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ORIGINAL ARTICLE

Effect of Mannan Oligosaccharides (MOS) to Ameliorate Adverse Effects of Aflatoxin on *In Vitro* Rumen Fermentation of a Buffalo Diet

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Abstract

The present investigation was undertaken to study the effect of mannan oligosaccharides (MOS) in ameliorating adverse effects of aflatoxin on rumen fermentation *in vitro*. Five treatment groups, viz. T_1: control (Basal feed); T_2: T_1+300 ppb Aflatoxin B_1 (AFB_1); T_3: T_2+0.05 MOS; T_4: T_2+0.1% MOS and T_5: T_2+0.2% MOS 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 aflatoxin contaminated group (T_2) was lower ($P<0.05$) than those of other treatment groups. The TDDM, TDOM, GP, MBP and PF values in control group (T_1) was higher than those of other treatment groups i.e. T_2 to T_4 and equal to T_5 barring TDDM. The values of these parameters improved with increasing concentration of MOS. The TDOM, MBP and PF value of group T_5 was statistically similar to that of control (T_1). The total volatile fatty acids (TVFA), 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 A:P ratio of T_2 was higher ($P<0.05$) than T_1. The A:P value of T_1 was statistically similar to those of T_3, T_4 and T_5. It was concluded that aflatoxin contamination of feed 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, total volatile fatty acids concentration and increased A:P ratio. Inclusion of mannan oligosaccharides to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on *in vitro* rumen fermentation parameters.

Keywords: Mannan oligosaccharides**,** Aflatoxin, Buffalo, Rumen fermentation, *In vitro.*

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1. Introduction

The worldwide contamination of foods and feeds with mycotoxins is a significant problem (Patil and Degloorkar, 2018). Studies have shown extensive mycotoxin contamination in both developing and developed countries (Raveendran *et al.,* 2020). The most widespread and most studied group of mycotoxins, aflatoxins are of great concern in warm and humid climatic conditions like Asian nations (Singh *et al*., 2010; Patil *et al*., 2017a, b). Aflatoxins contamination in feed is practically unavoidable (Coulombe *et al*., 2005). So it makes it necessary to evolve the practical and suitable methods to counteract the aflatoxicosis (Raveendran *et al.,* 2020; Sharma *et al.,* 2019a, b). Extensive research has been conducted to counter mycotoxicosis by physical, chemical, nutritional or biological approaches. Among them the most promising and applicable approach to reduce the toxicity of mycotoxin is to use the mycotoxin binders in the feed (Singh *et al.,* 2019a, b, c). The binder decontaminates the mycotoxin by binding them strongly so that absorption of mycotoxin through intestine is prevented. The clay-based adsorbents are typically used at high concentrations in animal feed (>1.0% of the diet), resulting in decreased nutrient density (Zaghini *et al.,* 2005). Binders have been evaluated using both *in vitro* and *in vivo* systems. *In vivo* studies have generally used performance responses or biological markers, such as tissue residues or changes in biochemical parameters, to determine the

effectiveness of binders. Numerous binders can bind aflatoxin efficiently but few could be used for other mycotoxins (EFSA, 2009). 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. Mannan oligosaccharides (MOS), present in the cell wall of *Saccharomyces cerevisiae*, is believed to be responsible for the beneficial effects observed with *S. cerevisiae* (Raju and Devegowda, 2000; Sharma *et al.*, 2019a, b; Singh *et al.*, 2019a, b, c). MOS was reported to have toxin-binding properties but little information is available on the potential of MOS to detoxify aflatoxin (Devegowda *et al*., 1996). Modified MOS derived from the cell wall of *S. cerevisiae* was reported to have higher binding capacity (95 percent) for aflatoxin (Mahesh and Devegowda, 1996). Generally probiotics and prebiotics acts by competition for substrates and competition for attachment sites in the GI tract and improves the gut health. They act as a biological adsorbent also and ameliorate the toxicity of the mycotoxin. Use of binders MOS and SC (at the rate 0.05%, 0.1%, 0.2%) and their combination ameliorated the effect of aflatoxin partially or completely in dose dependent manner (Khatke *et al*., 2012). The objective of the present investigation was to evaluate the efficacy of mannan oligosaccharides to ameliorate the adverse effects of aflatoxin on *in vitro* rumen fermentation.

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 sample (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 mannan oligosaccharides to get their desired concentration in the feed (Table 1).

2.3 Collection of Rumen Liquor

Fistulated male buffalo, fitted with permanent rumen cannula, about 3 years-old having 350 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 requirement 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.

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+0.05% Mannan oligosaccharides
T_4	T_2+0.1% Mannan oligosaccharides
T_5	T_2+0.2% Mannan oligosaccharides

2.4 In Vitro Incubation of Substrate and Gas Production

200 mg dry weight of 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 feeds 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 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°C overnight and weighed to determine true undigested residue. Residue was ashed at 500°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 Mannan Oligosaccharides to Ameliorate 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.

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

The TDDM and TDOM values in aflatoxin contaminated group (T_2) was lower ($P<0.05$) than those of other treatment groups. The TDDM values in control group (T_1) was higher than those of other treatment groups i.e. T_2 to T_5. The TDOM values in control group (T_1) were statistically similar to that of T_5. 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 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. Singh *et al*. (2020) also reported reduced TDDM and TDOM of 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 study indicated that inclusion of mannan oligosaccharides in feed significantly ($P<0.05$) improved the TDDM and TDOM in a dose dependent manner. However, inclusion of mannan oligosaccharide in feed even at highest level (0.2%) (T_5) could not reverse the TDDM value equivalent to that of control (T_1). In *in vitro* experiments Trenholm *et al*. (1994); Devegowda *et al*. (1996, 1998) observed that a commercial MOS binds aflatoxin B_1 and zearalenone. Mahesh and Devegowda (1996) in an *in vitro* study observed that the addition of 0.05% mannan oligosaccharides to a diet containing 200 ppb aflatoxins sequestered or bound 79% of these toxins. Modified MOS derived from the cell wall of *Saccharomyces cerevisiae* was reported to have even higher binding capacity upto 95% for AF (Mahesh and Devegowda, 1996). Khatke *et al*. (2012) also reported that use of binders MOS and SC (at the rate 0.05%, 0.1%, 0.2%) and their combinations in poultry feed ameliorated the effect of aflatoxin partially or completely in a dose dependent manner. The 0.2% level of MOS and SC was more effective than 0.05% and 0.1% level in counteracting the 300 ppb of aflatoxin in the feed. Generally, mannan oligosaccharides acts as prebiotics by competition for substrates and competition for attachment sites in the GI tract and improves the gut health. They act as a biological adsorbent also and ameliorate the toxicity of the mycotoxin.

Table 2: Effect of aflatoxin on rumen fermentation parameters

Treatments	TDDM %	TDOM %	GP ml/g DM	MBP mg/100mgDDM	PF
T_1	40.63±0.10^{e}	41.20±0.08^{d}	148.97±0.16^{e}	20.78±0.19^{b}	2.72±0.01^{c}
T_2	36.08±0.21^{a}	37.32±0.12^{a}	140.66±0.32^{a}	17.67±0.40^{a}	2.56±0.01^{a}
T_3	37.97±0.08^{b}	38.74±0.35^{b}	142.79±0.36^{b}	19.39±0.79^{b}	2.64±0.01^{b}
T_4	38.83±0.15^{c}	39.41±0.20^{c}	144.89±0.19^{c}	19.41±0.40^{b}	2.67±0.01^{bc}
T_5	39.66±0.17^{d}	40.76±0.19^{d}	147.30±0.41^{d}	20.84±0.50^{b}	2.71±0.03^{c}

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	6.16±0.04^{d}	4.47±0.04^{d}	1.25±0.01^{c}	0.52±0.02^{c}	3.57±0.02^{a}
T_2	5.15±0.05^{a}	3.81±0.04^{a}	0.93±0.01^{a}	0.36±0.01^{a}	4.09±0.02^{b}
T_3	5.69±0.14^{b}	4.04±0.08^{b}	1.07±0.02^{b}	0.39±0.01^{a}	3.75±0.10^{a}
T_4	5.92±0.04^{c}	4.26±0.04^{c}	1.15±0.03^{bc}	0.44±0.02^{b}	3.68±0.08^{a}
T_5	6.05±0.03^{cd}	4.35±0.05^{cd}	1.20±0.06^{c}	0.45±0.01^{b}	3.62±0.12^{a}

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

3.1.2 Gas Production and Microbial Biomass Production

The gas production (GP) value in control group (T_1) was higher (P<0.05) than those of other treatment groups i.e. T_2 to T_5. 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 in T_4 group was lower (P<0.05) than that of T_5. The results of the present investigation 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) 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 study, inclusion of mannan oligosaccharides to the aflatoxin contaminated feed significantly (P<0.05) ameliorated the adverse effects of aflatoxin on gas production in a dose dependent manner, however, even the highest level (0.2%) of mannan oligosaccharides could not reverse the gas production value equivalent to that of control. 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 lower (P<0.05) than those of other treatment groups i.e. T_3, T_4 and T_5. The MBP value of groupsT_3, T_4 and T_5 was statistically similar to that of control (T_1). The MBP value among groups T_3, T_4 and T_5 did not vary significantly. 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) who also reported significantly reduced microbial biomass production due to aflatoxin contamination of feed at 300 ppb level in the diet of buffalo. In the present study, inclusion of mannan oligosaccharides at any level (0.05 to 0.2%) (T_3 to T_5) to the 300 ppb aflatoxin contaminated feed reversed the MBP value equal to that of control (T_1).

3.1.3 Partitioning Factor (PF)

The partitioning factor value in control group (T_1) was higher (P<0.05) than those of T_2 and T_3. The PF value in aflatoxin contaminated group (T_2) was lower (P<0.05) than those of other treatment groups. The PF value in group T_3 was statistically similar to that of T_4. The PF value in group T_3 was significantly (P<0.05) lower than that of T_5. The PF value in group T_1 was statistically similar to that 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 that of Singh *et al*. (2020) who also reported reduced partitioning factor in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. The study further revealed that incorporation of mannan oligosaccharides at any level (0.05 to 0.2%) to the 300 ppb aflatoxin contaminated feed ameliorated the adverse effects of aflatoxin on PF value in a dose dependent manner. Inclusion of mannan oligosaccharides to the 300 ppb aflatoxin contaminated feed at the highest level (0.2%) reversed the PF value equivalent to 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).

3.1.4 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, P and B value in aflatoxin contaminated T_2 group was lower ($P<0.05$) than those of other treatment groups i.e. T_3 to T_5. The TVFA value in T_3 was lower ($P<0.05$) than that of T_4 and T_5. The TVFA value between groups T_4 and T_5 did not vary significantly. The TVFA value of group T_5 was statistically similar to that of T_1. The A, P and B value of T_3 was lower ($P<0.05$) than those of T_4 and T_5. The A, P and B value between groups T_4 and T_5 did not vary significantly. The A and P value of group T_5 was statistically similar to that of control (T_1). The B value of group 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 concentration was in agreement with Singh *et al*. (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 in aflatoxin contaminated group (T_2) was higher ($P<0.05$) than that of control (T_1). The A:P value of control group (T_1) was statistically similar to those of T_3, T_4 and T_5. The A:P ratio value among groups T_3, T_4 and T_5 did not vary significantly. This finding revealed that aflatoxin (300 ppb) contamination of feed resulted in increased A:P ratio value as compared to control. This result was in agreement with Singh *et al*. (2020) who also reported increased A:P ratio value in a buffalo diet when the diet was contaminated with 100 to 300 ppb aflatoxin. In the present study, inclusion of mannan oligosaccharides to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on VFA production in a dose dependent manner as the highest level (0.2%) of mannan oligosaccharides was most effective in ameliorating adverse effects of aflatoxin on total and individual volatile fatty acids production.

4. Conclusion

It was concluded that aflatoxin contamination of feed 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, total volatile fatty acids concentration and increased A:P ratio. Inclusion of mannan oligosaccharides to the aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxin on *in vitro* rumen fermentation parameters.

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