

The Adsorption of Veterinary Drugs to Mycotoxin Binders in a Feed-containing Buffered Matrix

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Introduction and Aims

Mycotoxin binders are feed additives which are mixed in the feed to adsorb mycotoxins and thereby reducing their toxic effects on animals. Interactions with orally administered veterinary medicinal products, such as antimicrobials, or other feed additives, such as coccidiostats, have been reported in animal studies.

In vitro models to assess the safety with respect to binding potential of medicinal products are very scarce and they are usually derived from models to assess efficacy (Devreese et al., 2013; De Mil et al., 2015). Furthermore, these models do not include feed, an element which is always present in the field situation and which is a major influencing factor of the bioavailability of a drug (Marasanapalle et al., 2011). Drugs usually have a lower bioavailability when administered with feed as the drug can undergo non-specific binding with feed compounds.

To the authors knowledge, no static models (with feed) are available to assess interactions between mycotoxin binders and veterinary drugs. Therefore, The aim of this study was to **develop a static adsorption model to assess the binding of veterinary drugs**, with two frequently used antimicrobials namely doxycycline (DOX) and tylosin (TYL) as model compounds, to **various mycotoxin binders in a buffered matrix with relevant pH ranges and containing feed**.

Materials and Methods

Experimental Design

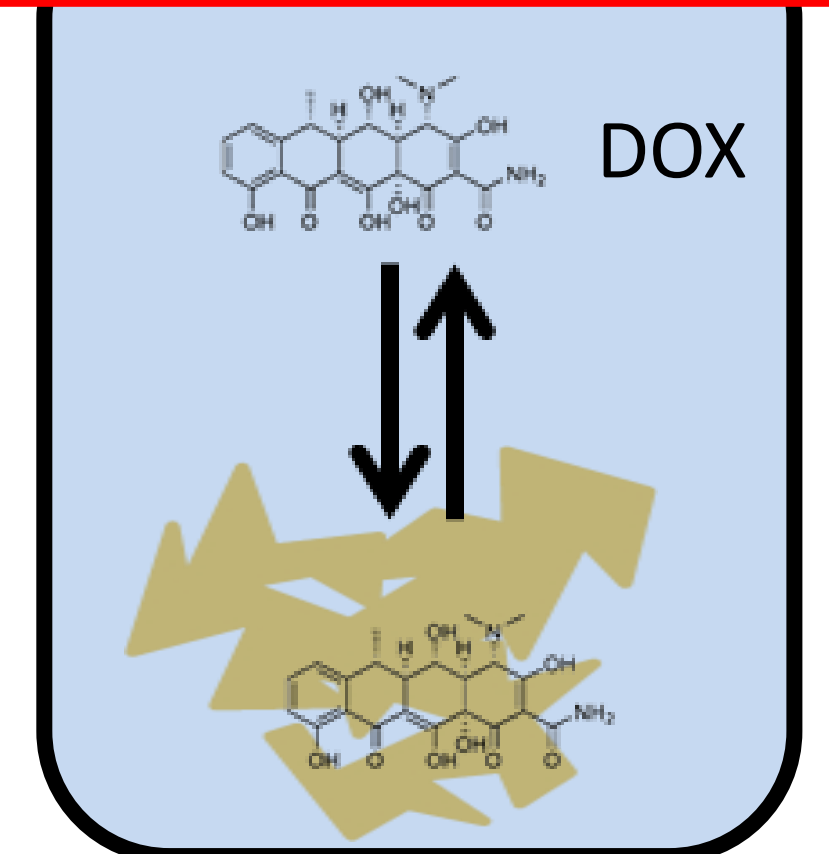
- Ten gram feed, supplemented with one of four mycotoxin binders (clay 1,2,3 and yeast 1) up to 0, 1, 2.5, 5, 10, 25, 50 and 100 g/kg feed
- Nineteen mL of PBS and 1 mL of 5 mg DOX/mL or 3 mg TYL/mL were added
- Three different acidity levels of PBS were used, pH 2.5 and 6.5 (DOX) and 6.5 and 8 (TYL), corresponding to pH levels of the gastro-intestinal tract in chickens
- The tubes containing feed, buffer and TYL or DOX were shaken on a horizontal roller mixer (150 rpm, 4 h, 37°C)
- Samples were centrifugated and 250 µL of the supernatant was transferred to an Eppendorf cup
- Experiments were conducted in triplicate per inclusion rate, per pH, per mycotoxin binder and per antimicrobial

Quantification of DOX and TYL by LC-MS/MS

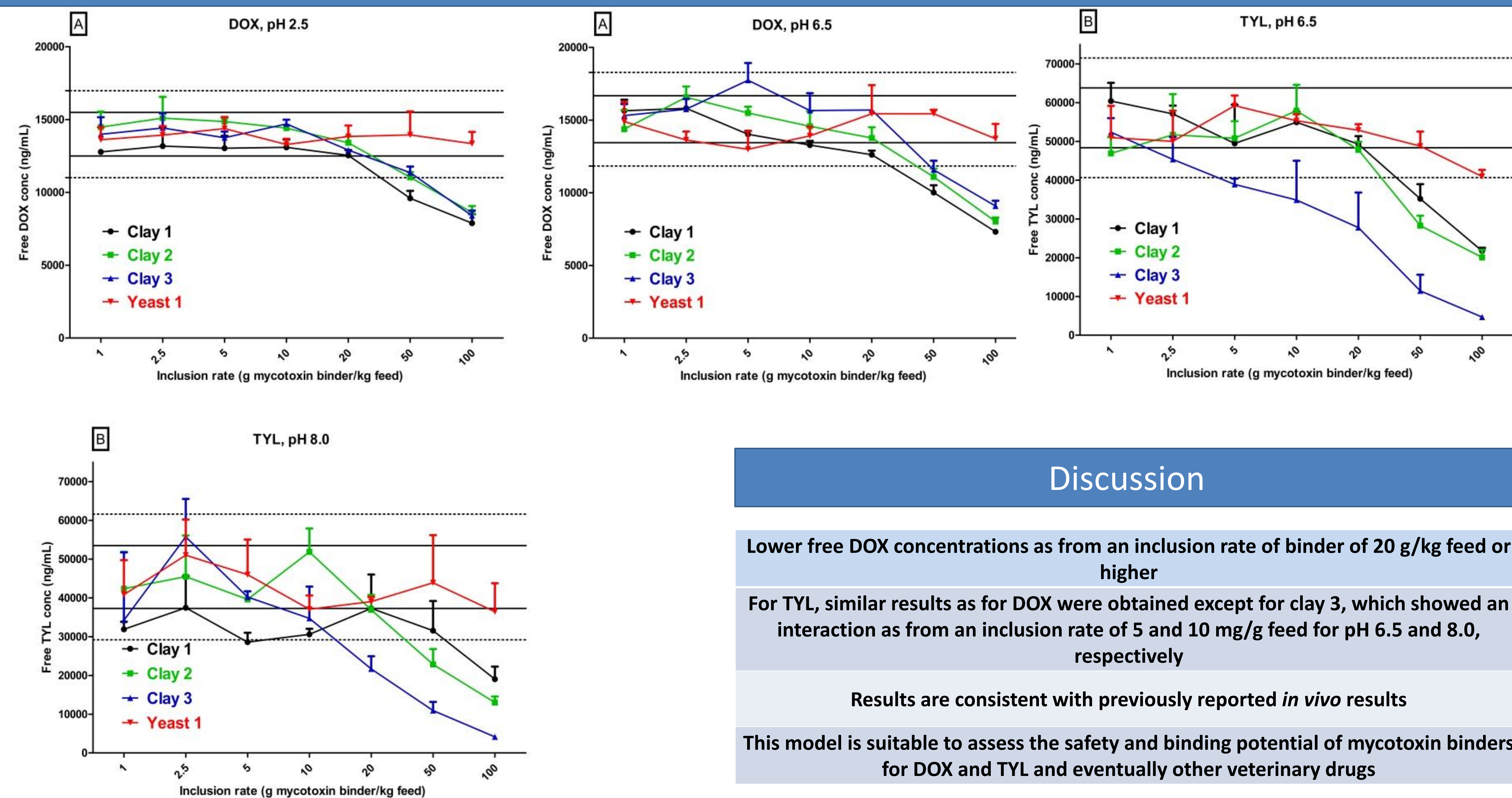
Quantification of DOX was performed as described by De Mil et al. (2015). Sample preparation consisted of addition of the internal standard (demethylchlortetracycline), 50 µL of methanol, 25 µL of trifluoroacetic acid followed by vortex mixing and centrifugation, For quantification of TYL, mobile phases consisted of acetonitrile + 0.01% formic acid and water + 0.01% formic acid. For TYL and the internal standard (valnemulin), following reactions were monitored for quantification: m/z 915.8>174.2 and m/z 564.2>263.1, respectively. Sample preparation consisted of addition of the internal standard and 950 µL acetonitrile followed by vortex mixing and centrifugation. Next, 25 µL of the supernatant was supplemented with 275 µL of an aqueous 0.01 M ammonium acetate buffer

Statistical Analysis

Each inclusion rate was analysed in triplicate per mycotoxin binder; the results were compared to the respective control (no binder) using one-way analysis of variance (ANOVA) followed by a post hoc, Bonferroni-corrected, LSD-test. Significance levels (p) below 0.05 were considered significant.



Results



Discussion

Lower free DOX concentrations as from an inclusion rate of binder of 20 g/kg feed or higher

For TYL, similar results as for DOX were obtained except for clay 3, which showed an interaction as from an inclusion rate of 5 and 10 mg/g feed for pH 6.5 and 8.0, respectively

Results are consistent with previously reported *in vivo* results

This model is suitable to assess the safety and binding potential of mycotoxin binders for DOX and TYL and eventually other veterinary drugs

Further information and References

De Mil et al., 2016 – *J Feed Sci Technol* - submitted

Figure 1. Free doxycycline (DOX) (A) and tylosin (TYL) (B) concentrations for different inclusion rates of the respective mycotoxin binder at two different pH's (presented as mean + SD). The samples (n = 3 per inclusion rate per mycotoxin binder) were incubated for 4 h in 20 mL of phosphate buffered saline solution containing 10 g of finely grinded feed supplemented with mycotoxin binder at different inclusion rates. The horizontal lines represent the average free concentration ± 1 SD (full line) or ± 2 SD (dotted line) of DOX or TYL in the control samples without mycotoxin binder (n = 12).