

**Mycotoxin adsorption by Blue Pacific
and Zeotec zeolites**

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**A confidential report prepared for
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Executive Summary

Adsorption of Mycotoxins by Blue Pacific and Zeotec zeolites

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This study was performed to obtain information on the adsorption profile of Blue Pacific and Zeotec zeolites for several mycotoxins. Blue Pacific and Zeotec are New Zealand natural zeolites produced by Resource Refineries Ltd. They are sold for addition at up to 2% to livestock and poultry rations and for application to bedding or floor litter. When added to animal feeds zeolites are claimed to bind toxins, ammonia and other gases, and to aid pelleting and feed flowability and to improve mineral utilisation. Similar aluminosilicate products from Latin America and Asia are sold for inclusion into animal feeds for adsorption of mycotoxins. Mycotoxins are toxic fungal metabolites that can occur in grain, nuts and other animal feed sources throughout the world.

A brochure prepared by BOMAC Laboratories Ltd reports adsorption studies on the mycotoxin ochratoxin A by a New Zealand natural aluminosilicate, Zeosorb. The product was reported to adsorb ochratoxin A to a greater extent than two commercial adsorbants (MycoFix and Antitox) and similar to a third (Toxiban) at longer exposure times. Ochratoxin is generally reported to be mid-range with respect to adsorption on aluminosilicates, with mycotoxins such as aflatoxin highly adsorbed, and others such as zearalenone poorly adsorbed. This study was designed to provide information on the adsorptive activity of Blue Pacific and Zeotec zeolites on the mycotoxins nivalenol (NIV) and deoxynivalenol (DON) and the aflatoxins B1, B2, G1 and G2.

There were three aspects to the study. The first involved an *in vitro* adsorption assay measuring the affect of Blue Pacific added at either 1% or 2.5% to an artificial gastric juice containing either 0.2 µg/ml or 1 µg/ml of each of NIV and DON. In the second part of the study Blue Pacific was added to an artificial gastric juice contained either 50 ng/ml or 100 ng/ml each of aflatoxin B1, B2, G1 and G2. The third aspect of the study involved comparison of two other zeolite adsorbants. Zeotec (from Resource Refineries Ltd) and Toxiban, with Blue Pacific. This comparison was at the lower addition rate (1%) and lower toxin rates (0.2 µg/ml for NIV and DON and 50 ng/ml for each aflatoxin).

The results of the assay showed that NIV and DON were not adsorbed by any of the three adsorbants in the study. On the other hand, addition of just 1% of Blue Pacific, Zeotec or Toxiban removed all free aflatoxin B1 and G1 in solution within 15 minutes. In the case of Blue Pacific, this was achieved at both toxin addition rates. With aflatoxins B2 and G2, only Toxiban removed them totally, with Blue Pacific giving 98% adsorption at 1% addition (at 15 minutes) and Zeotec giving 75-89% adsorption. The higher (2.5%) addition rate of Blue Pacific gave close to 100% adsorption.

This study has shown that Blue Pacific zeolite will adsorb aflatoxins in *in vitro* experiments to a level that is comparable with existing commercial products. The label claim for toxin adsorption is therefore reasonable. Further testing with a range of other mycotoxins, and including sorption-desorption testing, would be required to determine the robustness of the claim. The results suggest that Blue Pacific may have a beneficial effect on animal performance when selective mycotoxin-contaminated materials are used, but animal feeding studies would be needed to confirm this. The alternative material, Zeotec, was less effective than Blue Pacific.

Introduction

Mycotoxins are toxic fungal metabolites. They can occur widely in nature but are a particular concern in cereal grains and in food and animal feeds derived from them. One method of reducing the effect of mycotoxins in animal feeds has been the addition of aluminosilicate adsorbants which can bind up the toxins. Various mycotoxins bind to aluminosilicates to differing degrees. For example, the carcinogen aflatoxin B1 is generally reported to be highly adsorbed, ochratoxin A to be mid-range, with others such as zearalenone poorly adsorbed. Similarly, different aluminosilicates show differing adsorbant capabilities. Adsorption of ochratoxin A by a New Zealand natural zeolite, Zeosorb, was reported in a brochure prepared by BOMAC Laboratories Ltd.

This study was performed to evaluate the mycotoxin adsorbant ability of Blue Pacific and Zeotec zeolites, two materials made from New Zealand natural zeolites by Resource Refineries Ltd. The toxins used for the evaluation were aflatoxins B1, B2, G1 and G2, and nivalenol (NIV) and deoxynivalenol (DON), all mycotoxins that occur throughout the world.

Method

The tests were performed using an artificial gastric juice of 0.2% pepsin in 0.075N hydrochloric acid held at 37°C. Additions of mycotoxins and adsorbant were made on the basis that feed consumed and gastric juice would be in an approximately 1:1 ratio.

Blue Pacific was evaluated at 2 rates of addition and at two concentrations of toxins. The NIV and DON concentrations tested in the artificial juice were 0.2 and 1.0 µg/ml (equivalent to 0.2 and 1.0 mg toxin/kg feed respectively). The aflatoxins B1, B2, G1 and G2 concentrations tested were 50 and 100 ng/ml of each individual toxin (equivalent to 50 and 100 ng of each toxin/kg feed respectively). Blue Pacific was added to the solutions at 1% and 2.5% (equivalent to inclusion in the feed at 1% and 2.5%). Appropriate tests with zero addition of either toxin or zeolite were also included.

The test solutions were held at 37°C, and subsampled to measure the free toxin concentration at 0 min (before adding adsorbant) and at 15 min and 120 min after adding the adsorbant. All tests were performed in duplicate on solutions that contained toxins.

Comparative evaluation of Zeotec (produced by Resource Refineries Ltd) and Toxiban (a competitors product) were performed with 0.2 µg/ml NIV and DON and 50ng/ml each of aflatoxins B1, B2, G1 and G2 and at 1% addition of adsorbant.

All test solutions were analysed, using HPLC with UV detection for NIV and DON confirmed at two wavelengths and fluorescence detection for aflatoxins.

Results and Discussion

Results for duplicates of all measurements were averaged and these values presented in Tables 1 to 6.

In all tests it was observed that addition of the mycotoxins to the acidic pepsin solution resulted in an apparent loss of 20-30% of NIV and DON and 30-65% of individual aflatoxins, even without adding zeolite. Aflatoxin B1 showed the greatest losses (see Tables 1-6).

These concentration drops were thought to be due to the presence of the pepsin rather than the acid. The reason for this is unknown but is possibly due to pepsin acting as an adsorbant. The magnitude of the effect did not change over the 120 min of the test period for NIV and DON or for aflatoxins B2 and G2, therefore the adsorption of the aluminosilicate materials could be studied independently. In the case of aflatoxins B1 and G1 the apparent solution concentration continued to drop over 120 minutes even in the absence of zeolites, but the concentrations were still high enough to allow the added zeolite effect to be measured.

Results for addition of the 3 adsorbants to NIV and DON solutions are shown in Tables 1 and 2. There is no evidence of any adsorption of NIV and DON from Blue Pacific, Zeotec or Toxiban.

Results for aflatoxin adsorption is shown in Tables 3-6. Addition of 1% Blue Pacific, Zeotec and Toxiban removed all free aflatoxin B1 and G1 in solution within 15 minutes. Blue Pacific, the only adsorbant tested with the highest addition rate of aflatoxin B1, B2, G1 and G2 (100 ng/ml of each toxin) removed all aflatoxin B1 and G1 at even the 1% addition rate. The aflatoxins B2 and G2 were removed totally by 1% addition of Toxiban, but Blue Pacific (ca. 98% absorption at 1% addition) and Zeotec (75-89% adsorption) were less effective. Longer exposure time gave a greater reduction in concentrations of aflatoxin B2 and G2, but it should be noted that adsorption by the three adsorbants was close to maximum efficacy within 15 minutes.

The 1% inclusion rate used to compare the three test zeolite materials is within the recommended feed inclusion rate for some products but above that recommended for some other products (e.g. Mycofix recommends 0.15-0.5 %).

Conclusion

Blue Pacific, a product made from natural New Zealand zeolites, has been shown to adsorb aflatoxins B1, B2, G1 and G2 from an acid solution of pepsin. The majority of the free aflatoxin was adsorbed within 15 min of addition of 1% Blue Pacific. Toxiban adsorbed all aflatoxins present while Zeotec, another natural New Zealand zeolite material, adsorbed less than the Blue Pacific. None of the three products tested adsorbed NIV or DON.

The test has shown that a claim that Blue Pacific is suitable for toxin adsorption is reasonable. However, tests would be needed to establish the adsorptive capability of Blue Pacific relative to other products for a wider range of toxins, and also to establish the strength of binding by conducting sorption/desorption tests. Animal studies would be needed to determine whether the addition of Blue Pacific has a practical impact on the nutritive and performance value of feed diets.

Table 1: Mean^a measured solution^b concentrations of nivalenol (NIV) with and without addition of zeolite.

Sample	Adsorbant	NIV concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
plus 0.2 µg/ml Nivalenol	No adsorbant	0.14	0.15	0.15
	1% Blue Pacific	0.15	0.15	0.14
	2.5% Blue Pacific	0.15	0.16	0.17
	1% Zeotec	-	0.16	0.16
	1% Toxiban	-	0.18	0.16
	No adsorbant	0.69	0.67	0.79
plus 1.0 µg/ml Nivalenol	1% Blue Pacific	0.74	0.76	0.81
	2.5% Blue Pacific	0.70	0.73	0.78

^a Results for solutions containing mycotoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Table 2: Mean^a measured solution^b concentrations of deoxynivalenol (DON) with and without addition of zeolite.

Sample	Absorbant	DON concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
plus 0.2 µg/ml Deoxynivalenol	No adsorbant	0.15	0.15	0.14
	1% Blue Pacific	0.15	0.13	0.15
	2.5% Blue Pacific	0.16	0.16	0.17
	1% Zeotec	-	0.16	0.16
	1% Toxiban	-	0.16	0.15
	No adsorbant	0.75	0.69	0.77
plus 1.0 µg/ml Deoxynivalenol	1% Blue Pacific	0.72	0.75	0.79
	2.5% Blue Pacific	0.72	0.74	0.77

^a Results for solutions containing mycotoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Table 3: Mean^a measured solution^b concentrations of aflatoxin B1 with and without addition of zeolite.

Sample	Adsorbant	Aflatoxin B1 concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
	No adsorbant	17.98	14.57	10.10
plus 50 ng/ml Aflatoxin B1	1% Blue Pacific	17.85	0	0
	2.5% Blue Pacific	17.7	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
	No adsorbant	37.94	31.05	22.26
plus 100 ng/ml Aflatoxin B1	1% Blue Pacific	37.25	0	0
	2.5% Blue Pacific	36.74	0	0

^a Results for solutions containing aflatoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Table 4: Mean^a measured solution^b concentrations of aflatoxin B2 with and without addition of zeolite.

Sample	Absorbant	Aflatoxin B2 concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
	No adsorbant	25.59	23.29	23.5
plus 50 ng/ml Aflatoxin B2	1% Blue Pacific	25.41	0.14	0.07
	2.5% Blue Pacific	25.58	0	0
	1% Zeotec	-	2.67	1.20
	1% Toxiban	-	0	0
	No adsorbant	55.68	52.53	52.7
plus 100 ng/ml Aflatoxin B2	1% Blue Pacific	55.76	0.43	0.20
	2.5% Blue Pacific	56.35	0.02	0.02

^a Results for solutions containing aflatoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Table 5: Mean^a measured solution^b concentrations of aflatoxin G1 with and without addition of zeolite.

Sample	Absorbant	Aflatoxin G1 concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution ^b	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
plus 50 ng/ml Aflatoxin G1	No adsorbant	23.8	19.65	10.10
	1% Blue Pacific	24.4	0	0
	2.5% Blue Pacific	23.5	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
	No adsorbant	48.3	43.00	30.82
plus 100 ng/ml Aflatoxin G1	1% Blue Pacific	47.8	0	0
	2.5% Blue Pacific	48.1	0	0

^a Results for solutions containing aflatoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Table 6: Mean^a measured solution^b concentrations of aflatoxin G2 with and without addition of zeolite.

Sample	Absorbant	Aflatoxin G2 concentration (µg/ml) at times		
		0 min	15 min	120 min
pepsin solution	1% Blue Pacific	0	0	0
	2.5% Blue Pacific	0	0	0
	1% Zeotec	-	0	0
	1% Toxiban	-	0	0
plus 50 ng/ml Aflatoxin G2	No adsorbant	32.31	29.70	30.38
	1% Blue Pacific	32.24	0.80	0.39
	2.5% Blue Pacific	32.31	0.05	0.12
	1% Zeotec	-	7.63	4.82
	1% Toxiban	-	0	0
	No adsorbant	66.53	66.61	66.51
plus 100 ng/ml Aflatoxin G2	1% Blue Pacific	69.34	2.15	1.30
	2.5% Blue Pacific	69.75	0.25	0.19

^a Results for solutions containing aflatoxins are the mean of duplicate measurements.

^b 0.2% pepsin (activity 1:10,000) in 0.075 N HCl, held at 37°C.

Figure 1. Adsorption of aflatoxin B1, B2, G1 and G2 by Blue Pacific zeolite

Aflatoxin B1, B2, G1 and G2 added to acidic pepsin solution (37deg C) at 50ng/ml
Blue Pacific added at 1% feed equivalent

