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Saccharomyces cerevisiae partially to completely ameliorates the adverse effects of aflatoxin on the *in vitro* rumen fermentation of buffalo diet

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Abstract

The current study investigated how *Saccharomyces cerevisiae* ameliorates the adverse effects of aflatoxin on *in vitro* rumen fermentation. In this study, five groups (T_1: Control [basal feed]; T_2: T_1 + 300 ppb aflatoxin B_1 [AFB_1] and T_3, T_4, and T_5: T_2 with 0.05, 0.1, and 0.2% of *S. cerevisiae, respectively*) were prepared and incubated *in vitro*. The results revealed that truly degradable dry matter (TDDM), gas production (GP), microbial biomass production (MBP), truly degradable organic matter (TDOM), partitioning factor (PF), total volatile fatty acids (TVFA), acetate (A), propionate (P) and butyrate (B) values in the control group (T_1) were higher ($p < 0.05$) than those of the AFB_1 fed group (T_2). The A : P ratio in the control group (T_1) was reduced ($p < 0.05$) when compared to that of the T_2 group. The TDDM, TDOM, GP, TVFA, A, P, and B values of T_3, T_4, and T_5 improved with the increasing levels of *S. cerevisiae*; however, the values of group T_5 were lower ($p < 0.05$) than that of the control. The values of MBP, A : P ratio and PF in group T_5 were statistically similar to that of the control. It was concluded that the inclusion of *S. cerevisiae* (0.05 to 0.20%) to the AFB_1 (300 ppb) contaminated feed partially to completely ameliorated the adverse effects of AFB_1 on the *in vitro* rumen fermentation parameters.

Keywords: aflatoxin, *in vitro,* rumen fermentation, *Saccharomyces cerevisiae*

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Introduction

The genus *Aspergillus* produces aflatoxins that are usually from the feed and food with moisture and warm atmospheres conditions. Extreme occurrence rates of contamination in cereal grains and animal feed have been reported in Asia and some diverse substitutes such as the potential use of fermented phytogenic products to decontaminates and influences in performances has been ahead

drive in recently (Balasubramanian et al., 2017; Singh and Shrivastav, 2011a, 2011b; Singh, 2019). Therefore, the diet contaminations caused by mycotoxins and the compounds related to mycotoxins transmitted through the food have to be controlled properly. Production of buffalo is a crucial part of nation economical state and play an important role in affording of the increased quality and quantity food for the mankind. Few studies have suggested that the composition of milk, weight gain of the body, immune power and the reproduction capacity are affected in dairy ruminants by consuming aflatoxins contaminated feed (Battacone et al., 2003; Battacone et al., 2005; Xiong et al., 2015). The exposure of aflatoxins for a long period may lead to reduced body weight, reduced milk or production of egg, raised levels of susceptibility to diseases, lowered efficiency of feeding, teratogenicity and tumors (Streit et al., 2012). The aflatoxin B1 (AFB_1) contamination feed has been given to the lactating animals that resulted in the production of contaminated milk contains with mono-hydroxylated derivative aflatoxins M_1 (AFM_1) compound, which classified as group 2B by International Agency for Research on Cancer (IARC), probable carcinogenic substance to the human beings (IARC, 2002). One of the most common forms of aflatoxicosis is chronic toxicity, which may be due to the consumption of toxic compounds in little amounts for a long period of time. Liver has been observed to be the major target organ for toxicity, in which the toxicity maybe metabolized into various metabolites (Di Gregorio et al., 2014). Probiotic yeasts are widely used in animal feeding because of their effects on activity of rumen microbes (Chaucheyras-Durand et al., 2008). Along with the exceptional dietary value of the yeast, the glucomannans from the cell walls of the yeast have been found to significantly reduce (approximately -60%) the concentration of AFM_1 in the milk of cows consuming AFB_1 (55 $\mu g \cdot kg^{-1}$) contaminated diets (Diaz et al., 2004). In our earlier report shown that the adverse effects of AFB_1 through in vitro rumen fermentation (Singh et al., 2020). The adsorption of AFB_1 by the products of *Saccharomyces cerevisiae* is not totally a linear fact and may vary according to several factors, especially the amount of β-d-glucans and the yeast cell wall conformation that varies among the strains of the yeast (Jouany and Diaz, 2005). Therefore, the aim of the present experiment was to examine the ameliorative effects of *S. cerevisiae* on adverse effects of AFB_1 by *in vitro* rumen fermentation.

Materials and Methods

Production and determination of aflatoxin

The fungal strain of *Aspergillus flavus,* NRRL 6513 (U.S Department of Agriculture, Illinois, USA) produces aflatoxin, that can be used as inoculant and produced on liquid medium according to method described by Singh and Shamsudeen (2008). The culture was sub-cultured regularly on the slants of potato dextrose agar medium, in order to get the fresh spores and further stored at 5°C. Aflatoxin contents were finally quantified using UV-Spectrophotometry (Model no: 117, Systronics, Kolkatta, India). Plates were inspected for AFB_1 in a chromate-viewer to find the components by fluorescence. Quantification of AFB_1 was determined by visual comparison of fluorescence zone with the known quantity of zone formed by the standards of aflatoxin. AFB1 was separated by scratching the chromatogram.

Experimental design and substrate

Feed sample (wheat straw) was ground to go by a sieve of 1 mm and utilized for experimentation. The study categorized to one of the five groups as basal diet included with 0 (T_1), 300 ppb of AFB_1 (T_2), and T_2 mixed with 0.05% (T_3), 0.1% (T_4), 0.2%

Table 1. The chemical composition of wheat straw.

Compositions	Amount (% of diet DM)
Dry matter (DM, %)	93.7
Chemical composition	
Organic matter	91.18
Total ash	7.68
Crude protein	3.45
Ether extract	1.26
Neutral detergent fibre	84.92
Acid detergent fibre	63.78
Acid-detergent lignin	5.40

(T_5) of *S. cerevisiae* for further analysis. The wheat straw was examined for dry matter (DM), ash, ether extract (EE), organic matter (OM) and crude protein (CP), while Van Soest et al. (1991) method has been used to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF) and mentioned in Table 1.

Collection of rumen liquor

Fistulated male buffalo, fitted with permanent rumen cannula, about 2 years-old having 250 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 compensate the nutrients required for the maintenance. The animal was given free access to clean drinking. The rumen liquor (about 300 mL), has been accumulated from the reticulo rumen of various depths and directions, followed by the transfer into thermos flask, which is pre heated and strained using a fourfold cloth of muslin and flushed with CO_2. Rumen liquor was collected in the morning before feeding and watering of the animal as per standard procedure. The preparation of rumen fluid–medium mixture (inoculum) was carried out under constant flushing with CO_2 to maintain anaerobic condition.

In vitro incubation of substrate and gas production (GP)

About 200 mg, in dry weight of every feed was taken into the calibrated syringes of 100 mL, followed by the incubation for 24 h at 39℃ with the mixed rumen inoculums (30 mL) and the simultaneous incubation of blanks has also been done (Menke et al., 1979). 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 the period of incubation for 24 h, the production of gas was recorded by the piston displacement for the duration of incubation period for test substrate and blank syringes. The net production of the gas, as the result of fermentation of substrate was evaluated by deducting the value of production of gas in the blank syringes from that of test substrates.

In vitro dry matter degradability (IVDMD)

The syringe content has been extracted by boiling in 100 mL of neutral detergent solution (NDS) for an hour, which is then filtered on the pre-weighed gooch crucibles followed by the washing with distilled water, which is hot along with the acetone

for the proper recovery of undigested residue that is pure, by using the Van Soest et al. (1991) method. The above steps were carried out after the 24 h incubation period and the transfer of the obtained contents were done to the spotless beakers of 500 mL. The crucibles that contains the undigested residue has been subjected to overnight dry at 100℃ and then weighed for evaluating the true undigested residue. At 500℃, the residue was turned into ashes within 3 h in order to evaluate the true undigested organic matter (OM), that has been used for the blanks appropriately. After the 24 h of inoculation, a set of samples was evaluated *in vitro* truly degradable dry matter's (TDDM) true digestibility and truly degradable organic matter's (TDOM) true digestibility according to Van Soest and Robertson (1985) method, as the variation between incubated OM and the OM undigested that is recovered in the ND extraction residue. The microbial biomass production (MBP) and partitioning factor (PF) was evaluated as per the method of Blummel et al. (1997).

MBP = substrate truly degraded - (gas volume*stoichiometrical factor) (1)

The stoichiometrically factor was 2.20 for roughages.

Estimation of volatile fatty acid

After 24 h post incubation, 1 mL of the supernatant of each syringe content was taken in a micro centrifuge tube with 0.20 mL of metaphosphoric acid (25%, v/v) to analysis the total propionate, volatile fatty acids (TVFA), butyrate and acetate. The mixture was kept at the room temperature for 2 h followed by the centrifugation for 10 min at 5,000 g for 10 min to obtain the clear supernatant. The supernatant (1 μL) was injected into the flame ionization detector equipped gas chromatograph and chromosorb packed glass column according to the method described by Cottyn and Boucque (1968).

Statistical analysis

All data were evaluated in the statistical software SPSS, version 20.0 following one-way analysis. All the observations were observed at 95% ($p < 0.05$) level of significance.

Results and Discussion

Mycotoxins are secondary toxic metabolites formed by various hurtful fungi species, which are caused potential health effects on livestock animals and humans (Jeong et al. 2018a, 2018b). The average figures pertaining to TDDM, TDOM, GP, MBP, and PF as influenced by various treatments are presented in Table 2. The data pertaining total TVFA production are presented in Table 3. The TDDM and TDOM values in AFB_1 contaminated group (T_2) was reduced ($p < 0.05$) and higher in control (T_1) when compared to that of the other treatment groups. Further, the TDDM and TDOM values in T_3 group was lesser ($p < 0.05$) when compared to that of T_4 and T_5. The TDDM and TDOM values in T_4 group was lesser ($p < 0.05$) when compared to that of T_5. The results have indicated that inclusion of AFB_1 300 ppb in feed has decreased DM and OM degradability, in a significant ($p < 0.05$) manner when compared to that of the control. This finding was in concurrence with that of Westlake *et al.* (1989) who also suggested that the IVDMD of alfalfa hay was decreased by 50% with the addition of 1 $\mu g \cdot mL^{-1}$ AFB_1. Also, Mojtahedi et al. (2013) reported that the significantly reduced IVDMD ($p < 0.05$), in case of AFB_1 addition to the culture medium, and hence both the lowest IVDMD values as well as the highest IVDMD values were detected in AFB_1 treatments (900 and 0 $ng \cdot mL^{-1}$) as 0.54 vs. 0.68 respectively. Decreased IVDMD values with AFB_1 inclusion can be endorsed to the conciliated functions of rumen function by fibre digestion reduction and production of TVFA (Fehr and Delage, 1970; Helferich *et al.,* 1986a; Helferich *et al.,* 1986b).

Table 2. Effect of aflatoxin B_1 (AFB1) on rumen fermentation parameters.

Treatments	True digestibility of dry matter (%)	True digestibility of organic matter (%)	Gas production, (mL·g^{-1} DM)	Microbial biomass production (mg·100 mg^{-1})	Partitioning factor
T_1	40.63 ± 0.10e	41.20 ± 0.08e	148.97 ± 0.16e	20.78 ± 0.19c	2.72 ± 0.01d
T_2	36.08 ± 0.21a	37.32 ± 0.12a	140.66 ± 0.32a	17.67 ± 0.40a	2.56 ± 0.01a
T_3	37.78 ± 0.10b	38.38 ± 0.19b	142.51 ± 0.27b	19.02 ± 0.35b	2.63 ± 0.01b
T_4	38.75 ± 0.14c	39.25 ± 0.19c	144.78 ± 0.24c	19.09 ± 0.35b	2.67 ± 0.01c
T_5	39.58 ± 0.17d	40.28 ± 0.28d	146.65 ± 0.25d	20.22 ± 0.53bc	2.69 ± 0.01cd

Means values (3 replicates) bearing different superscripts in a column differ significantly ($p < 0.05$).
T1, control (basal feed); T2, T1 + 300 ppb AFB_1; T3, T2 with 0.05% of *Saccharomyces cerevisiae*; T4, 0.1% of *Saccharomyces cerevisiae*; T5, 0.2% of *Saccharomyces cerevisiae*; DM, dry matter.

Table 3. Effect of aflatoxin B_1 (AFB1) on volatile fatty acids production.

Treatments	Total volatile fatty acids (mM·100mL^{-1})	Acetate (mM·100mL^{-1})	Propionate (mM·100mL^{-1})	Butyrate (mM·100mL^{-1})	A : P ratio
T1	6.16 ± 0.04e	4.47 ± 0.04d	1.25 ± 0.01d	0.52 ± 0.02d	3.57 ± 0.02a
T2	5.15 ± 0.05a	3.81 ± 0.04a	0.93 ± 0.01a	0.36 ± 0.01a	4.09 ± 0.02d
T3	5.31 ± 0.03b	3.93 ± 0.02b	1.01 ± 0.01b	0.38 ± 0.01ab	3.89 ± 0.07c
T4	5.79 ± 0.04c	4.24 ± 0.02c	1.13 ± 0.01c	0.42 ± 0.02bc	3.75 ± 0.04b
T5	5.94 ± 0.07d	4.28 ± 0.06c	1.17 ± 0.03c	0.43 ± 0.02c	3.66 ± 0.06ab

Means values (3 replicates) bearing different superscripts in a column differ significantly ($p < 0.05$).
T1, control (basal feed); T2, T1 + 300 ppb AFB_1; T3, T2 with 0.05% of *Saccharomyces cerevisiae*; T4, 0.1% of *Saccharomyces cerevisiae*; T5, 0.2% of *Saccharomyces cerevisiae*.

However, some studies have suggested that the AFB_1 has no effect on *in vitro hay* DM disappearance (Pettersson and Kiessling, 1976; Jiang et al., 2012). Yeanpet et al. (2018) reported that the IVDMD and IVOMD were not significantly affected by AFB_1. In the present study, inclusion of *S. cerevisiae* in feed at any level significantly ($p < 0.05$) improved the TDDM and TDOM in a dose dependent manner as the *S. cerevisiae* has the binding ability towards aflatoxin. However, inclusion of *S. cerevisiae* in feed even at highest level (0.2%) could not reverse the TDDM and TDOM equivalent to that of control. This result was in agreement with earlier reports wherein Wu et al. (2009) suggested that *S. cerevisiae* has the capacity to bind to AFB_1 due to the oligomannanes presence in the cell wall of yeast. The chemical structure of oligomannanes has been found to have the 95% capability of binding to AFB_1. Bueno et al. (2007) has also stated that the enhanced capacity of *S. cerevisiae binding to the* AFB_1 because of the high availability of sites for binding. It has been understood that, the removal of toxins happens through the cell wall component adhesion, rather than by the covalent binding or the degradation of metabolites, even though the non-viable cells are binding to the toxins still (Shetty and Jespersen, 2006). Also, Devegowda et al. (1998) detected that the cell wall, which has been extracted from the yeast, *S. cerevisiae* was capable of binding to the wide range of mycotoxins, *in vitro*.

The GP value in the control group (T_1) was elevated ($p < 0.05$) and T_2 group was lesser ($p < 0.05$) when compared to that of other treatment groups. The GP value in T_3 was lesser ($p < 0.05$) than those of T_4 and T_5. The GP value in T_4 group ($p < 0.05$) was lower ($p < 0.05$) than that of T_5. The results indicated that the AFB_1 contamination of wheat straw at 300 ppb level

reduced the GP significantly ($p < 0.05$) when compared to that of control (T_1). The present result was in agreement with other reports wherein Mojtahedi et al. (2013) reported that, the raise of AFB_1 levels from 0 to 900 $ng \cdot mL^{-1}$ resulted in the reduction of production of gas from 0.071 to 0.051 as well as the cumulative production of gas has also reduced from 196.4 to 166.0 $mL \cdot g^{-1}$ DM. Similarly, Jiang *et al.* (2012) and Helferich et al. (1986a; 1986b) also stated that, the parameters of production of gas were decreased on the addition of AFB_1. These reductions in the production of gas production show that populations of microbes have been influenced by the contaminated feed that contains AFB_1. In the present study, inclusion of *S. cerevisiae* to the contaminated feed by aflatoxin improve the undesirable effects of aflatoxin on GP in a dose dependent manner. However, even highest level (0.2%) of *S. cerevisiae* could not reverse the gas production value equivalent to that of control. With respect to MBP, in control group (T_1) was elevated ($p < 0.05$) when compared to T_2, T_3, and T_4, statistically similar to that of group T_5. The MBP value in T_2 group was reduced ($p < 0.05$) when compared to the other treatment groups.

Similarly, the PF value in control group (T_1) was elevated ($p < 0.05$) and lesser ($p < 0.05$) in AFB_1 contaminated group (T_2) when compared to that of T_3 and T_4. The PF value in AFB_1 contaminated group (T_2) was lesser ($p < 0.05$) when compared to that of treatment groups. The PF value in group T_3 was lower than those of T_4 and T_5. The PF value between T_4 and T_5 groups did not vary significantly. The PF value of group T_5 was statistically similar to that of control (T_1). The findings of the current examination exposed that inclusion of AFB_1 to the feed at 300 ppb level resulted in significant decrease in the MBP and PF compared to that of control. Inclusion of *S. cerevisiae* at the highest level (0.2%) reversed the MBP and PF values equivalent to that of control.

The high PF in the feed, indicates that increased amount of degraded matter has been incorporated in the mass of microbes, which shows that efficiency of protein synthesis capacity of the microbe's id higher. More the PF in roughages, more the intake of DM has been observed (Harikrishna et al., 2012). The TVFA, acetate (A), propionate (P) and butyrate (B) values in control group (T_1) was elevated ($p < 0.05$) when compared to that of the other treatment groups i.e. T_2 to T_5. The TVFA, A, P and B value in AFB_1 contaminated T_2 group was lesser ($p < 0.05$) when compared to that of the treatment groups. The TVFA value in T_3 was reduced ($p < 0.05$) when compared to that of T_4. The TVFA value in T_4 was reduced ($p < 0.05$) when compared to that of T_5. The A and P value of T_3 was reduced ($p < 0.05$) when compared to that of T_4 and T_5. The A and P value between groups T_4 and T_5 did not vary significantly. The B value in group T_3 was lesser ($p < 0.05$) when compared to that of T_5. The B value between groups T_3 and T_4; and between T_4 and T_5 did not vary significantly. The A : P ratio in control group (T_1) was reduced ($p < 0.05$) when compared to that of other treatment groups i.e. T_2 to T_5. The A : P ratio in T_2 was higher ($p < 0.05$ when compared to that of other treatment groups i.e. T_1, T_3, T_4 and T_5. The A : P value of group T_3 was higher ($p < 0.05$) than those of T_4 and T_5. The A : P value between T_4 and T_5 groups did not vary significantly. The findings showed that the inclusion of AFB_1 at 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 Jiang et al. (2012) *who also stated that* the concentration of VFA reduced with the raise of dosage levels of AFB_1. Degradation of cellulose, synthesis of VFA and ammonia and proteolysis were lowered by 0.2 - 0.8 $mg \cdot kg^{-1}$ body weight of AFB_1 in acute bovine aflatoxicosis (Cook et al., 1986). Along with this, the VFA production has also been found to be inhibited irrespective to the nature of the substrate on raising the dosages of AFB_1 that has been consistent through the asymptotic gas volume reduction. The VFA level suppression along with the production of gas and ammonia has been concerned with the activity of microbes irrespective of the substrate. Contrastingly, a study by Edrington et al. (1994) has reported that, there have been no variations in the VFA concentrations of the rumen in the lambs growing with the given feed of AFB_1 (2.5 $mg \cdot kg^{-1}$ of diet). Another study by Helferich et al. (1986a) also stated that, the AFB_1 at the levels 60 - 600 ppb did not cause variations in the VFA production

in steers. In the follow-up study, of 0.714 μmol ingestion of AFB_1 per animal has not altered the VFA production in the rumen, in the goats that are lactating (Helferich et al., 1986b). In the present study, inclusion of *S. cerevisiae* to the aflatoxin contaminated feed partially ameliorated the adverse effects of AFB_1 on VFA production. However, inclusion of *S. cerevisiae* to the AFB_1 (300 ppb) contaminated feed caused reduction in A : P ratio. Increased A : P ratio due to aflatoxin contamination indicated that the fermentation has diverted towards more methanogenesis.

It was concluded that aflatoxin contamination of wheat straw based feed at 300 ppb level significantly affected the *in vitro* rumen fermentation in terms of reduced TDDM and TDOM, GP, MBP, PF, TVFA concentration and increased A : P ratio. Inclusion of *S. cerevisiae* to the contaminated feed partially to completely ameliorate the adverse effects of AFB_1 on *in vitro* rumen fermentation parameters.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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