Original in vivo model to test efficacy of aflatoxin B1 binders

by Danièle Marzin and Gaëlle Benzoni, Neovia, PO Box 394, 56009 Vannes cedex, France.

Aflatoxins are fungal secondary metabolites produced by certain Aspergillus species, among which aflatoxin B1 is the most harmful, as well as the most potent of the naturally occurring mycotoxins.

Aflatoxins are highly toxic to humans and animals, like broilers and turkeys. They cause a variety of effects, including poor performance, liver pathology, immunosuppression and changes in relative organ weights.

Several approaches have been investigated to reduce exposure of animals to aflatoxins in contaminated feeds. The addition of sequestering or binding agents to aflatoxin contaminated feedstuffs is one of the most used worldwide.

The effectiveness of a potential sequestering agent should be measured in vivo following in vitro evaluation. In fact, in vitro studies aiming at checking the binding capacity of mycotoxins are very useful for a first screening of potential candidates, provided they are made taking the digestive tract environment into account (pH variation for example).

However, it is difficult to assume that a product with good efficacy in vitro will for sure perform when fed to intoxicated animals.

The objective of the study is to develop the more ethical, rapid, not costly and reproducible in vivo method, which allows potential toxin binders to be efficiently screened.

Diet	Number of observations	Total protein (g/l)	Protection (%)	Cholesterol (g/l)	Protection (%)	Albumin (g/l)	Protection (%)
Negative control (NC)	8	33.3 (0.930[a]	100	1.6 (0.23)[a]	100	16.5 (0.76)[a]	100
Positive control (PC)	20	26.3 (2.32)[c]	0	1.3 (0.21)[b]	0	12.6 (1.57)[c]	0
PI 0.5%	19	30.3 (2.17)[b]	59.7	1.5 (0.28)[a]	66.7	15.2 (1.62)[b]	66.7
PI 0.75%	19	30.4 (2.31)[b]	61.2	1.5 (0.26)[a]	66.7	14.9 (1.31)[b]	59.0

Table 1. Plasmatic parameters.

Material and methods

Ducklings are considered the most sensitive species to mycotoxins among poultry, thus ducklings were selected as the potential animals to be used for the model.

In a preliminary study, a basal diet (standard duckling feed formula in mash form) was manufactured from aflatoxin-free raw materials.

This basal diet remained as a negative control diet. Pure synthetic aflatoxin B1 (Sigma Chemical Co., St. Louis, MO, USA) was added to the basal diet to manufacture the different contaminated diets, to achieve theoretical contamination levels of 50, 125, 250 and 500ppb.

Some 240 day-old male Pekin ducklings were housed in 10 wired cages (two replicates of 24 birds for each diet) and fed from day 1 to day 21. Ducks were observed twice a day and the mortality was recorded. Birds were individually weighed at days 7, 14 and 21. Feed intake per cage was also recorded. At day 21 all the animals were slaughtered.

Blood parameters were analysed (cholesterol, protein plasmatic rate, albumin) as intoxication biomarkers as they change very rapidly due to the reduction of the liver function, particularly inhibition of synthesis of protein and impairment of lipidic metabolism by the aflatoxins.

The slaughtered animals were dissected. Hearts and livers were individually collected and weighed.

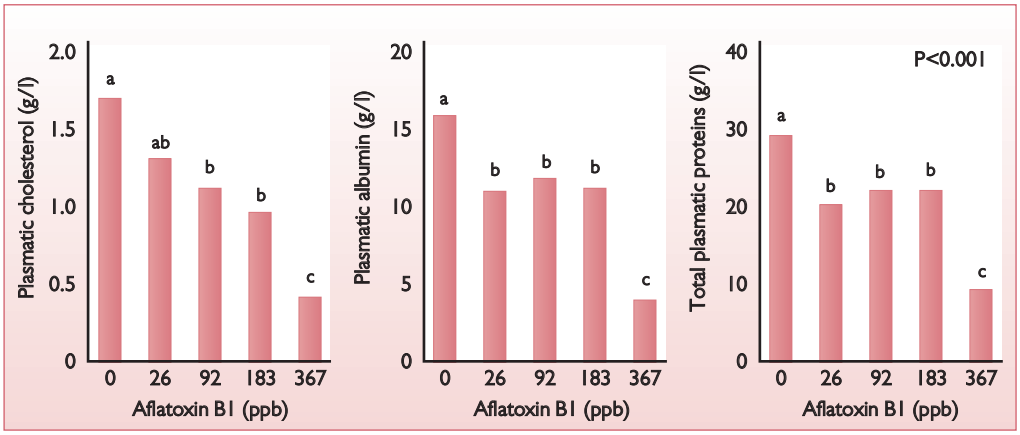
Weights were expressed as a percentage of body weight, thus obtaining the relative weight of the organs.

The protocol of the preliminary experiment was replicated in a second experiment to confirm the preliminary observation, and to evaluate the ability of the model to evaluate the protective effect of toxin binders.

For the second experiment, the same protocol was conducted but the trial duration was of 10 days instead of 21 days, in order to evaluate the possibility of shortening the trial. Moreover, four diets were compared: negative control without aflatoxin, positive control with 100ppb of aflatoxin B1, two groups receiving 100ppb of aflatoxin B1 and two doses of recognised aflatoxin binder from the market.

Once validated, the model aimed at screening different potential binders (clays, grounds, yeasts extracts) from different part of the world to select the most effective ones. 15 tests including up to 20 diets have been run on the model. More than 50 products have been tested. The results are presented as a compilation of these trials.

Fig. 1. Evolution of blood parameters according to the level of aflatoxin B1.



Results

1 **Preliminary experiment:**
The aflatoxin B1 diet content was analysed (Lareal, France) and revealed 26, 92, 183 and 367ppb of aflatoxin B1 respectively .

A significant ($p<0.05$) decrease in body weights and feed intake was observed at all ages at 367ppb of aflatoxin (highest dose). Relative weights increased significantly ($p<0.001$) for the heart at day 14 and day 21 at 92ppb, the spleen at day 7, day 14 and day 21 at the highest dose, the proventriculus at day

Continued on page 43

Continued from page 41

14 and day 21 at the highest dose and the gizzard at day 21 at the highest dose. Liver relative weight was not significantly affected. At day 21, total plasmatic protein and albumin were significantly lowered for every aflatoxin concentration. Total plasmatic cholesterol was significantly lowered from a level of 92ppb (Fig. 1). Analysis of the data according to the level of aflatoxin B1 ingested allowed the different parameters studied to be classified according to their sensitivity: total blood protein = albumin > spleen/heart relative weight > blood cholesterol > liveweight = feed intake > proventriculus relative weight > gizzard relative weight.

It was thus concluded that the plasmatic parameters (total blood proteins, albumin and cholesterol) were valuable indicators to study the possible prevention of the toxic effects of aflatoxin by toxin binders.

As protein plasmatic rates proved to have lower individual variation in between ducks, and as they were positively correlated to other plasmatic parameters (TP/albumin: r=0.98 and TP/cholesterol: r=0.9) it was retained as the intoxication biomarker for further studies.

The contamination level of 92 (100) ppb of aflatoxin was also validated as the reference for the positive control (contaminated). Even though this contamination could be considered low, compared to levels tested in some in vivo trials and to levels sometimes found in naturally contaminated raw materials, this dose was chosen as the best compromise between ethic, economics and sensitivity of the protocol.

A 10 day exposure was enough to observe the aflatoxin effect. As a consequence, it was decided to reduce the trial duration from 21 to 10 days for the next experiment.

1Second experiment:

The incorporation of 100ppb of aflatoxin B1 causes significant modifications in the biochemical plasmatic profile (Table 1), which were counteracted by the addition of 5 or 7.5mg of reference toxin-binder (based on clay, named P1) per g of contaminated diet.

The model was thus validated on ducklings during a 10 day period with 100ppb AFB1 in the diets, enabling significant differences on the three blood parameters to be tested.

The effect of the toxin binder is clearly seen on the different blood parameters.

Protective action is calculated on protein plasmatic rate (difference of total protein between NC and PC/ difference of total protein between test and PC).

Diet	No. of observations	Total protein (g / l)	Significance (p< 0.001***)	Protection (%)
Neg. control	30	30.17	a	100
Pos. control	30	18.97	c	0
T5X	30	29.57	a	95
1	30	26.6	ab	68
2	30	23.33	bc	39
3	28	22	cd	27
4	27	26.89	ab	71
5	30	26.47	ab	67
6	28	23.64	bc	42

Table 2. Plasmatic parameters.

Compilation of 15 studies

Some 15 tests including up to 20 diets have been run on the model. More than 50 products have been tested (Fig. 2).

One specific product was identified as the most potent one. Trials proved that it brought a repeatable and high protection against aflatoxin B1 contamination. 20 tests have shown that the protection was, on average, 87.6%, ranging from 75 to 100% (in five tests the protection was 100%). This product has led to the creation of a commercial product (T5X).

Final trials compared the selected product with different commercial and well known products on the market (Table 2).

The detrimental effect of AFB1 is clearly seen with the significant reduction of total protein plasmatic rate. The addition of binders affect the protein rate, allowing negative control levesl for some efficient products as T5X to come back.

Significant differences in efficacy can be shown among the products.

Conclusion

In vitro trials run in parallel to in vivo trials have shown the possible desorption phenomenon between tests at pH 3 and 7. Differences in ranking for efficacy have been noticed between in vitro and in vivo trials.

Some performing products in vitro failed in our in vivo model, confirming the need to validate the toxin binders in vivo.

The 'ducklings in vivo model' based on modification of physiological parameters requires low levels of toxins (realistic doses), low numbers of animals and short time of exposure. It is sensitive and reproducible and is a good compromise between ethical concerns and experimental needs. It enables potent or inefficient aflatoxin binders to be rapidly and economically differentiated. ■

References are available from the author on request

Fig. 2. Protection against aflatoxin B1. Compilation of 15 tests in the 'in vivo ducklings model'.