IN VITRO MYCOTOXIN BINDING STUDIES WITH A PROPRIETARY ADSORBENT

Final Report to Milwhite, Inc.

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Introduction

Mycotoxins have become an important issue for the grain industry, animal producers, grain companies, overseas buyers, and state and federal inspection agencies. With the advent of onsite testing using ELISA test kits for the various mycotoxins, contaminated grains can be readily identified and at low levels of contamination can be used in livestock and poultry feed. Grains with higher levels of contamination and many screenings from grain operations are unsafe for animal consumption and must be destroyed or alternate uses identified. If effective adsorbent clays can be identified that successfully prevent mycotoxicosis, these highly contaminated grains and screenings can be safely and economically utilized in the livestock and poultry industry.

The major advantages of adsorbents include expense, safety, and they can be easily added to animal feeds. However, not all adsorbents are equally effective in protecting livestock against the toxic effects of mycotoxins. In addition, several adsorbents have been shown to impair nutrient utilization. Recently, it has been noted that many of the adsorbents on the market today have not been adequately tested for *in vivo* efficacy, but are marketed solely on *in vitro* data. However, *in vitro* tests may not always be a reliable indicator of an adsorbents ability to bind a mycotoxin. Therefore, it is important that adsorbents be subjected to *in vivo* evaluation both with respect to efficacy and to determine if impaired nutrient utilization from diets occurs.

Experimental plan

Proprietary sorbent –a proprietary adsorbent was obtained from Milwhite, Inc on 5/5/2017. It was labeled: Zeolita Natural (San Luis Potosi) DOM 04/27/2017

Mycotoxin –aflatoxin B_1 and fumonisin B_1 were purchased from Sigma Chemical Co. A primary stock solutions (1,000 ppm) of aflatoxin B_1 and fumnisin B_1 were prepared in acetonitrile. Mycotoxin concentrations are based on the relative ease of analysis by HPLC and cost of mycotoxin rather than levels known to cause problems in livestock.

Initial binding study – The adsorbent was tested for its ability to bind aflatoxin B_1 and fumonisin B_1 at pH 3 and pH 7 using the following general procedure. Duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3 or pH 7, 10 mL) containing 2 ppm aflatoxin B_1 or 2 ppm fumonisin B_1 was added to 15 mL screw cap Falcon polypropylene tubes to which had been added 1 mg of aflatoxin B_1 or 25 mg of fumonisin B_1. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.1 M phosphate buffer plus 1 mg or 25 mg ofadsorbent to test tubes. Tubes were placed on a rotator shaker for 30 minutes at room temperature. Each mycotoxin test solution and control was centrifuged at 36,000 rpm for 5 minutes and 2 mL of the aqueous supernatant removed for analysis. An aliquot of the original buffered test solution was used as the HPLC standard.

Analysis - HPLC analyses were performed on a Hitachi L-7100 pump with a Hitachi L-7200 autosampler, fluorescence detection with a Hitachi L-7480 fluorescence spectrophotometer. Data were recorded and processed by a Hitachi D-7000 data acquisition package with ConcertChrom software on a microcomputer. Percent mycotoxin bound was calculated from the difference between the initial and final concentration in the aqueous buffered supernatant.

Results

The proprietary adsorbent was tested for *in vitro* binding of aflatoxin B_1 and fumonisin B_1 at pH 3 and pH 7. The percentage of the mycotoxin bound to the adsorbent is presented in Tables 1 and 2.

TABLE 1. ***In vitro*** **binding of aflatoxin B_1 by a proprietary adsorbent at pH 3 and pH 7.**

Sample #	**%Adsorption* Aflatoxin B1, pH 3**	**%Adsorption* Aflatoxin B1, pH 7**
Zeolita Natural	10%	3%

*average of duplicate analyses;

TABLE 2. ***In vitro*** **binding of fumonisin B_1 by aproprietary adsorbent at pH 3 and pH 7.**

Sample #	**%Adsorption* fumonisin B_1, pH 3**	**%Adsorption* fumonisin B1, pH 7**
Zeolita Natural	33%	< 1%

*average of duplicate analyses;

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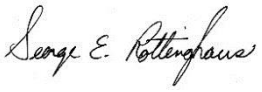
Please use case number 17-09207 when referring to billing of these tests. These preliminary results should be used only as an indicator of which adsorbents should be examined further in animal feeding trials. If we can be of further assistance, please contact me at 573-884-9240.

Signature: 

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