

**Pilot Study**

Effects of low dietary aflatoxin B1 on broiler liver concentration without and with Mycosorb® toxin binder

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**Summary**

A pilot study was conducted to evaluate the suitability of hepatic aflatoxin (AFB1) concentration as a biomarker to assess the in vivo efficacy of a mycotoxin binder in poultry when AFB1 dietary concentrations are low. Diets containing low doses of AFB1 without or with Mycosorb® (MTB), a mycotoxin binder, were fed to broilers from 7 to 21 days of age. The accumulation of AFB1 in liver was measured by high performance liquid chromatography with a detection limit of 5 ng/kg liver. In response to 10 and 50 µg AFB1/kg feed, hepatic AFB1 accumulation was 27 and 145 ng AFB1/kg liver, respectively. At each dietary concentration of AFB1, the inclusion of 5 g MTB/kg of feed reduced (P<0.1 for 10 µg AFB1/kg feed and P<0.05 for 50 µg AFB1/kg feed) hepatic AFB1 accumulation by at least 50%. These results suggest that hepatic AFB1 concentration is a suitable biomarker for evaluating mycotoxin binder efficacy in poultry fed the EU maximum dietary concentration of 10 µg of AFB1/kg feed.

**Keywords:** Aflatoxin B1: mycotoxin: broiler: liver: Mycosorb®

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**Introduction**

Mycotoxin sequestering agents, also called mycotoxin adsorbents or binders, can be used to inhibit the uptake of mycotoxins by the gut. The European Food Safety Authority (EFSA) has published guidelines for demonstrating the efficacy of mycotoxin inactivators (EFSA, 2010). The upper limit for aflatoxin B1 (AFB1) concentration in feed in the European Union is 10 µg/kg for young poultry (e.g. broiler chicks) and 20 µg/kg for older broilers. Most published studies on AFB1 in broiler chicks have been conducted using much higher toxin concentrations (0.07–10 mg/kg) as reviewed by Yunus et al. (2011). Magnoli et al. (2011) investigated the effect of 50 µg AFB1/kg feed, and found no significant effects on broiler productivity, biochemical parameters or relative liver weights. There are no published broiler studies demonstrating the effects of mycotoxin binders at AFB1 dietary concentrations as low as 10 µg/kg. Hence, a pilot study was conducted to evaluate the suitability of hepatic AFB1 concentration as a biomarker to assess the efficacy of mycotoxin binders in poultry when AFB1 dietary concentrations are low.

**Materials and methods**

Sixty-five, one-day-old male broiler (Ross 308) chicks, sourced from a commercial hatchery, were housed in 0.5 × 1 m pens with wood shavings litter. Birds had access to feed and water ad libitum throughout the experiment. Water was provided via nipple drinkers and feed
was provided in automatic feeders that were attached to the pens and gradually raised as the birds grew. The trial was conducted according to the EFSA guidance for demonstration of short-term efficacy of additives (EFSA, 2011). Pure AFB1 (Sigma-Aldrich, Saint Louis, MO) was used to prepare the toxin-contaminated dietary treatments. For uniform mixing of the toxin, AFB1 was first dissolved in acetonitrile (5 mL of acetonitrile/10 µg of AFB1) and then mixed into baking-grade wheat flour (25 g of flour per 10 µg of AFB1). The solvent was evaporated overnight, and the toxin-flour premix was carefully and gradually mixed with the wheat-soy basal diet. The mycotoxin binder Mycosorb® (MTB; Alltech Inc, Nicholasville KY, USA) was likewise first mixed into a premix with wheat flour before mixing into the wheat-soy basal diet. Experimental procedures were authorised by the National Animal Experiment Board of Finland.

During the first seven days, all the chicks were fed a wheat-soy diet containing no AFB1 or MTB. On day seven, 60 chicks were randomly allocated into five treatment groups with three replicate pens each, with four birds per pen (169.8 ± 0.5 g/bird). Between 7 and 21 days, each pen was allocated to one of five treatments: T1: Control diet (wheat-soy with no AFB1 or MTB); T2: Control + AFB1 10 µg/kg feed; T3: Control + AFB1 10 µg/kg feed + MTB 5 g/kg feed; T4: Control + AFB1 50 µg/kg feed or T5: Control + AFB1 50 µg/kg feed + MTB 5 g/kg feed. A sample of the control diet was quantitatively analysed for AFB1, ochratoxin A, and citrinin (BioCheck Laboratory, Leipzig, Germany). The concentration of each of these mycotoxins was below its corresponding detection limit of 1.7, 5.0, and 15 µg/kg feed, respectively. On day 21, all birds were euthanized by cervical dislocation. Livers were collected, placed in plastic bags, frozen with dry ice, and stored at −20 °C until analysis.

Liver samples were prepared for analysis of AFB1 according to the procedures of Tavcar-Kalcher et al. (2007) with minor modifications to the sample preparation. Ground liver sample (25 g) was mixed with 2.5 ml of 20% aqueous solution of citric acid and 5 g diatomaceous earth before extraction with dichloromethane. The extract was filtered and evaporated to dryness under nitrogen gas, then dissolved into a mixture of 30% acetonitrile, 20% methanol, and 50% water. The samples were applied to individual Vicam AflaTest WB SR immunoaffinity columns (Vicam, Milford, MA) and eluted with a mixture of 75% methanol and 25% water. High performance liquid chromatography (Waters Corp, Milford, MA) separation was performed in a Phenomenex Luna C18(2) 3 µm, 150 × 4.60 mm column and Gemini C18 4 × 3 mm SecurityGuard pre-column. After electrochemical post-column bromination in a Kobra cell (r-Biopharm, Darmstadt, Germany), fluorescence detection was conducted at an excitation wavelength of 362 nm and an emission wavelength of 425 nm. This method allowed quantification of AFB1 from the 25 mg liver samples within the linear range of 10–200 ng/kg. AFB1 concentrations between 5 and 10 ng/kg were detectable, but below the limit of quantification.

Data were analysed by Student’s t-test. Samples with no detected AFB1 were assigned a value of 2.5 ng/kg (half the AFB1 detection limit of 5 ng/kg). Samples with detected but not quantifiable AFB1 (between 5 and 10 ng/kg) were assigned the value as determined by analysis.

Results

In eleven of the twelve broilers fed the control diet, the concentration of AFB1 in liver was below the detection limit. The remaining control bird had a concentration of AFB1 in liver of 12 ng/kg. Feed analysis indicated that the control diet did not contain detectable concentrations of aflatoxin (<1.7 µg AFB1/kg feed), and that the AFB1 amendment to treatments two through five was the major source of AFB1 in the livers of birds at 21 days of age.

Broilers fed 10 or 50 µg AFB1/kg feed without MTB had average hepatic AFB1 concentrations of 27 or 145 ng/kg, respectively (Figure 1). Thus, a five-fold increase in AFB1 concentration in feed increased the hepatic AFB1 average concentration by a factor of 5.4. The hepatic AFB1 concentration in response to 50 µg AFB1/kg feed was the same order of magnitude as those reported by other investigators who studied similar concentrations of AFB1 in poultry feed (Bintvihok et al., 2006; Magnoli et al., 2011).

The MTB binder tested in the present trial was efficacious in preventing the absorption of AFB1 in broilers, as evidenced by at least a 50% reduction in AFB1 concentration in liver samples. Dietary addition of 5 g MTB/kg feed reduced the hepatic AFB1 concentration by 54.2% in birds fed 10 µg AFB1/kg feed (P < 0.10) and by 52.5% in birds fed 50 µg AFB1/kg feed (P < 0.05) (Figure 1). The effect of MTB in feed containing 10 µg AFB1/kg would most likely become statistically significant if a larger number of birds per replicate were used. Moreover, the sensitivity of the analysis could be increased and the linear range of quantification extended.
to 5 ng AFB1/kg feed if the liver sample size were increased to 50 g as described by Magnoli et al. (2011). Increasing liver sample size would necessitate either pooling the livers of two or three young birds or individually sampling the heavier livers of older broilers.

Conclusions

Hepatic concentrations of AFB1 in broilers increased in proportion to the dose (10 and 50 µg of AFB1/kg feed). Dietary MTB at 5 g/kg feed reduced hepatic AFB1 concentrations by at least 50% compared with AFB1-contaminated diet containing no binder, demonstrating its efficacy even at low toxin contamination levels. These results suggested that the efficacy of the mycotoxin binder MTB can be assessed by measuring the reduction in aflatoxin B1 accumulation in the liver of broilers fed the EU maximum dietary concentration of 10 µg of AFB1/kg feed.

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Declarations of Interest

C. Moran is an employee of Alltech France Ltd and A. Yiannikouris is an employee of Alltech Inc, USA.

References


