Indian J. Anim. Res., **40** (2) : 167 - 169, 2006

DETOXIFICATION OF ZEARALENONE BY CHEMISORBANTS AND YEAST IN POULTRY FEED

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ABSTRACT

Lyophilized culture of *Fusarium sporotrichioides* was cultured on oatmeal agar. Subsequently the fungus was grown on broken wheat for three weeks for production of zearalenone toxin on large scale. Quantification of the cultured toxin was done using High Performance Thin Layer Chromatagraphy and found to be 573. 12 ppm. This culture was added to poultry feed to obtain the required level of toxin (2 ppm). This zearalenone contaminated feed was then treated with different adsorbents : yeast culture, activated charcoal, hydrated sodium calcium alumino silicate (HSCAS) and polyvinyl poly pyrrolidone (PVPP) at different levels to study the levels of adsorption of the toxin. The quantity of unbound zearalenone was estimated by ELISA method. Yeast culture and activated charcoal at 0.2 % levels adsorbed toxin to a maximum extent (81.45 and 79.45 %, respectively) and proved better than HSCAS and PVPP (40.23 and 66.90%, respectively).

Zearalenone (ZEN) occurs as a natural mycotoxin contaminant in corn, wheat, barley, oats, sorghum (Mirocha *et al.*, 1982) to an extent of 1 ppb to 10.1ppm produced by different strains of Fusaria (*Fusarium graminearum, Fusarium avenarum, Fusarium equistm, Fusarium culmorum, Fusarium latreriticum*). ZEN is an estrogenic mycotoxin, which generally causes changes in genitalia. Swine are the most commonly affected animals (Miller *et al.*, 1973). Abortion, mummified piglets and spread leg syndrome of ZEN toxicosis have also been reported (CAST, 1989; Danicke and Gareis, 2001). Cattle, poultry and laboratory rodents are affected to a lesser degree (Christensen *et al.*, 1965). The dietary ZEN upto 800mg/kg diet has minimal effects on performance of 0-3 wk. old broiler chicks (Chi *et al.*, 1980).

Inorganic sorbent materials (chemisorbants or sequestrant) like zeolite, clay, hydrated sodium calcium alumino silicate (HSCAS), activated charcoal have been shown to alter the effects of mycotoxins in chicken (Chung *et al.*, 1990 and Huff *et al.*, 1992). Information on detoxification of ZEN using chemisorbants is scanty. Hence, an attempt was made to study the effect of activated charcoal, HSCAS, poly vinyl poly pyrrolidone (PVPP) and yeast culture as adsorbants to detoxify zearalenone.

F. sporotrichoides procured from, Institute of Microbial Technology, Chandigarh was subcultured on oatmeal agar to get maximum efficiency. Subsequently, 25 gm of broken wheat reconstituted to about 50% moisture (Bilgrami *et al.*, 1995) was taken in 250 ml conical flasks and autoclaved at 121°C at 15 psi for 20min. Each flask was then inoculated with loopful of *F. sporotrichoides* fungus culture and kept for incubation in CO_2 free condition for 21 days. The samples were then autoclaved for killing fungus at 121°C at 15 psi for 20 min and dried in oven at 50-55°C. The dried samples were then ground for further analysis.

The toxin was extracted using the methods of AOAC (1995). Fifty grams of ground sample was taken in 500ml conical flask and added 300ml of chloroform : water (10 : 1) and 25g celite. Flasks were shaken for

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Table 1. The effect of different adsorbants on zearalenone content in contaminated poultry feed

S.No.	Adsorbant	Level of adsorbant (%)	% of adsorption*
1.	Activated charcoal	0.1	$76.90^{b} \pm 1.23$
		0.2	$79.70^{c} \pm 0.97$
		0.4	$72.75^{a} \pm 1.54$
2.	Yeast culture	0.1	$79.45^{b} \pm 1.13$
		0.2	$81.21^{b} \pm 0.94$
		0.4	$70.75^{a} \pm 1.43$
3.	Polyvinyl poly pyrrolidone (PVPP)	0.1	$37.75^{a} \pm 1.53$
		0.2	$66.90^{b} \pm 1.37$
		0.4	$76.75^{c} \pm 1.04$
4.	Hydrated Sodium Calcium Alumino Silicate (HSCAS)	0.1	$21.25^{a} \pm 1.29$
		0.2	$40.23^{b} \pm 1.34$
		0.4	$38.25^{b} \pm 1.17$

* Mean of 3 values;

^{abc} Values bearing different superscripts within the column item wise differ significantly ($P<0.05$).

1 hour and filtered through Whatman paper I. Fifty milliliters of filtrate was loaded to column made of 5g sodium sulphate, 10g silica gel and 15g sodium sulphate in 2 x 30cm tube with glass wool bedding (Eppley, 1968). Column was equilibrated with chloroform and hexane (150 ml) and zearalenone was extracted with 5:95 acetone : benzene solvent mixture (250 ml). The solvent was then dried and transferred with hexane to separating funnel and separated with acetonitrile 10 ml. This step was repeated twice. Combined acetonitrile extracts, dried and dissolved in 0.5 ml of benzene and quantified on HPTLC.

Later, poultry feed free from zearalenone was used to dilute the identified toxin so as to get 2 ppm toxin level. For binding studies, 25g contaminated feed in 250 ml conical flasks was used for different concentrations which involved treatment with various binders and 100 ml buffer (273 ml of 0.1 M citric acid + 227 ml 0.2 M disodium hydrogen phosphate + 500 ml distilled water) to get pH 4.5. The contents were mixed on shaker at 37^{0}C for 3 hrs, filtered and dried at 35-45^{0}C for 2 hrs and the residue was reextracted by the same extraction procedure for quantifying unbound toxin in feed. The estimation was done by ELISA kits (Ridascreen® Zearalenone) supplied by R-Biopharma AG Darmstadt, Germany.

The quantity of zearalenone obtained, when the culture was grown on broken wheat for 3 weeks, estimated by HPTLC method, was 573.12 ppm. This indicate that broken wheat is a suitable substrate for *F. sporotrichoides* for producing large quantities of toxin.

The percentage of toxin in contaminated feed adsorbed by different adsorbants at different levels were 76.90, 79.70 and 72.75 for activated charcoal, 79.45, 81.21 and 72.75 for yeast culture, 37.75, 66.90 and 76.75 for PVPP, 21.25, 40.23, and 38.25 for HSCAS, respectively at 0.1, 0.2 and 0.4 % level (Table 1). The toxin adsorption by activated charcoal was highest ($P< 0.05$) at 0.2% level indicating that the level of this adsorbant above 0.2% has no advantage. Yeast culture adsorbed maximum toxin at 0.1 and 0.2% levels which were at par indicating that 0.1% level of yeast culture is enough to adsorb adequate quantity of zearalenone. PVPP had highest adsorption at 0.4% level while HSCAS had highest adsorption at 0.2% level.

Activated charcoal and yeast culture showed maximum adsorption compared to

PVPP and HSCAS. Highest percentage of adsorption was recorded by activated charcoal at 0.2% level and yeast culture at 0.1% level. Further, *in vivo* experiment using activated charcoal at 0.2% and yeast culture at 0. 1% level as adsorbent is suggested for testing the efficacy of these two adsorbants in detoxifying zearalenone.

ACKNOWLEDGEMENT

The authors acknowledge the financial assistance from National Agricultural Technology Project, ICAR, New Delhi as a part of technical programme of Team of Excellence on Feed Technology and Quality Assurance.

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