VETERINARY RESEARCH INTERNATIONAL
Journal homepage: www.jakraya.com/journal/vri

ORIGINAL ARTICLE

Effect of Diatomaceous Earth (DE) to Ameliorate Adverse Effects of Aflatoxin B_1 on *In Vitro* Rumen Fermentation of a Buffalo Diet

Ram Singh* and Ashwani Kumar Saini

Buffalo Nutrition Division, ICAR-Central Institute for Research on Buffaloes, Hisar-125001 (Haryana) India.

Abstract

The present investigation was undertaken to study the effect of diatomaceous earth (DE) in ameliorating adverse effects of aflatoxin B_1 (AFB_1) on rumen fermentation *in vitro*. Five treatment groups, viz. T_1: control (Basal feed); T_2: T_1+300 ppb AFB_1; T_3: T_2+0.33% DE; T_4: T_2+0.50% DE and T_5: T_2+1.0% DE were prepared and incubated *in vitro*. The results revealed that truly degradable dry matter (TDDM), truly degradable organic matter (TDOM), gas production (GP), microbial biomass production (MBP) and partitioning factor (PF) values in control group (T_1) was higher ($P<0.05$) than those of other treatment groups i.e. T_2 to T_5. The TDDM, TDOM, GP, MBP and PF values in aflatoxin contaminated group (T_2) was lower ($P<0.05$) than those of other treatment groups. The values of these parameters improved with increasing concentration of DE. The total volatile fatty acids (TVFAs), acetate (A), propionate (P) and butyrate (B) values in control group (T_1) was higher ($P<0.05$) than those of other treatment groups i.e. T_2 to T_5. The TVFA, A, P and B value in aflatoxin contaminated T_2 group was lower ($P<0.05$) than those of other treatment groups. The TVFA, A, P and B values improved with increasing level of DE, however, these values were lower ($P<0.05$) than control. The A:P ratio among various treatment groups (T_1 to T_5) did not vary significantly. It was concluded that aflatoxin contamination of feed (wheat straw) at 300 ppb level significantly affected the *in vitro* rumen fermentation in terms of reduced truly degradable dry matter, truly degradable organic matter, gas production, microbial biomass production, partitioning factor and total volatile fatty acids concentration. Inclusion of diatomaceous earth to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on *in vitro* rumen fermentation parameters.

Keywords: Diatomaceous earth**,** Aflatoxin, Buffalo, Rumen fermentation, *In vitro.*

*Corresponding Author:

Ram Singh
Email: carirsingh@yahoo.co.in

Received: 02/05/2020
Accepted: 29/05/2020

1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by animals. The Food and Agriculture Organization (FAO) estimated that 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses (Wu, 2007). Feed is the major input in animal production. Supply of high quality feed is must to obtain optimum performance. Presence of mycotoxins in feed is one of the major constraints in maintaining feed quality because the mycotoxins are widely present in feedstuffs around the world and may affect animal production even in very low concentration (Patil *et al.*, 2017a, b; Patil and Degloorkar, 2018). Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties. The most wide spread and most studied group of mycotoxins, aflatoxins are of great concern in warm and humid climatic conditions like India (Singh *et al.*, 2010; Singh, 2019e). The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which particular crop is grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are more prone to aflatoxin contamination than those in temperate zones. The most commonly used technique for reducing exposure to mycotoxins is to decrease their bioavailability by the inclusion of various mycotoxin-binding agents or adsorbents, which reduce mycotoxin uptake and subsequent distribution to the blood and target organs (Singh, 2019a, b, c, d; Singh and Saini, 2020; Silambarsan *et al.*, 2013, 2015, 2016). Decreasing the bioavailability of AFB_1 by the inclusion of binding agents is particularly effective, as this group of toxins has a chemical structure which favours adsorption (Silambarsan *et al.*, 2013), especially by materials of mineral origin such as clay and zeolites. A

binder should significantly prevent toxicity in animals and there should be no serious side-effects or, at least, the detrimental effects should not outweigh the benefits. Costs should render its use practical and profitable. Diatomaceous earth is fine-grained, biogenic siliceous sediment, and is available in large quantities at low cost (Kamigasa and Kato, 2000). Diatomaceous earth has shown the potential *in vitro* to bind aflatoxin, sterigmatocystin, T-2 toxin, zearalenone and ochratoxin (Natour and Yousef, 1998). The objective of the present study was to investigate the ameliorative effects of diatomaceous earth on adverse effects of aflatoxin on *in vitro* rumen fermentation of a buffalo diet.

2. Materials and Methods

2.1 Production and Analysis of Aflatoxin

Aflatoxin was produced using the fungal strain *Aspergillus flavus* NRRL 6513 that was obtained from U.S Department of Agriculture, Illinois, U.S.A. To get the fresh spores the culture was regularly sub-cultured on Potato Dextrose Agar (PDA) medium slants and stored at 5ºC. Aflatoxin was produced on liquid medium as per the method of Singh and Shamsudeen (2008). Aflatoxin contents were finally quantified using UV-Spectrophotometry.

2.2 Experimental Design and Substrate

Feed (wheat straw) was ground to pass a 1 mm sieve and used for experimentation. The following dietary treatments were prepared by mixing the required quantity of aflatoxin B_1 and diatomaceous earth to get their desired concentration in the feed (Table 1).

Table 1: Experimental groups and treatments

Groups	Treatments
T_1	Basal feed (Wheat straw)
T_2	T_1+300 ppb AFB_1
T_3	T_2+0.33% Diatomaceous earth
T_4	T_2+0.50% Diatomaceous earth
T_5	T_2+1.0% Diatomaceous earth

2.3 Collection of Rumen Liquor

Fistulated male buffalo, fitted with permanent rumen cannula, about 5 years-old having 450 kg body weight was used as donor animal for collection of rumen liquor. The animal was fed a basal diet of wheat straw offered *ad lib* and a standard concentrate mixture containing 20% CP and 70% TDN to meet the nutrient requirements for maintenance. The animal was given free access to clean drinking water. Approximately, 300 ml of rumen liquor was collected from different depths and directions of reticulo rumen and transferred into pre heated thermos flask, strained through a fourfold muslin cloth and flushed with CO_2. Rumen liquor was collected in the morning before feeding and watering of the animal as per standard procedure. Rumen fluid-medium mixture (inoculum) was prepared under continuous flushing with CO_2 to maintain anaerobic condition.

2.4 *In Vitro* Incubation of Substrate and Gas Production

200 mg dry weight of each feed substrate was weighed into 100 ml calibrated syringes and incubated with 30 ml of mixed rumen inoculum at 39^{o}C for 24h with parallel incubation of blanks (Menke *et al*., 1979; Menke and Steingass, 1988). Each substrate was incubated in triplicate. The syringes were regularly shaken by hand during the incubation period for proper mixing of feed with rumen inoculum. After 24 h of incubation period, the gas production was recorded by the displacement of piston during incubation period for test substrate and blank syringes. The net gas produced due to fermentation of substrate was calculated by subtracting the value of gas produced in blank syringes from that of test substrates.

2.5 *In Vitro* Dry Matter, Organic Matter Degradability and Microbial Protein Synthesis

After 24h of incubation period, the content of the syringes was transferred to 500 ml spoutless beakers, which was extracted in 100 ml of neutral detergent solution (NDS) by boiling for one hour, followed by filtration on preweighed gooch crucibles (G1), and washing in hot distilled water and acetone to recover true undigested residue as per the method of Van Soest *et al*. (1991). Crucibles with undigested residue were dried at 100^{o}C overnight and weighed to determine true undigested residue. Residue was ashed at 500^{o}C for 3 h to determine true undigested OM, which was corrected for the appropriate blanks. The TDOM was calculated as the difference between OM incubated and the undigested OM recovered in the residue of ND extraction. Truly degradable dry matter (TDDM) and truly degradable organic matter (TDOM) was estimated and microbial biomass production (MBP) and partitioning factor (PF) was calculated as per the method of Blummel *et al*. (1997).

Microbial biomass production (MBP) = Substrate truly degraded - (gas volume*stoichiometrical factor).
For roughages, the stoichiometrical factor was 2.20.

2.6 Estimation of Volatile Fatty Acid

After 24 h incubation 1 mL of the supernatant of each syringe content was taken in a micro centrifuge tube containing 0.20 mL metaphosphoric acid (25%, v/v). The mixture was allowed to stand for 2 h at room temperature and centrifuged at 5,000 × g for 10 min to get clear supernatant. The supernatant (1µL) was injected into gas chromatograph equipped with flame ionization detector (FID) and glass column packed with chromosorb as per the method described by Cottyn and Boucque (1968).

2.7 Statistical Analysis

All data were statistically analyzed using SPSS software package version 20.0 following one way analysis. All the observations were recorded at 95% ($P<0.05$) level of significance.

3. Results and Discussion

3.1 Efficacy of Diatomaceous Earth in Ameliorating Adverse Effects of Aflatoxin During *In Vitro* Rumen Fermentation

The data pertaining to truly degradable dry matter (TDDM), truly degradable organic matter (TDOM), gas production (GP), microbial biomass production (MBP) and partitioning factor (PF) as influenced by various dietary treatments are presented in Table 2. The data pertaining to volatile fatty acids (VFAs) production are presented in Table 3.

3.2 Truly Degradable Dry Matter (TDDM) and Truly Degradable Organic Matter (TDOM)

The TDDM and TDOM values of control group (T_1) was higher ($P<0.05$) than that of aflatoxin contaminated group (T_2). The TDDM and TDOM values in T_3 group was lower ($P<0.05$) than those of T_4 and T_5. The TDDM and TDOM values in T_4 group was lower ($P<0.05$) than that of T_5. The TDDM and TDOM values of control group (T_1) was higher ($P<0.05$) than those of T_3, T_4 and T_5. The results indicated that inclusion of 300 ppb aflatoxin in feed significantly ($P<0.05$) decreased the DM and OM degradability compared to that of control (T_1). This result was in agreement with that of Singh *et al*. (2020); Singh and Saini (2020), who also reported reduced TDDM and TDOM in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. Similar results were also reported by Westlake *et al*. (1989) wherein IVDMD of alfalfa hay was reduced by 50% with inclusion of 1 µg/ml AFB_1. Also, Mojtahedi *et al*. (2013) reported that IVDMD decreased significantly ($P<0.05$) with inclusion of AFB_1 in culture medium, so that the lowest and the highest IVDMD values were observed in treatments with 900 and 0 ng/ml AFB_1, respectively (0.54 vs. 0.68). Decreased IVDMD with AFB_1 addition can be attributed to compromised ruminal function by reducing fibre digestion and volatile fatty acid production (Fehr and Delage, 1970; Helferich *et al*., 1986a, b). However, some studies reported no effect of AFB_1 on *in vitro* dry matter disappearance of hay (Jiang *et al*., 2012; Pettersson and Kiessling, 1976). Yeanpet *et al*. (2018) also reported that IVDMD and IVOMD were not significantly affected by AFB_1. The present investigation revealed that addition of diatomaceous earth in 300 ppb aflatoxin contaminated feed (T_3 to T_5) significantly ($P<0.05$) ameliorated the adverse effects of aflatoxin on the TDDM and TDOM in a dose dependent manner. However, inclusion of diatomaceous earth in aflatoxin contaminated feed even at highest level (1.0%) (T_5) could not reverse the TDDM and TDOM value equivalent to that of control (T_1). Ameliorative effects of diatomaceous earth on adverse effects of aflatoxin are also reported in literature. The diatomaceous earth is a powerful natural adsorbent and it might adsorb the toxins effectively through their polar ends of toxin (Gowda *et al*., 2008). El-Husseiny *et al*. (2008) reported that feed intake of chicks fed with diatomaceous earth significantly increased the body weight. Modirsanei *et al.* (2008) observed that diatomaceous earth might be beneficial in reducing toxic effect of aflatoxin in broiler; they further concluded that it was possible to include DE as an alternative to other mycotoxins binders at levels of 30 mg/kg in the diets of broilers between the age of 1 and 42 days. They also observed that DE significantly increased body weight gain (9.51%), feed intake (7.44%), and improved feed conversion ratio (2.08%) as well as productive efficiency index in the birds that subjected to AFB_1. Silambarsan *et al*. (2013, 2015, 2016) also reported that diatomaceous earth, sodium bentonite and zeolite either at 0.5% or 1% level were partially to completely effective in ameliorating the different adverse effects of aflatoxin in broiler chickens. Among three mycotoxin adsorbents tested, diatomaceous earth least effective in comparison to sodium bentonite and zeolite. However, combination of the binders at a time was the most effective in ameliorating the adverse effects of aflatoxin B_1 in broiler chickens.

3.3 Gas Production and Microbial Biomass Production

The gas production (GP) value in control group (T_1) was higher ($P<0.05$) than that of aflatoxin contaminated group (T_2). The GP value in T_2 group was lower ($P<0.05$) compared to other treatment groups. The GP value in T_3 was lower ($P<0.05$) than those of T_4 and T_5. The GP value between T_4 and T_5 groups was statistically similar. The results of the present study indicated that aflatoxin contamination of wheat straw at 300 ppb level significantly ($P<0.05$) decreased the gas production compared to that of control (T_1). This result was in agreement with Singh *et al*. (2020); Singh and Saini (2020), who also reported reduced gas production in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. Also, Mojtahedi *et al*. (2013) reported that by increasing the level of AFB_1 from 0 to 900 ng/ml, the gas production rate decreased from 0.071 to 0.051 and cumulative gas production decreased from 196.4 to 166.0 ml/g DM, respectively. Similarly, Jiang *et al*. (2012); Helferich *et al*. (1986a, b) also reported that the gas production parameters were reduced when AFB_1 was added. These depressions in the gas production suggest that microbial populations are altered by AFB_1 contamination of feed. In the present investigation, addition of diatomaceous earth to the aflatoxin contaminated feed significantly ($P<0.05$) –

Table 2: Effect of aflatoxin on rumen fermentation parameters

Treatments	TDDM %	TDOM %	GP ml/g DM	MBP mg/100mgDDM	PF
T_1	40.85±0.08^{e}	41.23±0.08^{e}	148.98±0.16^{d}	20.70±0.20^{c}	2.73±0.01^{c}
T_2	36.02±0.12^{a}	37.11±0.16^{a}	140.25±0.32^{a}	17.37±0.47^{a}	2.56±0.01^{a}
T_3	36.83±0.07^{b}	38.09±0.18^{b}	142.01±0.37^{b}	18.61±0.25^{ab}	2.59±0.01^{a}
T_4	37.82±0.11^{c}	38.58±0.13^{c}	143.86±0.29^{c}	18.34±0.38^{b}	2.62±0.01^{b}
T_5	38.56±0.16^{d}	39.31±0.13^{d}	144.59±0.06^{c}	18.84±0.37^{b}	2.65±0.02^{b}

Values bearing different superscripts in a column differ significantly (P<0.05).

Table 3: Effect of aflatoxin on volatile fatty acids production

Treatments	TVFA mM/100ml	Acetate mM/100ml	Propionate mM/100ml	Butyrate mM/100ml	A:P ratio
T_1	6.26±0.04^{c}	4.51±0.09^{c}	1.27±0.01^{c}	0.50±0.01^{c}	3.55±0.08^{a}
T_2	4.99±0.04^{a}	3.56±0.02^{a}	0.90±0.01^{a}	0.35±0.01^{a}	2.97±0.96^{a}
T_3	5.10±0.04^{a}	3.52±0.03^{a}	0.93±0.01^{a}	0.38±0.01^{b}	3.75±0.04^{a}
T_4	5.55±0.09^{b}	3.95±0.06^{b}	1.05±0.03^{b}	0.40±0.01^{b}	3.77±0.13^{a}
T_5	5.66±0.13^{b}	4.03±0.04^{b}	1.06±0.02^{b}	0.40±0.01^{b}	3.78±0.10^{a}

Values bearing different superscripts in a column differ significantly (P<0.05).

ameliorated the adverse effects of aflatoxin on gas production in a dose dependent manner, however, even at the highest level (1.0%) of diatomaceous earth (T_5) the gas production value was significantly (P<0.05) lower than that of control (T_1). With respect to microbial biomass production (MBP), the MBP value in control group (T_1) was higher (P<0.05) than that of aflatoxin contaminated group (T_2). The MBP value in T_2 group was statistically similar to that of T_3 group. The MBP value in T_2 group was lower (P<0.05) than those of T_4 and T_5. The MBP value between groups T_4 and T_5 did not vary significantly. The MBP value of groups T_3, T_4 and T_5 was significantly lower (P<0.05) than that of control (T_1). The results of present investigation revealed that aflatoxin contamination of feed at 300 ppb level resulted in significant decrease in the MBP compared to that of control. This result was in agreement with that of Singh *et al*. (2020); Singh and Saini (2020), who also reported significantly reduced microbial biomass production due to aflatoxin contamination of feed at 100 to 300 ppb level in the buffalo diet. In the present study, inclusion of diatomaceous earth at 0.5% level (T_4) to the 300 ppb aflatoxin contaminated feed ameliorated the adverse effects of aflatoxin on MBP. However, even at the highest level (1.0%) of diatomaceous earth (T_5) inclusion to the aflatoxin contaminated feed, the MBP value was lower (P<0.05) than that of control (T_1).

3.4 Partitioning Factor (PF)

The partitioning factor value in control group (T_1) was higher (P<0.05) than those of all other treatments. The PF value in aflatoxin contaminated group (T_2) was lower (P<0.05) than those of other treatment groups barring group T_3. The PF value in groups T_2 and T_3 was lower (P<0.05) than those of T_4 and T_5. The PF value between groups T_4 and T_5 did not vary significantly. In the present study, aflatoxin contamination of feed at 300 ppb (T_2) level resulted in significant decrease in the PF value compared to that of control (T_1). This finding was in agreement with those of Singh *et al*. (2020); Singh and Saini (2020), who also reported reduced PF value in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. In the present study, inclusion of diatomaceous earth at 0.5% level (T_4) to the 300 ppb aflatoxin contaminated feed significantly (P<0.05) ameliorated the adverse effects of aflatoxin on PF value. However, even the highest level (1.0%) of diatomaceous earth (T_5) inclusion to the aflatoxin contaminated feed, the PF value was lower (P<0.05) than that of control (T_1). A feed with higher PF value means that proportionally more of the degraded matter is incorporated into microbial mass, i.e., the efficiency of microbial protein synthesis is higher. Roughages with higher PF have been shown to have higher dry matter intake (Harikrishna *et al*., 2012).

3.5 Volatile Fatty Acids (VFAs) Production

The total volatile fatty acids (TVFAs), acetate (A), propionate (P) and butyrate (B) values in control group (T_1) was higher (P<0.05) than that of aflatoxin contaminated group (T_2). The TVFA, A, and P value in aflatoxin contaminated T_2 group was similar to that of T_3. The B value of T_2 was lower (P<0.05) than T_3. The TVFA, A and P value in T_3 was lower (P<0.05) than those of T_4 and T_5. The TVFA, A, P and B value between groups T_4 and T_5 did not vary significantly. The TVFA, A, P and B value of groups T_3, T_4 and T_5 was significantly (P<0.05) lower than that of control (T_1). The results of the present investigation revealed that aflatoxin contamination @ 300 ppb in feed significantly decreased the TVFA, A, P, and B production compared to that of control. This finding of reduced VFA due to aflatoxin contamination was in agreement with Singh *et al*. (2020); Singh and Saini (2020); Jiang *et al*. (2012) who also reported that the VFA concentration decreased with the increase of

AFB_1 dose level. Cellulose degradation, VFA production, ammonia production, and proteolysis were decreased by AFB_1 at 0.2-0.8 mg/kg body weight in acute bovine aflatoxicosis (Cook *et al*., 1986). Also, the production of VFA irrespective of substrate was inhibited by the increasing dose levels of AFB_1, which was consistent with the reduction in the asymptotic gas volume. The suppression of VFA, gas production and ammonia N implicated that microbial activity was inhibited regardless of substrate used. Contrary to this, Edrington *et al*. (1994) found no differences in ruminal VFA concentrations in growing lambs fed 2.5 mg AFB_1 per kg diet. Helferich *et al*. (1986a) also reported that AFB_1 at 60-600 ppb did not influence the production of VFA in steers. In another experiment, ingestion of 0.714 µmol AFB_1 per animal did not influence the ruminal VFA production in lactating goats (Helferich *et al*., 1986b). With regard to A:P ratio, the A:P ratio value in various treatment groups T_1 to T_5 varied from 2.97 (T_2) to 3.78 (T_5). The A:P ratio value in various treatment groups (T_1 to T_5) did not vary significantly. The present study revealed that aflatoxin contamination of feed at 300 ppb level (T_2) did not produce any significant effect on A:P ratio as compared to that of control (T_1), however, the A:P ratio value in aflatoxin contaminated group (T_2) was numerically lower than that of control. Inclusion of diatomaceous earth to the aflatoxin 300 ppb contaminated feed (T_3 to T_5) did not produce any effect on the A:P ratio compared to that of aflatoxin contaminated group (T_2), however, the A:P ratio of groups T_3 to T_5 was numerically higher than that of aflatoxin contaminated group (T_2). This result revealed that aflatoxin (300 ppb) contamination of feed did not change the A:P ratio significantly. However, Singh *et al*. (2020); Singh and Saini (2020), reported increased A:P ratio value in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. Also, in the present study, inclusion of diatomaceous earth to the aflatoxin contaminated feed did not produce any significant effect on the A:P ratio.

4. Conclusion

It was concluded that aflatoxin contamination of feed (wheat straw) at 300 ppb level significantly affected the *in vitro* rumen fermentation in terms of reduced truly degradable dry matter, truly degradable organic matter, gas production, microbial biomass production, partitioning factor and total volatile fatty acids concentration. Inclusion of diatomaceous earth to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on *in vitro* rumen fermentation parameters.

References

Blummel M, Makkar HPS and Becker K (1997). *In vitro* gas production: a technique revisited. *Journal of Animal Physiology and Animal Nutrition*, 77: 24-34.

Cook WO, Richard JL, Osweiller GD and Trampel DW (1986). Clinical and pathologic changes in acute bovine aflatoxicosis: rumen motility and tissue and fluid concentrations of aflatoxins B_1 and M_1. *American Journal of Veterinary Research*, 47: 1817-1825.

Cottyn BG and Boucque CV (1968). Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*, 16: 105-107.

Edrington TS, Harvey RB and Kubena LF (1994). Effect of aflatoxin in growing lambs fed ruminally degradable or escapes protein sources. *Journal of Animal Science*, 72: 1274-1281.

El-Husseiny OM, Abdallah AG and Abdel-Ltif KO (2008). The influence of biological additives on broiler performance. *International Journal of Poultry Science*, 7(9): 862-871.

Fehr PM and Delage J (1970). Effect de l'aflatoxinesur les fermentations dans le rumen. *Comptes Rendus de l'Académie des Sciences Paris (Series D)*, 270: 550-553.

Gowda NKS, Ledoux DR, Rottinghaus GE, Bermudez AJ and Chen YC (2008). Efficacy of turmeric (*curcuma longa*), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. *Poultry Science*, 87: 1125-1130.

Harikrishna C, Mahender M, Ramana Reddy Y, Gnana Prakash M, Sudhakar K and Pavani M (2012). Evaluation of *in vitro* gas production and nutrient digestibility of complete diets supplemented with different levels of thermotolerant yeast in Nellore rams. *Veterinary World*, 5(8): 477-485.

Helferich WG, Baldwin RL and Hsieh DPH (1986b). (14C)-Aflatoxin B_1 metabolism in lactating goats and rats. *Journal of Animal Science*, 62: 697-705.

Helferich WG, Garrett WN, Hsieh DPH and Baldwin RL (1986a). Feed lot performance and tissue residues of cattle consuming diets containing aflatoxins. *Journal of Animal Science*, 62: 691-696.

Jiang YH, Yang HJ and Lund P (2012). Effect of aflatoxin B_1 on *in vitro* ruminal fermentation of rations high in alfalfa hay or ryegrass hay. *Animal Feed Science and Technology*, 175: 85-89.

Kamigasa S and Kato H (2000). Recent conditions and prospects of diatomite resources. *Energy Resources*, 21: 166-172.

Menke KH and Steingass H (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research Development*, 28: 7-55.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science*, 93: 217-222.

Modirsanei M, Mansoori B, Khosravi AR, Kiaei MM, Khazraeinia P, Farkhoy M and Masoumi Z (2008). Effect of diatomaceous earth on the performance and blood variables of broiler chicks during experimental aflatoxicosis. *Journal of the Science of Food and Agriculture*, 88: 622-632.

Mojtahedi M, Danesh Mesgaran M, Vakili SA and Hayati-Ashtiani M (2013). Effect of Aflatoxin B_1 on *in vitro*

rumen microbial fermentation responses using batch culture. *Annual Review and Research in Biology*, 3(4): 686-693.

Natour RM and Yousef SM (1998). Adsorption efficiency of diatomaceous earth for mycotoxin. *Arab Gulf Journal of Scientific Research*, 16: 113-127.

Patil RD and Degloorkar NM (2018). Ameliorative efficacy of commercial mycotoxin binder (Bantox®) against ochratoxin A-induced microscopic pathology in broiler birds. *Journal of Poultry Science and Technology*, 6(2): 26-30.

Patil RD, Degloorkar NM and Pawar PK (2017a). Ameliorating effects of Bantox® on clinical manifestation and growth performance of broiler chicken fed with ochratoxin A. *Journal of Poultry Science and Technology,* 5(3): 22-27.

Patil RD, Degloorkar NM and Pawar PL (2017b). Effects of ochratoxin a feeding on organ weights and gross pathological changes in broiler chicken and its amelioration with Bantox®. *Journal of Poultry Science and Technology*, 5(4): 44-51.

Pettersson H and Kiessling KH (1976). Metabolism of aflatoxin by intact rumen fluid, rumen protozoa and rumen bacteria. *Swedish Journal of Agricultural Research*, 6: 161.

Silambarasan S, Singh R and Mandal AB (2013). Evaluation of the ability of adsorbents to ameliorate the adverse effects of aflatoxin B_1 in broiler chickens. *Indian Journal of Animal Sciences,* 83(1): 73-77.

Silambarasan S, Singh R and Mandal AB (2015). Efficacy of certain adsorbents on carcass traits and livability of broiler chickens fed aflatoxin B_1 contaminated diet. *Indian Journal of Poultry Science*, 50(1): 113-117.

Silambarasan S, Singh R and Mandal AB (2016). Evaluation of adsorbents to ameliorate the adverse effects of aflatoxin B_1 on blood biochemicals, immune response and histopathology of liver in broiler chickens. *Indian Journal of Poultry Science*, 50(3): 267-271.

Singh R (2019a). Effect of dietary inclusion of Mycodetox B_2 on liveability, immunity and organ pathology during aflatoxicosis in turkey poults. *Livestock Research International,* 7(2): 55-61.

Singh R (2019b). Effect of dietary supplementation of Mycodetox B_2 on production performance, organ weights and serum biochemicals during aflatoxicosis in turkey poults. *Livestock Research International,* 7(1): 32-39.

Singh R (2019c). Effect of dietary Mycodetox B_2 on production performance, organ weights and biochemical parameters during aflatoxicosis in broiler chickens. *Journal of Poultry Science and Technology,* 7(2): 38-47.

Singh R (2019d). Ameliorative efficacy of Mycodetox B_2 on liveability, immune response and liver pathology during aflatoxicosis in broiler chickens. *Journal of Poultry Science and Technology,* 7(2): 48-55.

Singh R (2019e). Mycotoxins contamination of animal feeds and feed ingredients available in Haryana. *Livestock Research International,* 7(4): 250-254.

Singh R and Saini AK (2020). Effect of Mannan Oligosaccharides (MOS) to ameliorate adverse effects of aflatoxin on *In vitro* rumen fermentation of a buffalo diet. *Veterinary Research International*, 8(2): 78-84.

Singh R and Shamsudeen P (2008). Aflatoxigenic potential of *Aspergillus parasiticus* MTCC 411 and *Aspergillus parasiticus* NRRL 2999 under laboratory conditions. *Indian Journal of Poultry Science,* 43(2): 245-246.

Singh R, Park S, Koo JS and Balasubramanian B (2020). Influence of various concentrations of aflatoxin B_1 on *in vitro* rumen fermentation of a buffalo diet. *Korean Journal of Agricultural Science*, 47(1): 131-138.

Singh R, Shrivastava HP and Shrivastav AK (2010). Mycotoxin contamination in maize as poultry feed. *Indian Journal of Poultry Science,* 45(1): 108-110.

Van Soest PJ, Robertson JB and Lewis BA (1991). Methods for dietary fibre, neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74: 3583-3597.

Westlake K, Mackie RI and Dutton MF (1989). *In vitro* metabolism of mycotoxins by bacterial protozoal and ovine ruminal fluid preparations. *Animal Feed Science and Technology*, 25: 169-178.

Wu F (2007). Measuring the economic impacts of Fusarium toxins in animal feeds. *Animal Feed Science and Technology*, 137(3-4): 363-374.

Yeanpet C, Thamrongyoswittayakul C, Wachirapakorn C, Songsermsakul P, Somphon N and Wongnen C (2018). Efficacy of mycotoxin adsorbents on Aflatoxin B_1 decontamination and *in vitro* rumen fermentation. *Prawarun Agriculture Journal*, 15(1): 260-268.