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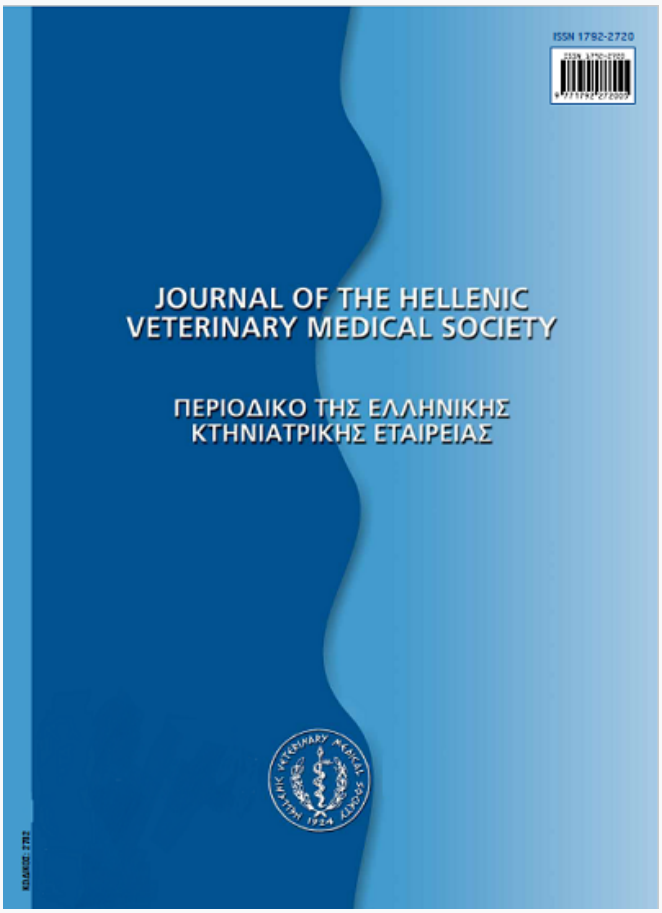


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# Effects of yeast based toxin binder supplementation on growth performance and intestinal microarchitecture in male buffalo calves (*Bubalus bubalis*) exposed to different concentrations of Aflatoxin B1.

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**ABSTRACT.** The purpose of study was to analyze the effects of yeast based toxin binder (YTB) on growth performance and intestinal microarchitecture of buffalo calves exposed to different concentrations of aflatoxin B1 (AFB1). Male buffalo calves (n=72) having no disease exposure in the last four months, aged 10 ± 1 months and weighing 150 ± 12.22 kg (Mean ± standard deviation)were purchased from a buffalo calf fattening farm and divided in 8 equal groups (n=9 animals in each group). Animals that were fed common basal diet and basal diet along with yeast cell wall based toxin binder (YTB) and had no exposure to AFB1 were served as controls. Different concentrations of AFB1 (6mg, 8mg and 10mg per kg of feed) were given to 27 animals, similarly 2mg of YTB (per kg of contaminated feed) was added to concentrate with different AFB1 concentrations and fed to another 27 animals. Average daily gain of each animal was recorded. Animals were slaughtered after 27 days and intestinal segments from duodenum, jejunum, ileum and colon were collected which were stained with hematoxylin/eosin or combined alcian blue and periodic acid schiff stain.

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Average daily gain and intestinal morphometric parameters (villus height, villus width, crypt depth and surface area) in all the selected intestinal segments decreased (P<0.05) whereas, intraepithelial lymphocytes, goblet cells (having acidic and neutral mucins) and proliferative cells increased (P<0.05) with AFB1 exposure. It was also observed that YTB neutralized (P<0.05) the harmful effects of AFB1 on intestinal morphology, boosted activity of goblet cells and decreased (P<0.05) number of proliferative cells. It was concluded that YTB supplementation is advantageous in eliminating the negative effects of AFB1 on gut of buffalo calves.

***Keywords:*** Buffaloes, aflatoxin B1, yeast based toxin binder supplementation, growth performance, intestinal histology.

## INTRODUCTION

Aflatoxins (AF), a cluster of intoxicating mycotoxins are secondary metabolites from certain strains of filamentous fungi specially *Aspergillus Flavus* and *Aspergillus Parasiticus* (Rashid et al., 2012). These fungi colonize on feed and food products and produce AF whose presence may lead to economic losses and raise public health concerns which at times can prove to be fatal (Mendoza et al., 2011). Harmful outcomes of AF which include teratogenic, mutagenic, growth inhibitory and carcinogenic effects have been widely studied (Oguz et al., 2000, Sur and Celik, 2003). Aflatoxins suppress the immune system and cause both macro and micro pathological alterations in the normal structure of liver, kidney and spleen making animal more susceptible to infectious agents (Magnoli et al., 2011).

To decrease the toxic effects of AF toxin binders (TB) are being used (Schatzmayr et al., 2003). Inclusion of TB in feed contaminated with AF has been the most promising approach for reducing the detrimental effects of AF (Galvano et al., 2001). In-vivo and in-vitro trails have been conducted over the last decade with TB for estimating their efficacy against toxins but results from both the trials do not correlate very well (Doll et al., 2004; Diaz et al., 2004). Many complex indigestible carbohydrates (polysaccharides in yeast cell wall and cellulose) and bacteria (glucomannans and peptidoglycans) have been reported to absorb AF among these yeast cell wall based toxin binder (YTB) is being preferred for its better nutritional properties and superior binding activity than other commercially available toxin binders (Huwig et al., 2001).

Among several types of AF aflatoxin B1 (AFB1) is most commonly encountered and is considered most potent than any other type (Yunus et al., 2011), causing stern health concerns for animal populations (Sirajudeen et al., 2009). Negative effects of AF have been an active area of research (Yunus et al., 2011), and studies have revealed that the primary site for absorption of AF is small intestine (predominantly duodenum) (Mendoza et al., 2011). To the best of our knowledge influence of YTB supplementation on the quantification and differentiation of goblet cells (GC), role of intraepithelial lymphocytes (IEL) as an indicator of mucosal damage and immunohistochemistry of proliferative cells in intestine of buffalo calves exposed to different concentrations of AFB1 has not been assed. This study will be pivotal in recognizing the structural alterations in buffalo gut associated with AFB1 and YBT supplementation as it aims to evaluate the effects of different concentrations of AFB1 on intestinal microarchitecture (morphometric variables, IEL count, GC quantification and differentiation and immunohistochemistry of proliferative cells) in gut of buffalo calves and efficacy of YTB to counter toxic effects of AFB1 on intestinal morphology.

## MATERIALS AND METHODS

### Experimental design and grouping of animals

A total of 72 having no disease exposure in the last four months, aged 10 ± 1 months and weighing 150 ± 12.22 kg (Mean ± standard deviation) were purchased from a buffalo calf fattening farm and divided in 8 equal groups (n=9 animals in each group). These animals were then acclimatized for 15 days in an environmentally control shed having an optimum temperature of 22 ± 2 ºC and a relative humidity of 55 ± 10 % before the start of trial during which they were given ad-libitum corn silage (Table 1). Silage was tested for the presence of AFB1 on weekly basis by high performance liquid chromatog-

raphy. Animals were dewormed and vaccinated for foot and mouth disease and hemorrhagic septicemia (UVAS-FMD+HS-VAC, Pakistan). At the start of trail all animals were individually housed in separate pens made of stainless steel and were offered corn silage (free of AFB1, 15 kg per animal) and concentrate (1 kg per animal) (Table 1) per day and there refusal were weighed daily. Animals were divided into eight groups with each group having (n = 9) animals. Basal diet (BD) and BD plus YTB (BD-YTB) were not exposed to AFB1. For investigating the harmful effects of AFB1 different concentrations of AFB1 were given to animals of 3 groups which included AFB1 6mg per kg of concentrate (AFB1 0.6), AFB1 8mg per kg of concentrate (AFB1 0.8) and AFB1 10mg per kg of concentrate (AFB1 1.0) after being formulated in the concentrate (Table 1) for 27 days. Furthermore for analysis the effects of YTB during aflatoxicosis in buffalo calves, 2mg per kg of YTB (Fixar viva dry yeast based, Bentoli Agrinutrition Asia Pte. Ltd, USA) was mixed in feed having different concentrations of AFB1 and given to another 3 groups of buffalo calves (AFB1 0.6-YTB, AFB1 0.8-YTB, and AFB1 1.0-YTB). Throughout the trail animals had open access to water. This study was conducted according to the guidelines of Animal Care and Use Committee, University of Veterinary and Animal Sciences (UVAS), Lahore.

**Production of AFB1,preparation of diet and safety measures**

Aflatoxin B1 being used in the trial was produced in Microbiology laboratory UVAS using toxic strains of *Aspergillus Flavus* IMI-90, obtained from International Mycology Institute, London as described by Tessari et al., (2006). Briefly, chloroform (30 ml chloroform per 10 grams of culture) was used to extract coconut culture after shaking for 30 minutes. Contents post filtration via filter paper (Whatman#1) was evaporated to dryness. Densitometry was applied for quantification and water bath (WNE 14 water bath, Memmert, USA) was used for evaporation of chloroform solution having AFB1 at 60 ºC. Contents were resuspended in fish oil that had been proven negative for AF presence. This fish oil with different concentrations

**Table 1.** Ingredients (as fed basis) and nutrient composition (% dry matter basis) of concentrate and corn silage fed to male buffalo calves.

| **Ingredients (Concentrate)** | **% unless indicated** |
|---|---|
| Corn Meal | 22.8 |
| Sugarcane molasses | 2 |
| Wheat midds | 65.8 |
| Soyabean meal | 3.3 |
| Limestone | 3.6 |
| Fish oil | 1 |
| Salt | 1 |
| Premix* | 0.4 |
| **Chemical composition (Concentrate)** | |
| Dry matter | 86 |
| Crude protein | 16.8 |
| Crude fat | 4.2 |
| Crude fiber | 12.3 |
| Neutral detergent fiber | 38.6 |
| Metabolizable energy | 2.6 M cal/kg |
| **Chemical composition (Corn Silage)** | |
| Dry matter | 35.7 |
| Crude protein | 7.3 |
| Crude fat | 1.3 |
| Crude fiber | 26.4 |
| Neutral detergent fiber | 66.2 |
| Metabolizable energy | 2.2 M cal/kg |

*M cal, Mega calories.*
** Each kg of premix contained; vitamin A, 495 IU; vitamin E, 0.33 IU; vitamin D3, 165 IU; zinc, 16mg; copper, 4mg; selenium, 0.1mg; iodine, 0.6mg; and cobalt, 0.6mg.*

(6mg, 8mg and 10mg) of AFB1 was used in the concentrate formulation (Table 1). Final concentrations of AFB1 in concentrate to be offered were confirmed using procedure used by Shephard et al., (1990).

Aflatoxin B1 is a toxic substance therefore it was manipulated as solutions to avoid formation of aerosol and dust. Aprons, masks and nitrile gloves were used at every step of manufacturing and manipulation (Corcuera et al., 2011).

**Growth performance, sampling and histological morphometry**

Animals were weighed on weekly basis and after completion of trail were slaughtered by Halal slaughter method as described by Gregory et al., (2008), and 3cm intestinal segments at midpoints of duodenum, jejunum, ileum and colon were collected. Samples were washed with physiological saline solution, opened longitudinally and immediately fixed in 10% buffered formaldehyde solution for 48 hours. Intestinal segments were then rinsed with water and after dehydration with graded series of absolute alcohol (50%, 60%, 70%, 80%, 90% and 100%) were cleared twice with benzene. Sections were embedded with paraffin and 4 µm thin sections were obtained which were stained with hematoxylin/eosin (Wang et al., 2009). Slides were observed under light microscope (Labomed CXL, New York Microscope Co, USA) fitted with camera (Moticam CMOS Digital Camera, New York Microscope Co, USA) and measurements were made with commercial morphometric program (Pixel Pro, Labomed, USA). Double blind analysis was done in triplicate on 5 well oriented villi that were selected on the basis of intact lamina propria. Variables that were measured included villus height (from tip of villus to villus crypt junction), villus width, crypt depth (from base to the transition region between villus and crypt), surface area (2 × villus width/2 ×villus length) (Solis de los Santos et al., 2007), and IEL (rounded cell that had central or slightly eccentric nucleus with scant cytoplasm) number.

**Histo-chemistry of goblet cells**

Slides obtained from paraffin embedding technique were subjected to alcian blue and periodic acid Schiff staining methods (Bancroft and Stevens, 2007), to evaluate goblet cells containing acidic and neutral mucin types respectively. Acidic mucins were stained blue by AB whereas neutral mucins were stained magenta by PAS (Leknes, 2010).

**Immunohistochemistry of proliferative cells**

Proliferative cells were counted in all the groups with antibody MIB-1 (Sigma-Aldrich, St. Louis, MO., USA) directed against proliferation marker Ki-67. Regarding antigen accessibility sections were incubated in boiling 0.01 M citrate buffer having pH 6 and washing was carried out in phosphate buffered saline (PBS) with 0.05% Tween (PBS-T). For blocking the endogenous peroxidase activity slides were treated with 1% hydrogen peroxide and to avoid binding of unspecific antibody slides were treated with 10% goat serum (Life technologies, Scientific supplies Ltd. Pakistan). At 4 ºC binding of MIB-1 (1:50 in PBS) was done overnight. Sections were then incubated with polyclonal goat anti-mouse antibody which had been labeled with horse reddish peroxidase. Visualization was done with 3,3′ diaminobenzidine solution (KPL Inc., Gaithersburg, Maryland USA). Quantification of proliferative cells positive to MIB-1 antibody was done as described by Masanatz et al., (2010) in the five consecutive crypts of selected intestinal segments and the average of the results were reported.

**STATISTICAL ANALYSIS**

Statistical differences were analyzed by using Statistical Package for Social Sciences (SPSS 13.3, SPSS, Chicago, USA). For estimating the normal distribution of data Shapiro- Wilk test was applied, data was found to be normally distributed. Data for comparison among groups were subjected to one way ANOVA keeping BD, different concentrations of AFB1, treatment of AFB1 contaminated groups with YTB and inclusion of YTB to BD as constant factors. Statistical differences among means were considered significant at $P < 0.05$ and calculated using Duncan's multiple range test.

**RESULTS**

Effects of feeding different levels of AFB1 on average daily gain and morphometric variables (villus

height, villus width, crypt depth, villus: crypt ratio and surface area) of selected intestinal segments are shown in Table 2. Exposure of buffalo calves to AFB1 resulted in lower (P<0.05) average daily gain and decreased (P<0.05) morphometric variables except for crypt depth which increased (P<0.05) with AFB1 for all the selected intestinal segments. Villus height, villus width, villus: crypt ratio and surface area decreased (P<0.05) whereas crypt depth increased (P<0.05) as AFB1 concentrations were increased. It was observed that by addition of YTB harmful effects of AFB1 on intestinal microarchitecture were neutralized as no difference was observed for morphometric variables in AFB1 0.6-YTB, AFB1 0.8-YTB and AFB1 1.0-YTB compared to BD. Moreover inclusion of YTB in BD not contaminated with AFB1 increased (P<0.05) villus height, villus width, and surface area.

A higher (P<0.05) IEL count was observed in all selected intestinal segments of male buffalo calves exposed to different concentrations of AFB1 compared to BD animals. Inclusion of YTB to contaminated diet decreased (P<0.05) IEL count of animals compared to those exposed to AFB1. Number of GC, GC having acidic mucin and neutral mucin increased (P<0.05) with exposure to AFB1 compared to BD and gradually increased (P<0.05) with increasing AFB1 concentrations in feed. Supplementation of YTB caused an increase (P<0.05) in GC having acidic mucin and neutral mucin in selected intestinal segments of animals exposed to AFB1 and BD-YTB animals.

For all the selected intestinal segments number of proliferative cells increased (P<0.05) with increas-

**Fig 1.** Acidic type of goblet cells in in the duodenum of calves fed AFB1 0.8 -YTB.

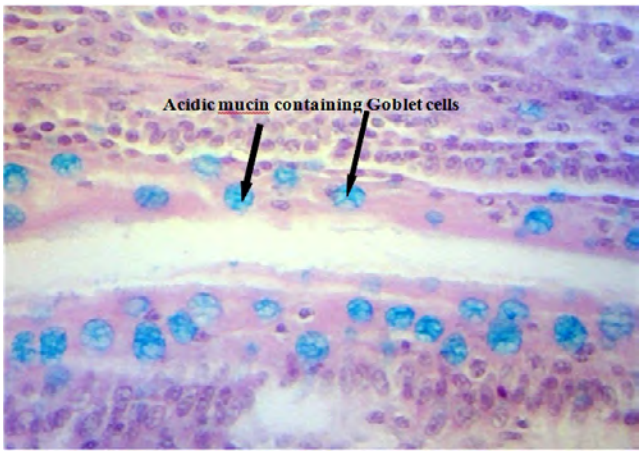



**Fig 2.** Mixed type of goblet cells in in the duodenum of calves fed AFB1 0.8 -YTB.

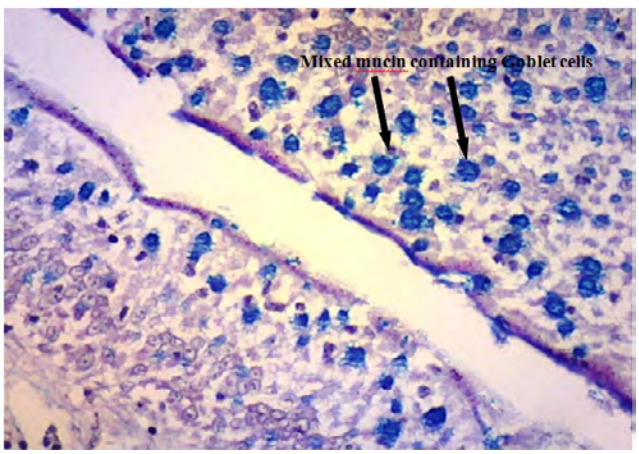



**Fig 3.** Acidic and mixed mucin having types of goblet cells in in the duodenum of calves fed AFB1 -0.8.

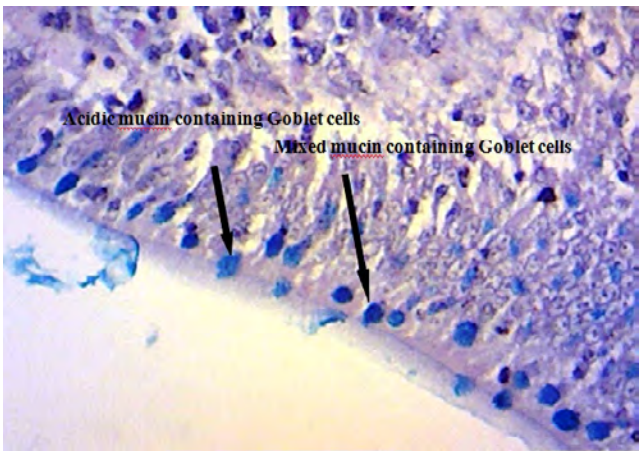



**Fig 4.** Intra epithelial lymphocytes in the Duodenum of calves fed AFB1- 0.8.

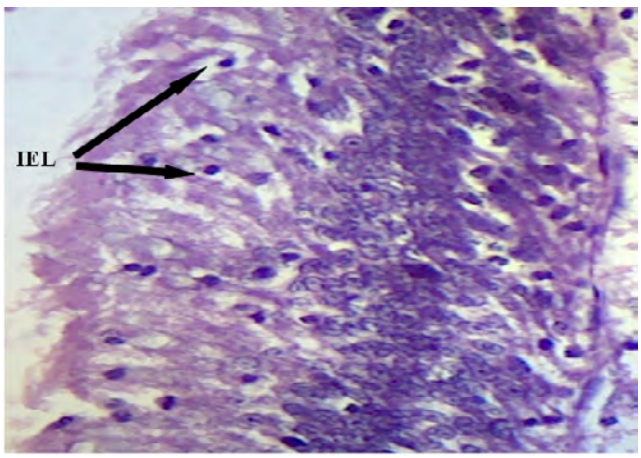


**Table 2.** Comparison of intestinal morphometric parameters and intraepithelial lymphocyte count in intestinal segments of male buffalo calves.

| Intestinal segments | Variables | BD | Contaminated | | | Treated | | | BD-YTB | SEM | *P*-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $AFB_1$ 0.6 | $AFB_1$ 0.8 | $AFB_1$ 1.0 | $AFB_1$ 0.6-YTB | $AFB_1$ 0.8-YTB | $AFB_1$ 1.0-YTB | | | |
| Duodenum | Average daily gain (grams) | $502^{d}$ | $411^{c}$ | $372^{b}$ | $310^{a}$ | $491^{d}$ | $505^{d}$ | $511^{d}$ | $557^{e}$ | 31.75 | < 0.01 |
| | Villus height (mm) | $0.73^{d}$ | $0.51^{c}$ | $0.45^{b}$ | $0.39^{a}$ | $0.72^{d}$ | $0.71^{d}$ | $0.68^{d}$ | $0.78^{e}$ | 0.04 | < 0.01 |
| | Villus width (mm) | $0.15^{d}$ | $0.11^{c}$ | $0.09^{b}$ | $0.06^{a}$ | $0.12^{d}$ | $0.11^{d}$ | $0.12^{d}$ | $0.18^{e}$ | 0.01 | <0.01 |
| | Crypt depth (mm) | $0.23^{a}$ | $0.35^{e}$ | $0.41^{d}$ | $0.50^{e}$ | $0.24^{a}$ | $0.21^{a}$ | $0.25^{a}$ | $0.27^{ab}$ | 0.04 | < 0.01 |
| | Villus: crypt ratio | $3.17^{e}$ | $1.45^{e}$ | $1.09^{b}$ | $0.78^{a}$ | $3.01^{e}$ | $3.38^{f}$ | $2.72^{d}$ | $2.88^{de}$ | 0.39 | < 0.01 |
| | Surface area ($mm^2$) | $0.37^{e}$ | $0.18^{c}$ | $0.15^{b}$ | $0.08^{a}$ | $0.27^{d}$ | $0.25^{d}$ | $0.26^{d}$ | $0.45^{e}$ | 0.04 | < 0.01 |
| | Intraepithelial lymphocytes | $24.61^{a}$ | $38.14^{d}$ | $42.73^{e}$ | $49.55^{f}$ | $27.32^{ab}$ | $30.24^{b}$ | $33.17^{b}$ | $24.18^{a}$ | 3.38 | < 0.01 |
| Jejunum | Villus height (mm) | $0.69^{d}$ | $0.44^{c}$ | $0.36^{b}$ | $0.25^{a}$ | $0.68^{d}$ | $0.71^{d}$ | $0.73^{d}$ | $0.79^{e}$ | 0.07 | < 0.01 |
| | Villus width (mm) | $0.14^{c}$ | $0.08^{b}$ | $0.07^{b}$ | $0.05^{a}$ | $0.13^{c}$ | $0.15^{c}$ | $0.15^{c}$ | $0.19^{d}$ | 0.01 | < 0.01 |
| | Crypt depth (mm) | $0.26^{a}$ | $0.38^{e}$ | $0.44^{d}$ | $0.48^{e}$ | $0.25^{a}$ | $0.27^{a}$ | $0.29^{a}$ | $0.31^{ab}$ | 0.03 | < 0.01 |
| | Villus: crypt ratio | $2.65^{d}$ | $1.15^{e}$ | $0.81^{b}$ | $0.52^{a}$ | $2.72^{d}$ | $2.62^{d}$ | $2.51^{d}$ | $2.54^{d}$ | 0.35 | < 0.01 |
| | Surface area ($mm^2$) | $0.32^{d}$ | $0.17^{c}$ | $0.08^{b}$ | $0.04^{a}$ | $0.28^{d}$ | $0.31^{d}$ | $0.34^{d}$ | $0.45^{e}$ | 0.05 | < 0.01 |
| | Intraepithelial lymphocytes | $22.61^{a}$ | $31.25^{d}$ | $35.43^{e}$ | $42.72^{f}$ | $23.69^{ab}$ | $25.57^{b}$ | $24.91^{ab}$ | $21.89^{a}$ | 2.64 | < 0.01 |
| Ileum | Villus height (mm) | $0.64^{e}$ | $0.41^{c}$ | $0.35^{b}$ | $0.33^{a}$ | $0.61^{de}$ | $0.58^{d}$ | $0.53^{d}$ | $0.71^{f}$ | 0.05 | < 0.01 |
| | Villus width (mm) | $0.13^{d}$ | $0.09^{c}$ | $0.06^{b}$ | $0.04^{a}$ | $0.11^{d}$ | $0.12^{d}$ | $0.11^{d}$ | $0.15^{e}$ | 0.01 | < 0.01 |
| | Crypt depth (mm) | $0.33^{a}$ | $0.39^{c}$ | $0.47^{d}$ | $0.53^{e}$ | $0.29^{a}$ | $0.31^{a}$ | $0.34^{a}$ | $0.36^{ab}$ | 0.03 | < 0.01 |
| | Villus: crypt ratio | $1.93^{ef}$ | $1.05^{c}$ | $0.74^{b}$ | $0.62^{a}$ | $2.10^{e}$ | $1.87^{e}$ | $1.55^{d}$ | $1.97^{ef}$ | 0.22 | < 0.01 |
| | Surface area ($mm^2$) | $0.28^{e}$ | $0.12^{c}$ | $0.07^{b}$ | $0.04^{a}$ | $0.21^{d}$ | $0.22^{d}$ | $0.19^{d}$ | $0.34^{f}$ | 0.03 | < 0.01 |
| | Intraepithelial lymphocytes | $27.19^{a}$ | $37.27^{d}$ | $44.31^{e}$ | $46.22^{f}$ | $29.64^{b}$ | $30.43^{b}$ | $28.88^{ab}$ | $26.91^{a}$ | 2.81 | < 0.01 |
| Colon | Villus height (mm) | $0.59^{d}$ | $0.45^{c}$ | $0.39^{b}$ | $0.32^{a}$ | $0.57^{d}$ | $0.55^{d}$ | $0.58^{d}$ | $0.67^{e}$ | 0.04 | < 0.01 |
| | Villus width (mm) | $0.11^{d}$ | $0.07^{c}$ | $0.05^{b}$ | $0.04^{a}$ | $0.11^{d}$ | $0.10^{d}$ | $0.12^{d}$ | $0.16^{e}$ | 0.01 | < 0.01 |
| | Crypt depth (mm) | $0.37^{a}$ | $0.43^{c}$ | $0.49^{d}$ | $0.57^{e}$ | $0.36^{a}$ | $0.39^{a}$ | $0.41^{a}$ | $0.43^{ab}$ | 0.02 | < 0.01 |
| | Villus: crypt ratio | $1.59^{e}$ | $1.04^{c}$ | $0.79^{b}$ | $0.56^{a}$ | $1.58^{c}$ | $1.41^{d}$ | $1.42^{d}$ | $1.55^{e}$ | 0.15 | < 0.01 |
| | Surface area ($mm^2$) | $0.22^{d}$ | $0.09^{c}$ | $0.06^{b}$ | $0.04^{a}$ | $0.19^{d}$ | $0.17^{d}$ | $0.21^{d}$ | $0.28^{e}$ | 0.03 | < 0.01 |
| | Intraepithelial lymphocytes | $31.11^{a}$ | $47.02^{e}$ | $54.55^{d}$ | $59.88^{c}$ | $35.62^{a}$ | $34.47^{a}$ | $35.23^{a}$ | $31.97^{a}$ | 3.92 | < 0.01 |

*Results are demonstrated as mean ± s.e.m.*
*Difference of superscripts (a-f) within a row indicates significance difference P < 0.05.*
*BD, basal diet (negative for AFB1); AFB1 0.6, aflatoxin B1 6mg per kg of concentrate; AFB1 0.8, aflatoxin B1 8mg per kg of concentrate; AFB1 1.0, aflatoxin B1 10 mg per kg of concentrate; AFB1 0.6- YTB, aflatoxin B1 6mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; AFB1 0.8- YTB, aflatoxin B1 8mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; AFB1 1.0- YTB, aflatoxin B1 10mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; BD-YTB, basal diet plus 2mg per kg of yeast based toxin binder; SEM, standard error of mean.*

**Table 3.** Goblet cells, histochemistry of goblet cells and immunohistochemistry of proliferative cells in intestinal segments of male buffalo calves.

| Variables | Intestinal segments | BD | Contaminated | | | Treated | | | BD-YTB | SEM | *P*-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $AFB_1$ 0.6 | $AFB_1$ 0.8 | $AFB_1$ 1.0 | $AFB_1$ 0.6-YTB | $AFB_1$ 0.8-YTB | $AFB_1$ 1.0-YTB | | | |
| Goblet cells (per villus) | Duodenum | 49.21$^{a}$ | 59.24$^{b}$ | 62.34$^{bc}$ | 65.37$^{c}$ | 67.52$^{d}$ | 69.98$^{d}$ | 73.29$^{e}$ | 99.86$^{f}$ | 5.56 | < 0.01 |
| | Jejunum | 55.82$^{a}$ | 68.13$^{b}$ | 71.14$^{b}$ | 75.02$^{bc}$ | 78.09$^{d}$ | 81.27$^{de}$ | 85.12$^{e}$ | 116.71$^{f}$ | 6.69 | < 0.01 |
| | Ileum | 71.65$^{a}$ | 79.84$^{b}$ | 80.23$^{b}$ | 83.26$^{b}$ | 89.17$^{c}$ | 93.45$^{c}$ | 96.15$^{c}$ | 141.34$^{d}$ | 8.12 | < 0.01 |
| | Colon | 88.91$^{a}$ | 93.23$^{b}$ | 95.77$^{b}$ | 98.65$^{c}$ | 101.24$^{d}$ | 105.36$^{d}$ | 108.91$^{d}$ | 168.79$^{e}$ | 9.65 | < 0.01 |
| Goblet cells having acidic mucin (per villus) | Duodenum | 18.14$^{a}$ | 31.86$^{b}$ | 34.25$^{c}$ | 36.97$^{c}$ | 41.69$^{d}$ | 44.26$^{d}$ | 49.87$^{d}$ | 51.27$^{e}$ | 3.59 | < 0.01 |
| | Jejunum | 26.61$^{a}$ | 30.74$^{b}$ | 33.37$^{b}$ | 35.42$^{c}$ | 39.19$^{d}$ | 43.84$^{e}$ | 44.36$^{e}$ | 58.17$^{f}$ | 3.43 | < 0.01 |
| | Ileum | 29.22$^{a}$ | 36.11$^{b}$ | 40.42$^{bc}$ | 41.24$^{c}$ | 45.13$^{d}$ | 48.27$^{d}$ | 51.86$^{d}$ | 62.88$^{e}$ | 3.87 | < 0.01 |
| | Colon | 33.15$^{a}$ | 38.21$^{b}$ | 40.07$^{b}$ | 41.14$^{c}$ | 49.11$^{d}$ | 51.24$^{d}$ | 53.62$^{d}$ | 74.16$^{e}$ | 4.84 | < 0.01 |
| Goblet cells having neutral mucin (per villus) | Duodenum | 15.26$^{a}$ | 22.17$^{b}$ | 23.79$^{b}$ | 25.32$^{c}$ | 21.35$^{d}$ | 22.13$^{d}$ | 23.03$^{d}$ | 48.13$^{e}$ | 3.71 | < 0.01 |
| | Jejunum | 24.27$^{a}$ | 27.21$^{b}$ | 28.53$^{b}$ | 30.79$^{b}$ | 32.84$^{c}$ | 34.21$^{c}$ | 36.18$^{c}$ | 49.27$^{d}$ | 2.97 | < 0.01 |
| | Ileum | 25.04$^{a}$ | 30.18$^{b}$ | 33.29$^{c}$ | 34.96$^{c}$ | 38.21$^{d}$ | 40.39$^{d}$ | 42.58$^{d}$ | 57.83$^{e}$ | 3.72 | < 0.01 |
| | Colon | 29.04$^{a}$ | 34.55$^{b}$ | 36.11$^{b}$ | 37.25$^{c}$ | 42.24$^{d}$ | 44.95$^{d}$ | 47.14$^{e}$ | 65.39$^{f}$ | 4.19 | < 0.01 |
| Proliferative cells (per mm of crypt) | Duodenum | 38.41$^{b}$ | 42.91$^{c}$ | 43.74$^{c}$ | 46.19$^{d}$ | 36.29$^{b}$ | 39.57$^{b}$ | 39.86$^{b}$ | 34.79$^{a}$ | 1.45 | < 0.01 |
| | Jejunum | 32.13$^{b}$ | 35.62$^{c}$ | 37.89$^{cd}$ | 39.27$^{d}$ | 30.61$^{b}$ | 31.46$^{b}$ | 32.87$^{b}$ | 29.28$^{a}$ | 1.36 | <0.01 |
| | Ileum | 27.57$^{b}$ | 31.73$^{c}$ | 33.54$^{d}$ | 37.28$^{e}$ | 26.12$^{b}$ | 28.99$^{b}$ | 29.15$^{b}$ | 24.85$^{a}$ | 1.54 | < 0.01 |
| | Colon | 25.38$^{b}$ | 28.25$^{d}$ | 30.42$^{d}$ | 34.67$^{e}$ | 24.11$^{bc}$ | 26.43$^{bc}$ | 27.49$^{c}$ | 21.09$^{a}$ | 1.53 | < 0.01 |

*Results are demonstrated as mean ± s.e.m.*
*Difference of superscripts (a-f) within a row indicates significance difference P < 0.05.*
*BD, basal diet (negative for AFB1); AFB1 0.6, aflatoxin B1 6mg per kg of concentrate; AFB1 0.8, aflatoxin B1 8mg per kg of concentrate; AFB1 1.0, aflatoxin B1 10 mg per kg of concentrate; AFB1 0.6- YTB, aflatoxin B1 6mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; AFB1 0.8- YTB, aflatoxin B1 8mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; AFB1 1.0- YTB, aflatoxin B1 10mg per kg of concentrate plus 2mg of yeast based toxin binder per kg of feed; BD-YTB, basal diet plus 2mg per kg of yeast based toxin binder; SEM, standard error of mean.*

ing AFB1 concentrations but YTB supplementation decreased (P<0.05) there number and bought it back to normal levels as no difference for proliferative cells was observed between animals of AFB1 0.6-YTB, AFB1 0.8-YTB, AFB1 1.0-YTB and BD groups. However, BD-YTB animals had the least number (P<0.05) of proliferative cells in the selected intestinal segments compared to animals of other groups.

## DISCUSSION

Outcomes of YTB supplementation in BD and feed contaminated with different concentrations of AFB1 on intestinal morphology were studied in male buffalo calves. Impairments in the intestinal mucosa caused by AFB1 decreases its nutrient absorbing ability (Liu et al., 2011). Villus surface area depends upon the dimensions of villus (Hou et al., 2012), and higher growth rates are associated with greater villus

surface area (Awad et al., 2009), and increased villus: crypt ratio (Wu et al., 2004), which is in agreement with our results. Aflatoxin B1 induces alterations in intestinal microarchitecture (Yang et al., 2012), such as short villi, deep crypts, reduced villus: crypt ratio and surface area (Wan et al., 2013). This study confirms previous reports by Dogi et al., (2011), and Motawe et al., (2014), that YTB supplementation nullifies the harmful effects of AFB1 on intestinal morphology. Yeast cell wall (YCW) binds with AFB1, reduces its absorption in intestine and thus is useful in protecting ruminants from detrimental effects of AFB1 (Firmin et al., 2011). Yeast improves intestinal microarchitecture by increasing concentration of useful microbes present in the intestine and suppressing the concentration of pathogenic bacteria thus increasing growth performance of animal (Gao et al., 2008). It was also observed that YTB supplementation improved intestinal microarchitecture in BD-YTB animals compared to BD, similar results were seen in duodenum and jejunum of broilers by Gao et al., (2008), and in duodenum of pigs by Shen et al., (2009), who reported that YCW supplementation increases villus height and villus: crypt ratio.

The IELs play an important role in the regulations of immune response as they form specialized lymphoid compartments and are the first cells to encounter antigens if intestinal lumen (Finamore et al., 2008). Increased number of IEL in all the selected intestinal segments are results of intestinal inflammatory response (Quinteiro-Filho et al., 2010), towards AFB1. This increase in IEL count may also be associated with lesions (O,Handley et al., 2001), caused by AFB1. Beta-glucans (ß-glucans) are carbohydrates having linked glucose molecules which are major components of yeast cell wall (Volman et al., 2008). These components have the ability to enhance mucosal immunity of intestine (Battilana et al., 2001), as after oral administration ß-glucans regulate immune cells of Peyer's patches and IELs (Suzuki et al., 1990;Tsukada et al., 2003). Supplementation of YTB decreased number of IEL in intestinal mucosa compared to AFB1. Intestinal motility is due to stimulation of parasympathetic nerves, since lymphocytes carry cholinergic receptors it can be assumed that stimulation of parasympathetic nerves then activate the mucosal immune system in the intestine leading to an increase in IEL number.

Highly viscous mucus layer that covers intestinal mucosa, helps in lubrication of ingested food and

**Fig 5.** Intra epithelial lymphocytes in the Duodenum of calves fed AFB1 0.8- YTB.

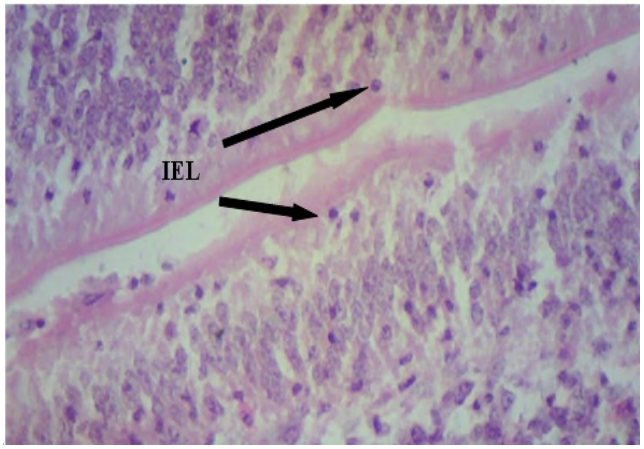



**Fig 6.** Histological section of duodenum showing villus height of calves fed AFB1 0.8- YTB.

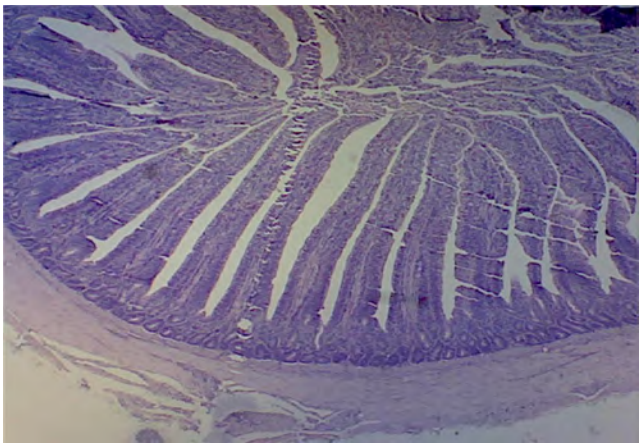
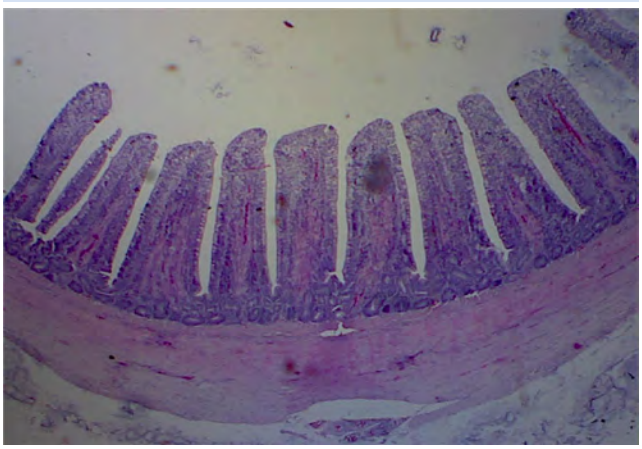


**Fig 6.** Histological section of duodenum showing villus height of calves fed AFB1 0.8.



protects intestinal epithelium by acting as a physical barrier to pathogens is secreted by GC (Kim and Ho, 2010). Goblet cells are responsible for secretion of mucin which after hydration becomes mucus (Cone, 2009). Number of GC in intestinal epithelium may vary with challenging substances and diet (Machado-Neto et al., 2013). Number of intestinal GC on exposure to AFB1 increased in our study, similar results were observed by Kenawy et al., (2009), who observed an increased GC number in intestine after exposure to AFB1 contaminated diet. Moreover, addition of YCW increases GC number in intestine (Chee et al., 2010; Morales-Lopez et al., 2010; Muthusamy et al., 2012), but there is no consensus on whether increase in GC number is considered an improvement in animal health or not (Lea et al., 2013).

To our knowledge no data is present regarding histochemistry of GC and immunohistochemistry of proliferative cells in male buffalo calves exposed to AFB1 or supplemented with YTB. Mucins secreated by goblet cells are either acidic, neutral or mixed in nature. In fish GC having acidic mucins protect intestinal epithelium against chemical agents while GC having neutral mucins provide protection against chemical agents (Cruz et al., 2014). Factors that lead to an increase in GC having acidic and neutral mucin under the influence of AFB1 or YTB supplementation are yet to be investigated. However increased number of GC having acidic or neutral mucin in response to YTB supplementation indicates greater intestinal protection against pathogens in male buffalo calves.

Amplified villus length is associated with an increase in number of proliferative cells as longer villi indicate faster proliferation in intestinal crypts (Wu et al., 2013). Decline in proliferative cells number reduces the amount of energy required for maintaining microarchitecture of gut (Masanetz et al., 2010). Yet the exact mechanisms that caused a diminution in proliferative cells number after YTB supplementation are needed to be explored.

## CONCLUSIONS

Collectively, results of the current study confirm that feeding aflatoxin B1 (AFB1) contaminated diet exerts deleterious effects on intestinal microarchitecture. Supplementation of 2mg/kg yeast cell wall based toxin binder (YTB) not only alleviates harmful effects on intestinal microarchitecture induced by AFB1 but also supports modulations in defense system of male buffalo calves. Therefore, use of YTB in AFB1 contaminated feed is beneficial and recommended.

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