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RESEARCH ARTICLE

# Dose Optimization of Zeolite and Its Effect on Aflatoxin Detoxification, Dry Matter Intake, Nutrients Digestibility, Production Performance and Milk Fatty Acids Profile of Nili Ravi Buffaloes

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**A B S T R A C T**

The study was conducted on dose optimization of zeolite and its effect on aflatoxin B1 detoxification, nutrients intake and digestibility and dairy performance of Nili Ravi buffaloes. A total of 20 Nili Ravi buffaloes with 500±30kg BW at same milking stage and identical age were distributed randomly into 5 groups (r= 4) fed on aflatoxin B1 (AFB1) and different doses of zeolite powder in total mixed ration (TMR). Diet A (TMR – Control), Diet B (TMR+25µg AFB1/kg feed), Diet C (TMR+25µg AFB1+10g zeolite powder/kg feed), Diet D (TMR+25µg AFB1+15g zeolite powder/kg feed), Diet E (TMR+25µg AFB1+20g zeolite powder/kg feed. The results showed that binding capacity of zeolite for AFB1 was significantly ($P<0.05$) higher (87.4%) at 15g supplementation as compared to 10 and 20g supplementation in faeces while it was 83.3% in urine. Higher DMI (14.5kg/animal/day) and nutrients digestibility (%) were observed at 15g zeolite supplementation. Blood profile (mg/dL) significantly ($P<0.05$) increased while HDL and total cholesterol decreased at 15g zeolite supplementation. Significantly ($P<0.05$) improved milk yield (13.3L) and composition and milk fatty acids profile were found at 15g zeolite supplementation. Carry-over rate of AFB1 to AFM1 in milk was significantly $P<0.05$) lower (0.14%) at 15g zeolite supplementation. It was concluded that lower carry-over rate of aflatoxin M1 in milk and improved dairy performance of Nili Ravi buffaloes are attributed to dietary supplementation of zeolite at 15g/kg feed.

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## INTRODUCTION

Zeolite is a natural mineral and its dietary supplementation to dairy animals mitigates economic losses due to mycotoxicosis in dairy industry to improve livestock health and subsequently performance of dairy animals. Livestock sector and particularly dairy industry which is a significant fragment of agriculture in Pakistan contributes about more than 14% to national GDP. More than 35% of income in Pakistan is created by rural engagement in livestock sector. According to (Economic Survey of Pakistan, 2024-2025), 53.4million (M) cattle, 43.7M buffaloes, 31.9M sheep and 82.5M goats are total dairy herd. Milk is one of the vital commodities with about 65.5million tones annual production, which is very low and putting Pakistan in the 4th number in global ranking. This low production is due to several reasons and vital significant factor is feed and its ingredients contamination with mycotoxin. Presently, this is an alarming situation in the feed industry because cost of animal feed accounts for more than 70% of total livestock productivity, which is indeed a question mark and necessitates a solution.

Mycotoxins are natural metabolites of toxigenic fungi which are toxic and infect food and feedstuffs and affect animal health and performance. *Aspergillus, Fusarium* and *Penicillium* are the foremost fungi producing mycotoxins. *Fusarium* affects crops in field and produce mycotoxins while *Asperigillus* and *Penicilium* affect cereals and feeds in storage (Hamad *et al.*, 2023). Climatic elements like stress on plants and damage of cereal grains commonly by rodents, pests, moisture

contents and pH of feed are responsible for mycotoxins formation (Mobashar, 2023). Consumption of contaminated feeds by animals may cause mycotoxicosis and consequently, huge livestock industry losses. Mycotoxin contamination of dairy feeds in Pakistan has been reported repeatedly and pose serious constriction to animal health as well as productivity (Kemboi *et al.,* 2020). The reason is that some mycotoxins like AFB1 and its metabolite (AFM1) are released into milk.

Different approaches of physical, chemical and biological nature are being practiced to mitigate drastic impacts of mycotoxins (Cui *et al.*, 2025). However, these methods have negative side effects such as toxic residues in products, changing nutritional value of feeds, undesirable flavor of treated commodities; environmental pollution aspects and also they are very costly because of the use of highly sophisticated equipment for their production (Pandey *et al.*, 2023; Llorens *et al.,* 2024). Therefore, the application of zeolite which is a natural mineral is the best substitution for prevention and control of fungal growth in dairy feed and subsequently formation of mycotoxins. The use of zeolite in dairy feed is safe, eco-friendly, economical, easy availability and there are no toxic residues in commodities (Wafaa *et al.,* 2022). Structurally zeolite is a crystal with three dimensions and is characterized by porosity, large surface area and total cations. These dynamic characteristics of zeolite make its greater affinity to adsorb aflatoxins and heavy metals on its surface and consequently reduce toxicity (Alotaibi *et al.,* 2025). Therefore, the present study was conducted on zeolite to investigate its detoxification potential dose optimization of zeolite and its effect on detoxification of AFB1, DMI, digestibility and dairy performance of Nili Ravi buffaloes.

## MATERIALS AND METHODS

**Location of Study, Duration and Ethical Statement:** The experiment was conducted at Military Dairy Farm, Peshawar-Pakistan. Due to availability of animals for trial, feeding and watering facility, and smooth carrying-out of the animal trials, this farm was selected for study. Geographically, Peshawar is located at 34.0151° N latitude and 71.5249° E longitude, with an elevation of 331meters (1,086feet) above sea-level. The region is characterized by a hot and humid tropical climate, with ambient temperatures ranging from 5°C in winter to 45°C in summer. The trial lasted for 45 days for data collection and 10 days for adaptation period. All animal handling and experimental protocols were conducted in accordance with ethical standards approved by the Biomedical Research Ethics Committee, The University of Agriculture, Peshawar, (UAP), Pakistan via letter no.10/FAHV&VS/2023.

**Animals, Treatment and Study Design:** Completely Randomized Block Design (RCBD) was used for the study to optimize zeolite dose for the best outcomes in vivo. A total of 20 Nili Ravi buffaloes with 500±30kg BW with same parity stage and age were randomly distributed into 5 groups (r=4). Each animal was individually housed in a pen size of 130–160sq. ft in a partially roofed system. Medication for ecto and endo parasites before starting the trial was completed for animals. Total mixed ration (TMR) was formulated according to (NRC, 2001). AFB1 was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and was mixed thoroughly with a small and uniform portion of feed using a high-shear mixer to form a premix. This premix was then systematically incorporated into the bulk ration using a mixer to ensure consistent distribution. Zeolite was purchased from SMA Syndicate Ltd, Faisalabad Pakistan and its chemical composition is given in Table 1. Same procedure for mixing of zeolite in diet was followed as for AFB1 mixing in diet. Each animal received 25µg AFB1/kg diet. There were five dietary treatments; Diet A (control- (basal diet only), Diet B (TMR+ 25µg AFB1/kg feed), Diet C (TMR+25µg AFB1+ 10g zeolite powder/kg feed), Diet D (TMR+25µg AFB1 + 15g zeolite/kg feed, Diet E (TMR+25µg AFB1+20g zeolite/kg feed). Experimental diets were offered to animals 2 times a day.

**Table 1:** Ingredients and chemical composition of total mixed ration for Nili Ravi buffaloes

| Ration ingredients | Incorporation level (%) |
|---|---|
| Green roughages (maize silage and green fodder) | 28-38 |
| Wheat straw | 8-17 |
| Corn grains | 16-21 |
| Wheat bran | 10-16 |
| Cotton seed cakes | 14-20 |
| Molasses | 4-6 |
| Mineral mixture (Ca, P and trace minerals) | 1.5-2.5 |
| Sodium chloride salt | 0.5-1.0 |
| Rumen protected fat | 1.5-2.5 |
| Chemical composition (%) | |
| Components | Percentage |
| DM | 50-70 (as fed basis) |
| CP | 16-18 |
| TDN | 60-70 |
| NDF | 32-38 |
| ADF | 20-24 |
| EE | 2.5-5.5 |
| Trace minerals | 5-8 |
| Ca | 0.6-0.8 |
| P | 0.40-0.45 |

**Table 2:** Chemical composition (%) and cation exchange capacity (mEq/100g) of zeolite

| Components % | Zeolite |
|---|---|
| $Al_2O$-Aluminum oxide | 13.5 |
| $SiO_2$-Silicate oxide | 64.5 |
| $Fe_2O_3$-Feric oxide | 2.32 |
| CaO-calcium oxide | 3.51 |
| MgO-Magnacium oxide | 1.02 |
| $Na_2O$- Sodium oxide | 1.38 |
| $K_2O$-Pottasium oxide | 1.35 |
| $TiO_2$-Titanium oxide | 0.14 |
| Cations Exchange capacity (CEC) | 152.2 |

**Dry Matter Intake and Nutrients Digestibility:** DM intake of individual animal was calculated by subtracting feed refusal from offered feed. Daily feed offered and feed leftover of each animal was weighed and recorded and feed samples were collected in polythene bags and stored. Nutrients digestibility was determined by using total collection method. Feed samples from individual animal were collected in polythene bags and labeled with treatment name, individual animal number and day. Sample collection was taken every day and also during last week of trial. To minimize variability, and reduce large feed sample in order to obtain representative and composite sample, sub-sample was taken from all spots of treatment. These

samples were mixed properly, carefully and systematically to obtain a representative sample for laboratory analysis. Feed samples on daily basis from all animals in each treatment were mixed. 5% of the representative feed sample was taken in polythene bags. Similarly, 10% representative faecal sample during last week of trial was taken in polythene bags. Both samples were stored at-20°C till analysis. Proximate analysis of feeds and faeces was conducted in laboratory of Department of Animal Nutrition, UAP. The feed samples were dried in oven at 60ºC. Dried samples were ground by Thomas Willy miller, using 1mm sieve and used for determination of DM,CP content and ether extract (AOAC, 2005) while NDF, ADF and ADL were analyzed using procedure of Van Soest (1991). The digestibility percent was calculated by equation (nutrients consumed-excreted) divided by total nutrient utilized multiplied by 100.

**Determination of AFB1 in Faecal and Urine Samples:** Urine was collected over 24hrs which was very difficult, but it was managed by two skilled workers performing alternatively during day and night. It is more precise and accurate compared to spot urine sampling. Daily urine excretion was about 4 to 8L/ animal. Faecal samples were obtained from the experimental animals in different treatments. Samples were oven dried for 24hrs. and used for AFB1 extraction. Extraction solution was prepared by adding 70 mL methanol and 30 mL of distilled water. Dried representative faecal sample was ground to a particle size of 1mm. A ground sample of about 20g was added in 100mL of extraction solvent. Ratio between sample and solvent was 1:5 (w/v). This mixture was sealed in a beaker for 2min to settle. Mixture was filtered using Whatman no.1 paper. 10mL of filtrate extract was collected and used for quantification for AFB1 using Elisa Kit (Ngaira *et al.,* 2013).

Pooled urine samples were taken into a beaker and stirred with a stir bar for 10min. After homogenization for 20min, sample was centrifuged at 807xg for 10min. Centrifuged urine samples were stored at -80ºC and thawed in a water bath. Blank urine samples/control were centrifuged at 807xg for 10min. Seven control urine samples of 2mL were weighed into 15mL conical tubes. 4, 8, 16, 40, 80 and 120µL of 250ng/mL standard solution of AFB1 and AFM1 were added to 2mL control urine. The obtained fortified samples were mixed by vortexing for 10min. For extraction, samples were added with 4mL 80/20 (v/v) methanol/water and vertexed at 349 xg for 5min. 2mL solution was pipetted into a 50mL plastic centrifuge tube and was diluted with 14mL 1X PBS solution and was vortexed for 5 min. AFB1 was analyzed in samples using TLC according to method used at VRI, Peshawar.

**Analysis of AFM1 in Milk:** Milk samples were analyzed for AFM1 contamination using an immunoaffinity column for clean-up according to method of Grosso *et al.* (2004). The milk samples (100mL) were centrifuged at 224 xg for 15min and the upper fat layer was discarded. The skimmed milk was passed through an immune-affinity column (AFLAPREP M, R-Biopharm Rhône Ltd). The column was washed with water (40mL) to remove extraneous non-specific material. Following, the AFM1 bound to the antibody was released by the elution with 2.5mL acetonitrile-methanol (3:2;v/v) and 2.5mL methanol. The elute was evaporated to dryness using a stream of N2. The TLC procedure was developed, dissolving the AFM1 residue in 150µL of toluene-acetonitrile (9:1;v/v). On the TLC plate (TLC aluminum sheets, 20x20 cm, Silica gel 60), 50µL of sample and spots of working standard were applied. The plate was developed in chloroform-acetone-isopropanol (87:10:3; v/v). After the plate had dried, it was examined under long wave light (366nm) and finally concentration and chemical identity of AFM1 was determined. Recovery tests were performed to determine the efficacy of analytical method by spiking aflatoxin raw milk samples with known amounts of AFM1 and submitting them to free extraction procedures in 5 replicates. AFM1 standard was purchased from Sigma Chemical Co (St Louis, MO, USA).

**Blood Profile:** It was analyzed using modifications in method of Teneva *et al.* (2021) used at National Veterinary Laboratory, Islamabad, Pakistan. About 10mL of blood sample was taken from jugular veins in EDTA tubes before morning feeding and was clotted for 24hrs at 4°C and to obtain serum, blood sample was centrifuged for 10min at 580xg. After freezing at−20°C, serum was analyzed for total protein, glucose, blood urea nitrogen (BUN), total cholesterol, low-density lipoprotein (LDL) and high-density lipoproteins.

**Milk Yield and Milk Composition:** From all experimental animals in each treatment, daily milk yield was recorded two times a day (5:00am and 5:00pm). The samples were hygienically collected without any foreign contamination from morning (05:00am) and evening milking (17:00pm). Morning and evening milk samples were mixed. Then 10-20mL representative milk sample from a particular day was taken and analyzed for AFM1 and milk composition. At each milking, milk yield was recorded. A small and well-mixed aliquot from that milking was taken. For obtaining a representative daily sample, milk aliquot from morning and evening was pooled in proportion to their respective milk yields**.** It was mixed thoroughly and split into subsamples for AFM1) analysis and milk composition. Similarly, faecal and urine sampling was collected over a 24-hour period (from 05:00 to 05:00 next day) by total collection (metabolic cages) at several time points across the day. Samples were pooled and homogenized from that period to form a single 24hrs composite sample per animal. An aliquot from the homogenized pool was taken for AFM1 analysis. All samples were analyzed at Center of Animal Nutrition, VRI, Peshawar for milk lactose, fat, protein, total-solids and solid not fat (SNF) using Lactometer. About 2-3mL of milk sample was taken in aluminum flask for analysis of total solids and was kept in oven for 3hrs at 60°C for drying, After cooling for 15min and weighing. Total milk solids were determined by dividing dried milk sample over fresh milk sample multiplied by 100.

SNF was analyzed using method of (Harding, 1995) by subtracting fat content from total solids.

**Milk Fatty Acids Profile:** It was determined at PCSIR Laboratory, Peshawar-Pakistan according to standard

procedure used at laboratory. Each four representative milk samples of animals from all treatments were kept in a water-bath at 40°C and stirred wisely to avoid frothing. Fatty acids were converted into FAME after extraction. Mixture prepared (2mL milk, 2mL $NH_4OH$, 1mL ethanol, and 50mg pyrogallic acid) was kept in water-bath for 20min at 70°C and added internal standard C10:1 cis-4 FA and 4mL hexane/isopropanol. Hydrolysate oscillated was centrifuged at10,000 × g, 4°C for 5min for lipid extraction. Extracted hexane was mixed with 2 mL methanolic NaOH, sealed, and heated at 50°C in a water bath followed by an acid-methylation at 80°C using 2mL acetyle chlorocarbinol. Hexane was added with 5 ml ultra-pure water and diluted and analyzed by GC-MS (Serafim *et al.,* 2019).

Agilent 7890A GC system equipped with a 7000B MS detector (Agilent Technologies) and a capillary DB-WAX UI column (30m×0.25mm×0.25µm) was used for fatty acids analysis. 1 µL was injected volume and for gas carrier, helium was used at 7psi pressure. The inlet temperature was 250°C while temperature in oven was maintained at 60°C for 2min, 120°C, 160°C for 40min, 200°C for 20min and finally at 240°C for 8min. Transfer line temperature was set to 250°C. MS ion source temperature was 230°C, and energy was 70 eV. FAME quantification and identification was based on retention time and characteristic ions. FAME was quantified using external and internal standards based on quantification ion peak area. FAME concentration was determined by GC-MS as FA mass percentages, %).

AFB1 binding efficacy of zeolite is computed by looking at how much AFB1 is consumed by an animal and how much AFB1 is recovered as AF in milk, faeces and urine. In our case, AFB1 intake (µg/day) in 24hrs by each animal was calculated as animal AFB1intake=DMI (kg/day)×AFB1 concentration in feed ( µg/kg DM). AFB1 recovered (µg/day) was calculated by summing Faecal AFB1, milk AFM1 and urine AFM1 while bound AFB1 was estimated by subtracting recovered AFB1 from animal AFB1 intake. Animal AFB1 binding efficiency (%) was calculated by dividing bound AFB1 by intake of AFB1 multiplied by 100. While binding capacity of zeolite was computed by dividing bound AFB1 over animal AFB1 intake.

Determination of AFB1 was carried out by Thin Layer Chromatography (TLC) according to standard procedure used at Veterinary Research Institute (VRI) Peshawar-Pakistan.

**Statistical Analysis:** The final data was recorded in Microsoft Excel Worksheets for sorting and basic statistics. Data was inserted in excel sheet and analyzed using Two Way RCBD ANOVA design in SPSS statistical program version-20. Mean values were compared by Tukey Test at 0.05 level of significance. Duncan multiple range tests were applied to separate the means. The following model was used: There were 4 replicates per treatment.

$Yij = \mu+Ti+\beta j+€ij$

Yij = Yield or Response of the Treatment

µ- Overall Mean

Ti- Treatment Effect

βj- Block Effect

€ij- Random Error

## RESULTS

In the present study, in comparison to control diet, addition of 25µg AFB1/kg feed caused clear reduction in animal production and physiological status of animal. Particularly, animals receiving only AFB1 depicted lower digestibility, reduced serum biochemistry, lesser milk yield, and impaired milk composition and fatty acid profile. Moreover, AFB1 detoxification was also adversely affected. These observations confirm the drastic effects of AFB1 contamination and highlight this group as an appropriate negative control group for investigating efficacy of different doses of zeolite.

With inclusion of zeolite in AFB1 contaminated diets, a general enhancement was observed in study parameters as compared to negative control group. Irrespective of zeolite inclusion level, its supplementation alleviated the negative effects of AFB1, indicating its vital role, which is possibly linked with adsorption and decreased AFB1 bioavailability. Further improvements were observed with increasing levels of zeolite supplementation up to some extent. Among the challenge doses, the inclusion of 15 g zeolite/kg feed showed the most predominant results. This moderate level resulted in increase in nutrient digestibility, improved blood parameters, greater AFB1 detoxification, and higher dairy performance, milk composition, and fatty acid profile. Though 10g/kg and 20g/kg presented key effects too compared to the negative control, the 15g/kg zeolite level seemed to be optimal in this study. Detailed results on these parameters are given in the following.

**Binding Capacity of Zeolite for Aflatoxin B1 *In Vivo*:** Binding capacity (%) of zeolite for AFB1 in faeces and urine significantly ($P<0.05$) varied among different dietary treatments fed to Nili Ravi buffaloes. Binding capacity of zeolite for AFB1 was higher in faeces (87.4%) and urine (83.3%), respectively at 15g zeolite supplementation/kg feed followed by 20g and 10g zeolite supplementation in diet (Table 3).

**Table 3:** Optimization of different doses of zeolite for binding capacity of aflatoxin B1 in vivo (means±SE)

| Treatment | Binding capacity % in faeces | Binding capacity % in urine |
|---|---|---|
| TMR-control diet | $4.0^{d}$±0.87 | $2.8^{d}$±0.17 |
| TMR+25µg AFB1/kg diet | $3.7^{d}$±0.23 | $2.5^{d}$±0.46 |
| TMR+25µg AFB1+10g zeolite/kg feed | $58.1^{c}$±2.29 | $24.4^{c}$±1.82 |
| TMR+25µg AFB1/kg diet+15g zeolite/kg feed | $67.3^{a}$±5.51 | $32.3^{a}$±3.2 |
| TMR+25µg AFB1/kg diet+20g zeolite/kg feed | $63.4^{b}$±3.95 | $28.4^{b}$±1.23 |
| P-value | 0.007 | 0.003 |

Mean values with different superscripts within same columns are significantly different at 0.05 significant level. SE, standard error, TMR, total mixed ration

**Dry Matter Intake and Nutrients digestibility:** Results on effects of different doses of zeolite on dry matter intake and nutrients digestibility of Nili Ravi buffaloes are given in Table 4. DMI (kg) and nutrients digestibility (%) significantly ($P<0.05$) increased among dietary treatments. Significantly higher DMI (14.5kg/day/animal) and improved nutrients digestibility were reported with 15g zeolite dietary supplementation (Table 4).

**Blood Profile:** Blood profile of Nili Ravi buffaloes was presented in Table 5. Blood urea nitrogen (BUN) (mg/dL), total blood proteins (mg/dL), blood glucose (mg/dL) significantly (P<0.05) improved. HDL and total cholesterol (mg/dL) significantly (P<0.05) reduced at 15g zeolite supplementation in diet followed by 10g zeolite supplementation (Table 4).

**Milk Yield and Milk Composition:** Milk yield (L) and milk composition (%) significantly (P<0.05) increased among the dietary treatments (Table 6). Higher milk yield (13.3L) and improved total solids (16.7%), solid not fat (SNF) (10.5%), fat content (6.8%) and lactose (5.1%) were observed at 15 g zeolite supplementation/kg feed while milk protein remained similar across the treatments (Table 6).

Effects of various doses of zeolite supplementation on milk fatty acids of Nili Ravi buffaloes are presented in Table 7. Concentration of saturated fatty acids (SFA%), momo unsaturated fatty acids (MUFA%), poly unsaturated fatty acids (PUFA%), medium chain fatty acids (MCFA%) and long chain fatty acids (LCFA%) significantly (P<0.05) changed among the dietary treatments. Improved concentration of SFA (65.4%), MUFA (25.3%), PUFA (4.8%), MCFA (57.9%), SCFA (7.8%) and LCFA (38.4%) was found in milk with 15g zeolite dietary supplementation/kg feed (Table 7).

**Carry-Over Rate of Feed Aflatoxin B1 to Milk Aflatoxin M1:** Carry-over rate of feed AFB1 to milk aflatoxin M1 (AFM1) is shown in Table 8. Carry-over rate (%) of AFB1 to AFM1 significantly (P<0.05) differed among the dietary treatments (Table 7). Carry–over rate ranged from (0.21 to 0.81%) among the treatments showing the lower carry-over rate (0.14%) of mycotoxin from feed to milk at 15g zeolite/kg feed supplementation as compared to other treatments (Table 8).

**Table 4:** Effect of different doses of zeolite on dry matter intake and nutrients digestibility of Nili Ravi buffaloes (Mean values± SE)

| Treatment | DMI kg | DMD% | CPD% | NDFD% | ADFD% |
|---|---|---|---|---|---|
| TMR-control diet | 13.2$^{c}$±0.35 | 65.4c±0.87 | 72.1$^{c}$±0.92 | 40.5c±0.96 | 25.8$^{c}$±0.75 |
| TMR+25µg AFB1/kg diet | 12.3$^{d}$±0.34 | 62.3$^{d}$±1.25 | 66.4$^{d}$±1.23 | 37.3$^{d}$±0.98 | 23.7$^{d}$±0.40 |
| TMR+25µg AFB1/kg diet+10g zeolite/kg feed | 14.1$^{b}$±0.40 | 67.1$^{b}$±1.27 | 72.6$^{b}$±1.04 | 42.0$^{b}$±1.09 | 27.1$^{b}$±0.87 |
| TMR+25µg AFB1/kg diet+15g zeolite/kg feed | 14.5$^{a}$±0.40 | 68.3$^{a}$±1.09 | 73.5$^{a}$±1.20 | 43.6$^{a}$±0.75 | 28.2$^{a}$±0.98 |
| TMR+25µg AFB1/kg diet+20g zeolite/kg feed | 13.4$^{c}$±0.46 | 64.5$^{c}$±0.92 | 71.8$^{c}$±1.52 | 41.3$^{c}$±0.98 | 26.3$^{c}$±0.69 |
| P-value | 0.006 | 0.03 | 0.05 | 0.04 | 0.02 |

Mean values with different superscripts within columns are significantly different at 0.05 significant level SE, standard error

**Table 5:** Effect of different doses of zeolite on blood profile of Nili Ravi buffaloes (Mean±SE)

| Treatment | BUN (mg/dL) | Total blood proteins (mg/dL) | Blood glucose (mg/dL) | LDL (mg/dL) | HDL (mg/dL) | Total Cholesterol (mg/dL) |
|---|---|---|---|---|---|---|
| TMR)-control diet | 26.4$^{b}$±1.47 | 7.6$^{c}$±0.82 | 45.6$^{c}$±0.31 | 83.6$^{c}$±1.25 | 46.4$^{b}$±1.18 | 110.4$^{b}$±1.14 |
| TMR+25µg AFB1/kg diet | 29.2$^{a}$±1.07 | 5.9$^{d}$±0.43 | 65.7$^{a}$±0.72 | 87.2$^{a}$±1.14 | 41.3$^{d}$±0.83 | 143.9$^{a}$±1.19 |
| TMR+25µg AFB1/kg diet+10g zeolite/kg feed | 24.3$^{c}$±0.79 | 8.5$^{b}$±0.36 | 46.3$^{c}$±0.81 | 84.2$^{c}$±1.17 | 45.7$^{b}$±0.81 | 95.2$^{c}$±0.74 |
| TMR+25µg AFB1/kg diet+15g zeolite/kg feed | 22.4$^{d}$±0.69 | 9.5$^{a}$±0.34 | 56.2$^{b}$±1.18 | 81.3$^{d}$±1.18 | 49.2$^{a}$±0.82 | 84.6$^{d}$±1.02 |
| TMR+ 25µg AFB1/kg diet+20g zeolite/kg feed | 25.7$^{b}$±0.73 | 8.0$^{c}$±0.32 | 38.1$^{d}$±1.22 | 85.3$^{b}$±1.27 | 43.2$^{c}$±1,28 | 109.5$^{b}$±0.84 |
| P-value | 0.006 | 0.03 | 0.05 | 0.04 | 0.02 | 0.008 |

Mean values with different superscripts within columns are significantly different at 0.05 significant level. Mean±SE, mean value± standard error
TMR, total mixed ration

**Table 6:** Effect of different doses of zeolite on milk yield and milk composition of Nili Ravi buffaloes (Mean± SE)

| Treatment | Milk yield/day/animal (L) | Total solids (%) | SNF (%) | Fat (%) | Protein (%) | Lactose (%) |
|---|---|---|---|---|---|---|
| Total mixed ration (TMR)-control diet | 12.3$^{b}$±1.23 | 15.4$^{c}$±.56 | 10.2$^{c}$±0.56 | 6.3b±0.8 | 3.7±0.73 | 4.2$^{b}$±0.80 |
| TMR+25µg AFB1/kg diet | 11.2$^{c}$±0.85 | 13.5$^{d}$±13.5 | 8.5$^{d}$±0.72 | 5.4$^{c}$±0.58 | 3.6±0.78 | 3.1$^{c}$±0.59 |
| TMR+25µg AFB1/kg diet+10g zeolite/cow/day | 12.2$^{b}$±1.22 | 15.9$^{b}$±0.78 | 9.8$^{b}$±0.70 | 6.1$^{b}$±0.60 | 3.5±0.76 | 4.2$^{b}$±0.57 |
| TMR+25µg AFB1/kg diet+15g zeolite/kg feed | 13.3$^{a}$±30.74 | 16.7$^{a}$±0.57 | 10.5$^{a}$±0.49 | 6.8$^{a}$±0.67 | 4.1±0.79 | 5.1$^{a}$±0.66 |
| TMR+25µg AFB1/kg feed+20g zeolite/kg feed | 12.1$^{b}$±0.59 | 14.8$^{c}$±0.69 | 9.6$^{c}$±0.61 | 5.9$^{b}$±0.54 | 3.9±0.75 | 4.4$^{b}$±0.63 |
| P-value | 0.003 | 0.01 | 0.05 | 0.04 | 0.08 | 0.01 |

Mean values with different superscripts within columns are significantly different at 0.05 significant level. SE, standard error
TMR, total mixed ration

**Table 7:** Effect of different doses of zeolite on milk fatty acids profile of Nili Ravi buffaloes (Mean±SE)

| Treatment | SFA (%) | MUFA (%) | PUFA (%) | SCFA (%) | MCFA (%) | LCFA (%) |
|---|---|---|---|---|---|---|
| Total mixed ration (TMR)-control diet | 62.7$^{b}$±0.83 | 24.4$^{b}$±0.67 | 3.7$^{b}$±0.63 | 6.3$^{b}$±0.65 | 49.6$^{b}$±0.79 | 31.5$^{b}$±0.68 |
| TMR+25µg AFB1/kg feed | 45.2$^{d}$±0.79 | 14.3$^{d}$±0.69 | 2.3$^{d}$±.67 | 5.2$^{d}$±0.63 | 34.3$^{d}$±0.74 | 20.1$^{d}$±0.72 |
| TMR+25µg AFB1+10g zeolite/kg feed | 53.1$^{c}$±0.73 | 21.5$^{c}$±0.66 | 3.3$^{c}$±0.62 | 6.6$^{c}$±0.66 | 44.2$^{c}$±0.49 | 26.7$^{c}$±0.68 |
| TMR+25ug AFB1+15g zeolite/kg feed | 65.4$^{a}$±0.66 | 25.3$^{a}$±0.54 | 4.8$^{a}$±0.69 | 7.8$^{a}$±0.63 | 57.9$^{a}$±1.02 | 38.4$^{a}$±0.89 |
| TMR+25µg AFB1+20g zeolite/kg feed | 63.2$^{b}$±0.69 | 23.8$^{b}$±0.77 | 3.5$^{b}$±0.42 | 6.1$^{b}$±0.61 | 50.2$^{b}$±0.75 | 32.1$^{b}$±0.76 |
| P-value | 0.03 | 0.01 | 0.004 | 0.01 | 0.006 | 0.02 |

SFA, saturated fatty acids; MUFA, momo unsaturated fatty acids; PUFA, poly unsaturated fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; SE, standard error.

**Table 8:** Effect of different doses of zeolite on carry-over rate of aflatoxin B1 to aflatoxin M1 in the milk of Nili Ravi Buffaloes

| Treatment | Carry-Over Rate (%) |
|---|---|
| TMR-control diet | 0.43$^{b}$ |
| TMR+25µg AFB1/kg diet | 0.81$^{a}$ |
| TMR+25µg AFB1+10g zeolite/kg feed | 0.21$^{c}$ |
| TMR+25µg AFB1+15g zeolite/kg feed | 0.14$^{d}$ |
| TMR+25ug AFB1+20g zeolite/kg feed | 0.23$^{c}$ |
| P-value | 0.04 |

Mean values with different superscripts within columns are significantly different at 0.05 significant level AFB1, aflatoxin B1

## DISCUSSION

Among mycotoxins, AFB1 is one of the most intoxicating mycotoxins affecting animals predominantly in tropical and sub-tropical regions, but polygastrics are relatively less affected as compared to simple stomach animals due to presence of toxin degraders (rumen microbes). In the present study, a dietary inclusion of 25µg/kg feed is in principle considered low to moderate

exposure which is not acutely toxic. With inclusion level, acute aflatoxicosis is not expected like hepatotoxicity, nephrotoxicity or sudden death. However, this inclusion level has more tendency for chronic toxicity in ruminants like reduced dry matter intake, decreased weight gain, low milk production and AFM1 contamination in milk.

Generally, 25µg/kg feed hysterics into real practice. Under normal conditions, low mycotoxin contamination <20–25µg/kg is well-managed in feeding systems and is considered safe level. Moderate mycotoxin contamination (20–50µg/kg), this is the range where 25µg/kg feed used in the present study fits. At this level, dairy animals show chronic effects, when they are exposed for prolonged period. With mycotoxin contamination greater than 100µg/kg, animals show acute aflatoxicosis, with clear clinical diseases and possibly high mortality. Therefore, A level of 25µg/kg feed does not induce acute toxicity, but it is above ideal safety thresholds, especially for dairy animals, and can contribute to chronic health and productivity issues if exposure persists (Álvarez-Días *et al.,* 2022: Ajmal *et al.,* 2022; Zental *et al.,* 2023).

In the present study, 10, 15 and 20g zeolite inclusions /kg feed were used to optimize its dose in buffaloes to evaluate its effect on aflatoxin B1 (AFB1) detoxification, digestibility and dairy performance of Nili Ravi buffaloes. Binding capacity of zeolite for AFB1 was higher in faeces (87.4%) and urine (83.3%) at 15g zeolite supplementation in diet (Table 3). In fact, zeolite works by binding the mycotoxin within GI tract, preventing AFB1 absorption into the bloodstream and subsequent excretion via the kidneys into urine. The mycotoxin-zeolite complex is then eliminated in the faeces (Yang *et al.,* 2025).

In acidic and alkaline pH medium of digestive system, zeolite uses its large surface area and porous structure to physically and chemically bind to AFB1 via mechanisms like ion-dipole interactions. Once binding occurs, AFB1 form stable complexes, which cannot be cleaved or absorbed through the intestinal walls. The mycotoxin-zeolite complex, being indigestible and unabsorbable, travels the length of the GI tract and is ultimately expelled from body in faeces. This increased fecal excretion is a direct indicator of successful mycotoxin binding and reduced bioavailability (Choi *et al.,* 2025; Vila-Donat *et al.,* 2025). Since the primary function of effective zeolite binder is to prevent this initial absorption, concentration of mycotoxins in urine is consequently lower compared to amount bound and excreted in faeces (Kihal *et al.,* 2022). The present results on AFB1 binding capacity in faeces and urine are in agreement with (Yang *et al.,* 2025; Kihal *et al.*, 2022), who also observed similar pattern of AFB1 excretion in faeces and urine.

Important mechanism of zeolite for AFB1 detoxification is to be highly negatively charged aluminosilicate framework having greater positive ions exchange capacity and large porous surface area. Although, AFB1 mycotoxin is nonpolar, it interacts with zeolite via H-bond, and micropores. Binding occurs in rumen and lowers mycotoxin bioavailability**,** decreases intestinal absorption of AFB1, reduces hepatic conversion of AFB1to AFM1 and reduces AFM1 contamination in milk (Diaz *et al.,* 2004; Kabak *et al.,* 2006; Jouany, 2007).

Zeolite is an important feed additive for dairy animals, primarily due to its ability to act as a mycotoxin binder and a rumen buffer. This leads to improved feed intake, enhanced nutrient digestibility, and positive effects on dairy performance when used at appropriate levels (Ghoneem *et al.,* 2022; Alghamdi and Ali, 2026). It functions as toxin binder through its unique physicochemical features and is highly porous aluminosilicate with a high cation exchange capacity (CEC) (Kordala *et al.,* 2024). It has a net negative charge that attracts and binds positively charged ions, including toxins like AFB1 and heavy metals. This prevents toxins from being absorbed into blood stream, allowing them to be safely excreted in feces. Honeycomb-like structure of zeolite contains pores of specific sizes allowing it to trap toxin molecules (Zhao *et al.,* 2022). At moderate inclusion levels; 10 to 15g/kg feed), zeolite generally does not negatively affect dry matter intake (DMI) and may even enhance appetite by improving feed palatability and utilization. However, high dose (over 15 or 20g/kg feed) decreases DMI, possibly due to reduced palatability (Ghoneem *et al.,* 2022). Zeolite improves nutrient digestibility, particularly crude protein (CP) and fiber. By binding excess ammonia and releasing it gradually, it provides a more stable nitrogen source for rumen microbes, enhancing microbial protein synthesis and fiber digestion. It also helps stabilize ruminal pH, favorable for microbial growth and activity. Zeolite supplementation, especially at moderate level upto 15g, has been shown to increase milk yield (Frizzarini *et al.,* 2024). This is often associated with improved energy status and enhanced feed conversion efficiency. Studies generally indicate that zeolite has no significant negative effect on major milk components like fat, protein, and lactose percentages. Some suggest a tendency for increased milk protein concentration or total fat and protein yields (as a result of higher milk volume). Importantly, zeolite use can reduce the concentration of heavy metals and AFM1 in milk, improving quality and safety of dairy products (Khaklouf *et al.,* 2018; Su *et al.,* 2025).

In the rumen, zeolite affects ammonia buffering and nitrogen efficiency. It binds ammonium ions by acting slow-release nitrogen reservoir. This harmonizes nitrogen and energy availability and enhances microbial protein synthesis. Zeolite also stabilizes rumen pH by its cations exchange capacity and buffering potential and therefore improves cellulolytic bacteria population and prevents acidosis**,** reduces toxin load via extensive fibrous microbes and therefore, greater NDF digestibility. Zeolite changes ionic equilibrium in rumen and subsequently enhances digestive efficacy and nutrients absorption **(**EFSA, (2025); Katsoulos (2016) and Papaioannou *et al.* (2005).

In the present study, higher DMI, improved nutrients digestibility, increased milk yield and composition and higher milk fatty acids profile were reported. Zeolite modifies the ruminal microbial flora; this together with the mammary gland activity affects the milk fatty acids' biochemical mechanisms like rumen microbial biohydrogenation and enhances mammary lipogenic properties and subsequently milk fatty acids level (Hossain *et al.,* 2018; Souza and Ribeiro, 2021). The present results on these parameters are in line with

Frizzarini *et al.* (2024); Ghoneem *et al.* (2022); Khaklouf *et al.* (2018) who also reported higher level of these parameters and reduced carry-over rate of AFB1 from feed to AFM1 in milk at moderate level of zeolite supplementation.

Zeolite has indirect impact on milk production via reducing mycotoxin stress, enhances rumen fermentation, improves nutrients digestibility. These factors increase milk production from higher microbial protein synthesis providing increased metabolizable protein and energy from fiber and enhance net energy for milk yield. Zeolite stabilizes rumen pH and causes increased acetate production, which is very vital precursor for milk fat synthesis. It also increases microbial protein flow to small intestine and therefore, reduces AFM1 in milk via mycotoxin binding (Alic Ural, 2014; Khachlouf *et al.,* 2019)

In the present study, reduced value of BUN, HDL and total cholesterol and improved total blood protein, blood glucose of Nili-Ravi buffaloes were reported at 15g zeolite supplementation. BUN indicates urea as a waste product generated during protein metabolism by liver. This urea is transported to kidneys, which filter it out and excrete in urine. High level of BUN suggests that kidneys are not filtering waste efficiently (Bui *et al.,* 2025). AFB1 enhances BUN levels by affecting liver and kidney function, which disrupts normal protein metabolism. This disruption leads to alter amino acid metabolism and reduce total protein content in the blood. AFB1 is toxic to renal cells and can cause physical damage, which decreases kidneys' ability to filter waste products effectively. This reduced filtration capacity results in the accumulation of urea and creatinine in bloodstream, thus increasing BUN levels (Bui *et al.,* 2025).

Zeolite supplementation in animal models has been shown to improve insulin levels and glucose regulation**.** It works by reducing oxidative stress and protecting pancreatic tissue. Natural and synthetic zeolite showed a significant rise in insulin levels, bringing them closer to those of healthy control groups. Exposure to aflatoxins is linked to elevated blood glucose levels in present study and a higher risk of developing diabetes. This is primarily due to the toxin's interference with normal carbohydrate metabolism and insulin function **(**MIynarska *et al.,* 2025).

LDL is unhealthy cholesterol, while HDL is useful cholesterol. LDL carries cholesterol to arteries, where high levels can lead to plaque buildup and increase risk of heart disease and stroke. HDL carries cholesterol back to liver to be removed from body, and high levels of HDL can lower risk of heart disease. In the present study, zeolite form complex with bile acids in intestine and blocks formation of micelles needed for cholesterol absorption. By hindering micellization, zeolite effectively reduces cholesterol absorbed by body (Demirel *et al.,* 2011; Shin *et al.,* 2025). Low bile acids absorption prompts liver to use more circulating cholesterol to synthesize new bile acids, which increases LDL cholesterol uptake from blood and lowers its serum concentration (Hussein-Nia *et al.,* 2018). The present results on blood profile are in line with (Hussein-Nia *et al.,* 2018; Hussein *et al.,* 2021; Bui *et al.,* 2025), who also found similar pattern of increase and decrease with zeolite supplementation.

**Conclusions:** Various studies showed potential of zeolite as feed additive and toxin binder. Present study presented higher binding capacity of dietary supplementation of zeolite at 15g/kg feed for AFB1 in faeces as compared to urine. Greater dry matter intake and greater nutrients digestibility of Nili Ravi buffaloes were observed at 15g zeolite supplementation. Blood urea nitrogen, total blood protein, blood glucose and low density lipoprotein (LDL) increased while high density lipoprotein (HDL) and total cholesterol decreased at 15g zeolite supplementation Milk yield, milk composition and milk fatty acids profile increased at 15g zeolite supplementation/kg feed. Therefore, zeolite dietary supplementation at 15g/kg feed may potentially be used to reduce detoxification, improve nutrients intake and digestibility, blood profile, milk yield, composition and milk fatty acids profile of Nili Ravi buffaloes.

**Ethics Approval:** This research received ethical clearance from the Ethical Review Committee of the Faculty of Animal Husbandry and Veterinary Sciences at University of Agriculture Peshawar-Pakistan. under reference number.10/FAHV&VS/2023.

**Conflicts of Interest:** The authors do not have any conflicts of interest to declare.

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**Authors Contribution:** AK performed the research work, collected data on different parameters, MM designed research and original draft, and conducted the analysis of data. FA assisted in the statistical analysis and script of the manuscript. MM assisted in organization of data. NA made English corrections.

## REFERENCES

Alghamdi RA and Ali AQD, 2026. Effects of natural zeolite on growth performance, antioxidant status, and NRF2 and TGF-β1 gene detection in broilers. Pakistan Veterinary Journal 46(4):1008-1017.

Alotaibi AM, 2025. An overview of zeolites: From historical background to diverse applications. Molecules 30 (4): 1-17.

AOAC Assn. of Official Analytical Chemists, 2005. Official methods of analysis of Association of Official Analytical Chemists International 1-28.

Bui DH, Nguyen VT, Nguyen GTP *et al.,* 2025. Dietary *Saccharomyces cerevisiae* ameliorates the adverse effects of aflatoxin b1 on growth performance, haematological and biochemical parameters in broiler chickens. Applied Microbiology 5(3): (1-12).

Choi H, Garavito-Duarte Y, Gormley AR *et al.,* 2025. Aflatoxin B1: Challenges and Strategies for the Intestinal Microbiota and Intestinal Health of Monogastric Animals. Toxins 17 (4):1-31

Cui R, Pan A, Wang T, Liang Y and Yu HF, 2025. Aflatoxin B1 in animals: metabolism and immunotoxicity. Pakistan Veterinary Journal 45(3):923-934.

Demirel R, Yokus B, Demirel DE *et al.,* 2011. Effects of dietary zeolite on serum contents and feeding performance in rats. International Journal of Agriculture and Biology 13(3): 346-350.

Frizzarini WS, Monteiro PLJ, Campolina JP *et al.,* 2024. Mechanisms by which feeding synthetic zeolite A and dietary cation-anion difference diets affect mineral metabolism in multiparous Holstein cows: Part I. Journal of Dairy Science 107(7): 5204-5221.

Ghoneem WM, El-Tanany RR and Mahmoud AE, 2022. Effect of natural zeolite as a rumen buffer on growth performance and nitrogen

utilization of barki lambs. Pakistan Journal of Zoology 54(3):1199-1207.

Harding F, 1995. Milk quality. London: Blackie Academic & Professional Education pp: 165.

Hamad GM, Mehany T, Simal-Gandara J *et al.,* 2023. A review of recent innovative strategies for controlling mycotoxins in foods. Food Control 144 (2): 1-11.

Hossein-Nia B, Khorram S, Rezazadeh H *et al.,* 2018. The effects of natural clinoptilolite and nano-sized clinoptilolite supplementation on lipid profile, food intakes and body weight in rats with streptozotocin-induced diabetes. Advanced Pharmaceutical Bulletin 8(2): 211-216.

Khachlouf K, Hamed H, Gdoura R *et al.,* 2018. Effects of zeolite supplementation on dairy cow production and ruminal parameters–a review. Annals of Animal Science 18(4):857-877.

Khachlouf K, Hamed H, Gdoura R *et al.,* 2019. Effects of dietary Zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows. Journal of Applied and Advanced Research 47(1): 54–62.

Kemboi DC, Antonissen G, Ochieng PE *et al.,* 2020. A review of the impact of mycotoxins on dairy cattle health: Challenges for food safety and dairy production in sub-Saharan Africa. Toxins 12: 1-25.

Kihal A, Rodríguez-Prado M and Calsamiglia S, 2022. The efficacy of mycotoxin binders to control mycotoxins in feeds and the potential risk of interactions with nutrient: a review. Journal of Animal Science 100(11):1-14.

Kordala N and Wyszkowski M, 2024. Zeolite properties, methods of synthesis, and selected applications. Molecules 29(5):14-25.

Llorens P, Juan-Garcia A., Juan C *et al.,* 2024. Mycotoxins contamination and their possible mitigation strategies in plant-based meat alternatives (PBMAs): A review. Food Bioscience 62 (4):1-14.

Młynarska E, Grabarczyk M, Leszto K *et al.,* 2025. Environmental Insults to Glucose Metabolism: The Role of Pollutants in Insulin Resistance. International Journal of Molecular Sciences 26: 1-38.

Mobashar M, 2023. Mycotoxins incidence in animal feeds, their prevention and control measures. One Health Triad 2, 242-250.

Ngaira VM, Oduho GW, Masinde JW *et al.,* 2013. Assessment of aflatoxin in dairy feeds, milk, blood and evaluation of mitigation measures in North of Rift Valley counties, Kenya. African Journal of Education, Science and Technology 1(3): 211-216.

Pandey AK, Samota MK, Kumar A *et al.,* 2023. Fungal mycotoxins in food commodities: present status and future concerns. Frontiers in Sustainable Food Systems 7(1):1-21.

Serafim V, Tiugan DA, Andreescu N *et al.,* 2019. Development and validation of a LC–MS/MS-based assay for quantification of free and total omega 3 and 6 fatty acids from human plasma. Molecules 24(2): 1-11.

Shin MJ, Lee JH, Jang Y *et al.,* 2005. Micellar phytosterols effectively reduce cholesterol absorption at low doses. Annals of Nutrition and Metabolism 49(5): 346-351.

Souza JGD and Ribeiro CVDM, 2021. Ruminal biohidrogenation and main impact on met the fatty acid profile: a review. Research, society and Development 10 (13): 1-13.

Su X, Huang P, Guo Y and Cao J, 2025. Zeolite for preventing periparturient hypocalcemia in dairy cows: mechanisms and application strategies. Frontiers in Veterinary Science 12(3):1-15.

Teneva A, Stoimenov G, Hristov K *et al.,* 2021. Analysis of blood biochemical profiles of dairy cows and their calves from Bulgarian Brown Cattle Breed. Tradition and Modernity in Veterinary Medicine 6(2):78–83.

Van Soest PV, Robertson JB and Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583-3597.

Vila-Donat P, Sánchez D, Manyes L *et al.,* 2025. A. Impact of Bioactive Ingredients on the Fecal Excretion of Aflatoxin B1 and Ochratoxin A in Wistar Rats. Molecules 30, (647): 1-15.

Yang B, Deng H, Jia Y *et al.,* 2025. Validation of a Methodology for the Quantification of DON in Feces and Feedstuffs by UPLC as Possible Strategy to Evaluate the Detoxifying Efficacy of a Mycotoxin Adsorbent In Vivo. Toxins 17(7): 322.

Zhao M, Ma X, Chen D & Liao Y, 2022. Preparation of honeycomb-structured activated carbon–zeolite composites from modified fly ash and the adsorptive removal of Pb (II). American Chemical Society Omega 7(11): 9684-9689.