



Comparative Efficacy of Sodium Bentonite and Yeast as Toxin Binders for Mitigation of Mycotoxicosis in Broilers

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MM conceptualized and designed the study. MAJ conducted the experiments. SBK and MI reviewed manuscript. MM analyzed the data. NI and MNK interpreted the results. MM and MI have written the manuscript. FAK edited the tables and references in the manuscript. All the authors approved final version.

Key words

Aflatoxins, binding capacity, broilers, growth performance, sodium bentonite, dressing percentage

ABSTRACT

The study was conducted at the University poultry farm using completely randomized design to evaluate comparative efficacy of inorganic and organic toxin binders for mitigation of aflatoxicosis in broiler birds. A total of 150 days old chicks were distributed into five treatment groups, each containing 10 birds as replicates. Diets were prepared with different inclusions of aflatoxins B1 and B2 (AFB1 and AFB2), sodium bentonite (SB) and yeast. Five treatment groups were: Control (basal diet only), basal diet + 80ppb AFB1 and 80 ppb AFB2, basal diet+80ppb+AFB1+80ppb AFB2+2.5% SB, basal diet+80ppb AFB1+80ppb AFB2+2% yeast and basal diet+80ppb AFB1+80 ppb AFB2+2.5%+2% yeast. Results showed that growth performance improved significantly ($P<0.05$) with treatment containing SB and yeast in combination as compared to other treatment groups. Significant variations ($p<0.05$) were observed in binding capacity of SB for AFB1 and AFB2 at pH3 and pH7. Binding capacity of SB for AFB1 at pH3 and pH7 was 92.2 and 96.4%, respectively. It was 81.1 and 85.5% for AFB2 at pH3 and pH7, respectively. Dressing percentage was also significantly ($P<0.05$) increased (61.54 %) with treatment including SB and yeast in combination. Weight of breast, thigh and leg was significantly higher with treatment including SB and yeast in combination. Treatment with SB and yeast in combination also showed no mortality of the broiler birds. It was concluded that dietary inclusions of SB and yeast in combination depicted better results on improved growth performance, increased dressing percentage and higher binding capacity for aflatoxins.

INTRODUCTION

Poultry sector is one of the most dynamic and coruscating sector of agriculture and contributes 1.4% to national GDP of Pakistan. Currently, poultry industry is the 2nd largest industry in Pakistan providing employment to more than 1.5 million people. Pakistan has become 11th largest poultry producer in world with production of 1.02 billion broilers, 48.83 million layers and 11.8 million breeding stock annually (Hanif, 2023).

In spite of its challenges in terms of disease outbreaks and mycotoxins contamination of feed, its vital contribution towards national economy is acknowledged. Economic losses annually to poultry industry due to mycotoxicosis are estimated to be over Rs. 500 million (Mobashar, 2023). Mycotoxicosis reduces feed intake, weight gain and egg production in poultry birds. Among mycotoxins, aflatoxins cause more loses in poultry like high mortality, reduced egg production and quality (Bryden *et al.*, 2021), carcass condemnation and lower reproduction (Wu *et al.*, 2021).

Various physical, chemical and microbial strategies have been used to combat mycotoxins in livestock, but they are expensive and have side effects. Alternatively, a local mycotoxin binder was developed by extracting from local clay to mitigate mycotoxicosis in poultry. Sodium bentonite (SB) is a layered aluminum silicate with hydrophilic and colloidal nature and has high swelling capability and act as enterosorbant in livestock feed and can bind and reduce absorption of mycotoxins (Ghazalah *et al.*, 2021). This study was therefore planned for the development of local

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toxin binder for mitigation mycotoxicosis in poultry.

MATERIALS AND METHODS

Collection of clay and extraction and chemical analysis of SB

Clay was collected from Shagia region, district Karak and was dried in oven at 100°C for 7 days and ground and diluted with HCl at 70°C for 90 min. Solution was stirred at 300 rpm and washed with distilled water repeatedly to attain a normal pH. Washed solution was heated at 600°C to eliminate Cl bond on bentonite resulting in sodium bentonite (SB). Chemical analysis of SB clay showed the following chemical components (mg/g DM): silica, 51.45; calcium, 2.89; sodium, 3.64; aluminum, 10.35; iron, 12.78; magnesium, 0.38; potassium, 2.31 and moisture, 5.49.

Mycotoxin binding capacity of SB

In vitro trial was conducted with a slight modification in the method of Diana Di Mavungu *et al.* (2009) for evaluation of binding capacity of product for AFB1 and AFB2. Mycotoxin was determined by using Vicam mycotoxin analyzer. Binding capacity was calculated as.

$$\text{Binding capacity(\%)} = \frac{100 \times (C_i - C_f)}{C_i}$$

where C_i and C_f are initial and final mycotoxin concentrations.

Birds' husbandry and experimental design

Study was conducted at University Poultry Farm with 150 broilers randomly selected in completely randomized design (CRD). Birds were distributed into 5 treatments, each with 3 replicates of 10 birds each. Treatment 1 control (basal diet only), treatment 2 (basal diet+ 80 ppb AFB1+80 ppb AFB2), treatment 3 (basal diet+80 ppb AFB1+80 ppb AFB2 + 2.5% SB), treatment 4 (basal diet +80 ppb AFB1+80 ppb AFB2+2% yeast) and treatment 5 (basal diet +80 ppb AFB1+80 ppb AFB2+2.5% SB +2% yeast). Study lasted for 42 days.

Table II. Effect of SB and yeast on growth performance of broiler birds fed on aflatoxins B1 and B2 (Means±SE).

S.	Treatment	Feed intake (g/bird)	Feed conversion ratio (FCR)	Gain in body weight
1	Control (commercial basal diet)	3067±17.5 ^a	1.52±0.01 ^a	1995±46.3 ^a
2	Basal diet+80 ppb AFB1+80 ppb AFB2	2931±19.3 ^d	2.1±0.11 ^d	1433±69.1 ^d
3	Diet of treatment 2 + 2.5% SB	2995±15.9 ^b	1.61±0.06 ^b	1852±58.4 ^b
4	Diet of treatment 2 + 2% yeast	2974±14.2 ^c	1.68±0.04 ^c	1765±35.2 ^c
5	Diet of treatment 2 + 2.5% SB +2% yeast	3059±13.73 ^a	1.54±0.02 ^a	1985±23.5 ^a
	P-value	0.040	0.007	0.020

Mean values within same column with different superscripts are significantly different at 0.05 level of significant.

Table III. Effect of SB and yeast on dressing percentage of broiler birds fed on aflatoxins B1 and B2 (Means±SE).

Growth performance

Feed consumption was calculated on daily basis while gain in body weight and FCR on weekly basis.

$$\text{FCR} = \text{Total feed intake/Total weight gain}$$

Mortality of birds was checked and recorded. Live weight of one randomly selected bird from each replicate was recorded. Birds were slaughtered and skinned off.

$$\text{Dressing (\%)} = \text{Carcass weight/ live weight} \times 100$$

Statistical analysis

Data was analyzed by using completely randomized design (CRD) in SPSS Inc., version. 20.

RESULTS

Mycotoxin binding capacity of SB

Table I shows that mycotoxin binding capacity of sodium bentonite for AFB1 and AFB2 mycotoxins was significantly higher ($P < 0.05$) at pH3 compared to pH7. Mean values of binding capacity for AFB1 and AFB2 at pH3 were 96.7 and 85.5%, respectively. While mean values of binding capacity for AFB1 and AFB2 at pH7 were 92.2 and 81.1%, respectively.

Table I. Binding capacity of SB clay for mycotoxins at pH3 and pH7 (Mean±SE).

Mycotoxins	Binding capacity (%) at		P value
	pH3	pH7	
AFB1	96.4±0.05 ^a	92.2±1.09 ^b	0.007
AFB2	85.5±0.03 ^a	81.1±1.12 ^b	0.004

SB, sodium bentonite; AFB1, aflatoxins B1. Mean values within row with different superscripts are significantly different at 0.05 significant level.

Effect of SB and yeast on growth performance

Table II show the effect of SB and yeast on growth performance of broiler birds growth performance was significantly improved ($P < 0.05$) due to inclusion of SB and yeast across the treatments. Among different treatments,

Treatment	Dressing (%)	Breast weight	Thigh weight	Leg weight	Mortality (%)
1	61.69±0.85 ^a	291.41±4.01 ^a	81.62±0.89 ^a	60.23±1.23 ^a	0
2	51.35±0.54 ^d	217.43±3.12 ^d	63.41±0.64 ^d	41.34±0.65 ^d	3
3	58.41±0.64 ^b	273.31±4.06 ^b	77.51±0.57 ^b	54.31±0.93 ^b	1
4	55.32±0.55 ^c	261.29±3.47 ^c	72.12±0.42 ^c	50.47±0.79 ^c	1
5	61.54±0.75 ^a	290.57±4.18 ^a	79.69±0.39 ^a	59.08±0.55 ^a	0
P-value	0.040	0.009	0.044	0.037	-

Mean values within same column with different superscripts are significantly different at 0.05 level of significant.

feed intake, FCR and gain in body weight ranged from 2931 to 3067 g, 1.5 to 2.1 and 1433 to 1995 g, respectively. Growth performance improved with control treatment and treatment containing SB and yeast in combination followed by treatments containing SB and yeast alone. Synergetic action of sodium bentonite and yeast showed better results of growth performance as compared to their inclusion in separate treatment.

Effect of SB and yeast on dressing percentage and mortality

Table III shows the effect of SB and yeast on dressing percentage and mortality of broiler birds dressing percentage, mortality and weight of edible organs were significantly influenced ($P < 0.05$) due to inclusion of SB and yeast in different treatments. Among different treatments, dressing percentage ranged from 51.35 to 61.69%, showing the highest dressing percentage with control treatment (61.69%) and treatment with SB and yeast in combination (61.54%). Moreover, treatment with SB alone depicted better results on dressing percentage as compared to treatment containing yeast alone. No mortality was recorded in control treatment and treatment with SB and yeast in combination. However, mortality of three birds in treatment with AFB1 and AFB2 without toxin binder and mortality of one bird in each treatment containing SB and yeast alone was recorded. Weight of breast, thigh and leg was significantly ($P < 0.05$) higher with treatment with SB yeast in combination followed by treatments with SB yeast alone.

DISCUSSION

In the present study, binding capacity of sodium bentonite for mycotoxins AFB1 and AFB2 was evaluated at pH3 and pH7. These pHs were selected to represent pH working medium in proventriculus and intestine of the poultry birds, respectively. Comparatively higher mycotoxin binding capacity of SB was observed at pH3. pH 3 and pH7 were maintained by using citrate and phosphate buffer solutions, respectively used in the current research to obtain *in vitro* medium conditions well-suited with GIT

of poultry birds. Citrate buffer solution was composed of 4.27g of trisodium citrate 2-hydrate in 0.9 lit of DW and 17.96g of citric acid in 1 liter of DW. Phosphate buffer solution was composed of 35.814g of sodium phosphoric acid in one liter of DW. The pH 3 presents high acidity used in the present study can be established in the gizzard and proventriculus of poultry birds for a little time while pH 7.0 is observed in many segments of GIT for prolong time with great relevancy (Hajati, 2018). SB which was extracted from clay and is inorganic mycotoxin binder represents the largest class of mycotoxin binders, and most of the studies on the alleviation of mycotoxicosis by the use of adsorbents have been focused on these clays (Nviiri *et al.*, 2022; Santos *et al.*, 2011).

The properties of mycotoxin adsorbent like SB and composition of feed play an important role in binding of mycotoxins and adsorbent activity (Luo *et al.*, 2020). The physicochemical properties of the adsorbent like amount of charge and its distribution, number and size of the pores on the surface of adsorbent and pH are important determinants of binding efficacy (Gupta *et al.*, 2021; Lemke *et al.*, 2001). In addition, polarity, solubility, type and molecular weight of mycotoxin cannot be ignored to evaluate binding effectiveness of an adsorbent. European food safety authority (EFSA) stated that along with efficacy testing of mycotoxin binders; their safety should also be investigated because the toxin binders added to the feeds are thought to make non-specific bindings (Additives and Feed, 2010). *In vitro* investigation gives significant data for the adsorption mechanism of a binder and then its further validity in *in vivo* studies. In the past, various methods have been used to evaluate *in vitro* mycotoxin binding or adsorption potential (Appell *et al.*, 2023; Kihal *et al.*, 2020; Ledoux and Rottinghaus, 1999). Although the *in vitro* studies for mycotoxin binding or adsorption do not give authentic results and may not always be a reliable for consideration for binding of specific mycotoxins in *in vivo* studies, however, these are used to estimate mechanism for adsorbent with the identification and approximate dosage requirements for the adsorbent to be used.

In the present study, primary advantage of conducting

an *in vitro* test was to validate if a sequestering agent has little or no affinity for AFB1. In case, the agent has no binding capacity for AFB1 *in vitro*, it is unlikely to bind in *in vivo* environment. As *in vitro* preliminary tests of mycotoxin adsorption are regarded as a potent tool for screening potential mycotoxin-detoxifying agents since if no adsorption occurs *in vitro*, little or no chance exists to do so *in vivo* (Boudergue *et al.*, 2009; Pappas *et al.*, 2014). Based on working experience with *in vitro* studies on binding of mycotoxins, mycotoxin adsorbents with a binding ability higher than 80%, under *in vitro* conditions, may be considered for *in vivo* evaluation for binding of mycotoxin in the feed (Yalcin *et al.*, 2018). According to this thumb rule, results obtained on mycotoxin binding capacity which is between 81 to 96%, may be used as an inorganic binder in the diet of livestock in general and poultry in particular to bind aflatoxins *in vivo*.

Effect of SB and yeast on growth performance

Socially and economically this study has a great impact on control of mycotoxicosis in poultry for local farming community in province through development of local toxin binder. In the present study, combined action of SB and yeast improved growth performance in broiler birds as compared to their inclusions separately. This may be disturbance in the normal metabolism that results in decreased appetite and hepatic degeneration (Azizpour and Moghadam, 2015; Espina *et al.*, 2023; Zhang *et al.*, 2022). Adsorbent like SB mixed in feed has high economical competence value for ducks by adding 0.5 to 1% sodium bentonite in feed, which enhanced ($P < 0.05$) feed consumption (Fatouh *et al.*, 2012). The present results indicated that SB clay possibly has absorptive and selective character that improved digestion and therefore caused improvement in growth performance.

Addition of non-nutritional adsorbents in the diet that bind mycotoxins in the gastrointestinal tract and reduce their bioavailability is one of the vital approaches. These binding agents do not undergo any changes in the digestive system. When they are used in feed in different levels, they prevent mycotoxins from being absorbed through the digestive system. They also bind AF molecules and reduce their toxic effects (Benkerroum, 2020; Bhatti *et al.*, 2017; Mobashar, 2023; Sipos *et al.*, 2021).

Inorganic mycotoxin binders include commonly clays of Na and Ca and are the largest class of mycotoxin binders, and most of the studies on the alleviation of mycotoxicosis by the use of adsorbents have been focused on these clays (Santos *et al.*, 2011). The organic binders include indigestible carbohydrates, cell walls of yeast, and bacteria such as glucomannans, peptidoglycans, and others (Ćolović *et al.*, 2019; Greco *et al.*, 2022; Oguz,

2016). *Saccharomyces cerevisiae* initially used as a growth promoter and was also found to induce beneficial effects on growth performance in broilers exposed to mycotoxins. The beneficial effects of yeast have been attributed to mannan in the yeast cell wall. By using only yeast cell walls (composed of beta-glucans and mannan oligosaccharides) instead of the whole cell, mycotoxin binding can be enhanced (Karaman *et al.*, 2005).

Dressing percentage, mortality and carcass weight

In the present study, dietary inclusion of SB and yeast improved dressing percentage and controlled mortality of broiler birds. The highest dressing percentage was found with treatment including SB and yeast in combination. No mortality was recorded in control treatment and treatment with SB and yeast in combination. Mean dressing percentage was lower ($p < 0.05$) in birds raised on aflatoxin contaminated feed alone. Zahid and Durrani (2007) reported similar findings on feeding different levels of herb (milk thistle) to broilers and found significantly higher dressing percentage. In the present study an increase of more than 6% in dressing percentage was recorded while Zahid and Durrani (2007) reported about 4% improvement in dressing percentage. Furthermore, Afzal and Zahid (2004) and El-Katcha *et al.* (2017) also found similar results on dressing percentage and mortality of broiler birds fed on yeast. In the present study carcass weight (breast, thigh and leg weights) improved with treatment including both SB and yeast in combination. Similar results have been reported by Gümüş (2023).

CONCLUSIONS

It was concluded from the present study that binding capacity of SB for AFB1 and AFB2 was significantly higher ($P < 0.05$) at pH3 as compared to pH7. Best results were obtained on improved growth performance, increased dressing percentage and less mortality of the broiler birds with treatment including both SB and yeast in combination in the diet. Further research needs to be conducted to explore the role of different inclusions of sodium bentonite and yeast in combination on egg production and reproductive efficiency of layer birds.

DECLARATIONS

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Ethical statement

Study was conducted in compliance with Animal Care and Ethical Regulations of FAH&VS, UAP, 2019.

Statement of conflict of interest

The authors have declared no conflict of interest.

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