coccidiostat, when the additive is intended to be used in poultry/rabbits, are measured. Such studies should be performed with the highest recommended dose of the additive'. In compliance with these guidelines, Neovia has had such trials performed with the products in the T5X range, in a well-defined *in vivo* model with roosters in cages at the InVivo NSA Research department.

No significant difference was observed for any of the four components: crude protein, vitamins A and B1 and narasin were not affected by the addition of any of the three T5X products at their highest recommended dosage. Combined with the previous results on aflatoxin binding efficiency, assessed in *in vivo* models, this trial confirms the safety of the in-feed use of the T5X range at their highest dosage to efficiently protect livestock from the effect of mycotoxins. **AAF**

*Lamic, the Laboratory of Mycotoxicological Analysis of the Federal University of Santa Maria, Brazil is one of the world's largest independent mycotoxin laboratories.

Danièle Marzin presented the benefits of the 'duckling model' in evaluating mycotoxin-binding products to over 200 attendees, nutritionists, distributors and feed manufacturers, at the Mycotoxins Forum held in March 2011 in Bangkok, Thailand.

