Association between aflatoxin M1 excretion in milk and indicators of rumen fermentation in bovines

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Abstract
Aflatoxins and its metabolites negatively impact the ruminant health and production. The present cross-sectional study was aimed to determine the effect of aflatoxins on rumen fermentation by deducing the correlation between the aflatoxin M1 (AFM1) excretion in milk and indicators of rumen fermentation in bovines. The indicators of rumen fermentation were taken into account and correlated with AFM1 concentration in milk of 120 bovines (cattle ($n = 82$) and buffalo ($n = 38$)). The AFM1 in milk samples ($n = 120$) was quantified by ELISA kit. The correlation analysis revealed that with increase in excretion of AFM1 in milk, the pH ($r = 0.38$), methylene blue reduction time (MBRT) ($r = 0.43$), sedimentation activity time (SAT) ($r = 0.31$) and ammonia nitrogen content ($r = 0.34$) of rumen liquor increased, whereas the total volatile fatty acid (TVFA) content ($r = −0.25$), total bacterial count (TBC) ($r = −0.43$) and total protozoal count (TPC) ($r = −0.14$) of rumen liquor decreased. The results of the present study suggest that the presence of aflatoxins in rumen could have negative effect on the process of rumen fermentation. Therefore, the prevention of primary entry point(s) of AFB1 through the feed of bovines is important for the animal health as well as public health.

Keywords Aflatoxin M1 · Bovines · ELISA · Milk · Rumen fermentation

Introduction
Aflatoxins are toxic fungal metabolites mainly produced by Aspergillus flavus and A. parasiticus (Okoth et al. 2018; Frisvad et al. 2019). Aflatoxins are mutagenic, genotoxic, teratogenic and carcinogenic (group 1) with aflatoxin B1 (AFB1) being most toxic (McCullough and Lloyd 2019). The contaminated crops are the primary entry point of AFB1 in the food chain. The ingestion of AFB1 by dairy animals leads to the hydroxylation of the AFB1 toxin to a lesser toxic metabolite in the liver, i.e. aflatoxin M1 (AFM1), which excretes through the milk and urine of the animals (Sharma et al. 2020). On ingestion, aflatoxins are first encountered by the ruminal fauna and flora which acts as the first line of defence against aflatoxins. Several bacterial and yeast strains isolated from rumen liquor of cattle have been shown to have some detoxifying effect on aflatoxins in vitro (Intanoo et al. 2018). In addition, adult ruminants are considered as more resilient to the adverse effects of aflatoxins than monogastric animals and young/immature ruminants (Mathur et al. 1976; Upadhaya et al. 2009; Khodabandehloo et al. 2019). After ingestion, AFB1 is degraded in rumen into aflatoxicol and the remaining is absorbed in the digestive tract and converted to AFM1 in the liver, the same being excreted through milk (Nidhina et al. 2017). On the downside, the degraded aflatoxicol can almost entirely convert back into its more toxic parent compound AFB1 or AFM1 in the liver (Benkerroum 2020). The AFB1 and AFB2 can also be produced by rumen microflora in ruminal environment; therefore, the rumen can also act as a source of AFM1 in milk of ruminants (Nidhina et al. 2017).

Efforts to reduce human and animal exposure to aflatoxins have resulted in the establishment of regulatory limits for AFM1 in milk worldwide. The Food Safety and Standards Authority of India (FSSAI) and Codex Alimentarius of Food and Agriculture Organization (FAO)/World Health Organization (WHO) have established the
maximum permissible limit (MPL) of 0.5 µg/L of AFM1 in bulk milk, while the European Union established the MPL at 0.05 µg/L (EC 2010; FSSAI 2011 and Codex Alimentarius Commission 2017). The available physical and chemical methods of mycotoxin detoxification are restricted by problems concerning safety issues, possible losses in the nutritional quality, limited efficacy and cost implications (Intanoo et al. 2018), but recent studies suggest the use of gastro-intestinal microbials in detoxification process as a promising alternative (Var and Kabak 2009; Nguyen et al. 2020). However, limited information is available on the interactions between rumen microbes and aflatoxins, though the impact of rumen on degradation of aflatoxins has been reported (Var and Kabak 2009). In this context, the present study was designed to determine the possible effect aflatoxins have on rumen fermentation by finding the correlation between the AFM1 excretion in milk and indicators of rumen fermentation in bovines.

Materials and methods

Sampling procedure

The present cross-sectional study was carried out across the 22 districts of Punjab State of India during the period of July 2019 to February 2020. A total of 120 bovines (cattle \(n = 82\) and buffalo \(n = 38\)) were selected in the study (Fig. 1). The consent of the dairy farmers for enrollment in the study was obtained and the samples were collected in the presence of regional veterinary doctors. The ethical permission for the collection of rumen liquor samples was taken from the Institutional Animal ethics Committee (IAEC) (IAEC/2019/188–221).

From each animal, 50 mL of milk and 15 mL of rumen liquor samples were collected aseptically under the supervision of field veterinarians. The milk collection was done in sterile collection tubes which were transported to the laboratory in ice-box and stored at \(-20\) °C until further analysis. The rumen liquor was collected in sterile collection tubes by needle puncture of rumen at the left paralumbar fossa using 20 mL syringe with 16 gauge needle under aseptic conditions. The collected samples were strained through double layer of muslin cloth as described by Lengemann and Allen (1955) and labeled properly as ‘strained rumen liquor’ (SLR) with unique identification number. The preservation of rumen liquor was done in saturated solution of mercuric chloride and in 8% formaldehyde solution separately. For analysis of rumen metabolites, 2–3 drops of mercuric chloride were added per 4–5 mL of rumen liquor sample and for rumen microflora count, 1 mL of formaldehyde was added per 1 mL of rumen liquor sample (Singh et al. 2018). The samples were immediately stored in ice box after collection and in \(-20\) °C upon reaching laboratory for further analysis. The district wise details of the collected samples are presented in Fig. 1.

AFM1 detection in milk samples by ELISA

All the collected milk samples \(n = 120\) were subjected to commercial competitive Aflatoxin M1 ELISA (EuroProxima
B.V., The Netherlands) having the limit of detection (LOD) of $5 \times 10^{-6}$ µg/mL for milk samples. All the procedures were performed as per the instructions provided by the manufacturer. The milk samples were prepared for ELISA testing by carrying out centrifugation at $2000 \times g$ for 10 min at 4 °C after being thawed to room temperature and the separated fat layer was removed by using spatula. The optical density (O.D.) values for each sample were recorded immediately at 450 nm by using microplate spectrophotometer. The concentration of AFM1 in milk samples was calculated by using regression equation obtained from calibration curve built by plotting values of % maximal absorbance of standards versus the analyte’s equivalent concentration (µg/mL).

\[
\text{%maximal absorbance} = \frac{\text{O.D. of standard or sample}}{\text{O.D. of zero standard}} \times 100
\]

The O.D. of the standards and samples were averaged and corrected by subtracting the mean O.D. of blank wells before computation. The O.D. is inversely proportional to the AFM1 concentration in the sample.

**Analysis of rumen liquor**

To study the correlation of aflatoxins on rumen fermentation, the rumen liquor parameters selected as indicators of rumen fermentation were as follows: (1) Physico-chemical parameters (colour, odour and consistency, pH, methylene blue reduction time (MBRT) and sedimentation activity time (SAT)); (2) Rumen metabolites (total volatile fatty acids (TVFAs) and ammonia nitrogen); and (3) Rumen microflora count (total bacterial count (TBC) and total protozoal count (TPC)).

**Physico-chemical parameters**

**Colour, odour and consistency**

After the collection of rumen liquor, the colour, odour and consistency of rumen liquor samples were immediately recorded as described by Garry (2002). The observations were grouped into 2 categories: normal and abnormal (Supplementary Table 1). The normal group included greenish-yellow to yellowish-brown colour, aromatic odour and slightly viscous consistency. The observations recorded other than normal were grouped under abnormal category.

**pH of rumen liquor**

The pH of rumen liquor was determined immediately after collection by using digital pH meter.

**Methylene blue reduction test (MBRT)**

Methylene blue reduction test was performed as per the method described by Dirksen (1979). A total of 0.25 mL of 0.03% methylene blue solution were added in 5 mL of freshly collected rumen liquor in a test tube and incubated in water bath at room temperature. The time taken for the reduction of methylene blue by the microbial constituents of rumen liquor, i.e. for discoloration of sample, was recorded by using a plain rumen fluid as a basis for comparison.

**Sedimentation activity test (SAT)**

Sedimentation activity test was performed according to the method described by Dirksen (1979). The freshly collected rumen liquor was observed in glass test tubes kept in water bath at 39 °C. The time required for sedimentation and floatation was referred to as sedimentation activity time.

**Rumen metabolites**

**Total volatile fatty acids**

Total volatile fatty acid concentration in rumen fluid was estimated by the method of Barnett and Reid (1957). A total of 1 mL of SRL were transferred into Markham’s micro-Kjeldahl distillation apparatus and 1 mL of scaribrick buffer (10% potassium oxalate and 5% oxalic acid in equal volumes) was added. The cup was made air tight with stop cork and by adding some water in it. The steam distillation was carried and approximately 75 mL distillate was collected. To the distillate, few drops of phenolphthalein indicator were added and titrated against standard 0.01 N NaOH solution.

\[
\text{TVFAs (mEq/L) = Amount of standard base used (mL) × 10}
\]

**Ammonia nitrogen (NH₃-N)**

The Conway micro-diffusion technique (Conway 1957) was used to estimate NH₃-N in SRL. In the inner chamber of Conway cell, 1 mL of 2% boric acid solution containing mixed indicator was taken and 1 mL of clear SRL was pipetted into the outer compartment. Furthermore, 1 mL of 50% potassium carbonate solution was added slowly into the outer compartment opposite to SRL. After covering the micro-diffusion cell, it was gently rotated clockwise and anticlockwise at a horizontal plain to mix contents of outer chamber followed by incubation for 1 h. Thereafter, contents of the inner chamber were titrated against standard 0.01 N H₂SO₄ solution. Simultaneously, a blank of 1 mL distilled water was also titrated against standard acid.
Ammonia nitrogen \( \frac{\text{mg}}{\text{dL}} \) = \( \frac{\text{Amount of standard acid used} \times 0.14}{\text{Volume of sample taken (mL)}} \times 100 \)

**Rumen microflora count**

**Total bacterial count**

Total bacterial count was determined by using Nigrosin slide technique as per the method described by Gall et al. (1949). In brief, the preserved sample of SRL was thawed and shaken vigorously in order to separate microbes from feed particles and to break microbial clumps. The thawed rumen liquor sample was centrifuged at 3000 rpm for 5 min and the supernatant was serially diluted in 1:10,000 ratio with distilled water. The diluted bacterial suspension was mixed well and 0.01 mL of diluted suspension was taken onto a clean grease free glass slide. A loopful of saturated Nigrosin stain was added to 0.01 mL of diluted suspension. The sample was mixed and uniformly spread over 2 × 2 cm area of glass slide with the platinum loop. The smear was dried immediately over a preheated (about 60 °C) hot plate. The bacterial counting was done in total 30 microscopic fields from 2 × 2 cm area of stained smear under 100 x objective of microscope. The total bacterial count per mL of rumen liquor was calculated by the formula given below:

\[ \text{TBC per mL of sample} = \frac{\text{Number of bacteria per field} \times \text{Number of microscopic field (1000)} \times \text{Dilution factor (10^6)}}{1000} \]

**Total protozoal count**

The rumen protozoal count was done as per the method described earlier by Naga and El-Shazly (1969). A total of 5 mL of sample were taken through wide bore (3.5 mm) pipette into a test tube. Then 15 mL of normal saline solution (0.85%) was transferred and thereafter 5 mL Lugol’s iodine was added. The solution was mixed gently and 0.1 mL of sample was transferred swiftly to a dry clean slide and spread under a glass cover of known area (24 × 60 mm). A total of 30 fields were counted per slide both for ease and accuracy and total protozoal count per mL of rumen liquor was calculated by the formula given below:

\[ \text{TPC per mL of sample} = \frac{\text{Number of protozoa per field} \times \text{Number of microscopic field (1000)} \times \text{Dilution factor (100)}}{1000} \]

**Statistical analysis**

All the data entry and the computation of mean, standard deviation, maximum and minimum concentrations were carried out using Microsoft® Excel 2010. The correlation analysis was taken as the measure for the degree of association between AFM1 excretion in milk and indicators of rumen fermentation computed as Pearson correlation coefficient \( r \) by using Microsoft® Excel 2010. The correlation coefficient was categorized into weak (0–0.25), fair (0.25–0.5), good (0.5–0.75) and excellent (0.75–1) correlation based on the \( r \) value as described by Cohen (2013).

**Results**

**Occurrence of AFM1 in bovine milk samples from Punjab**

Covering all the districts of Punjab \( n = 22 \), a total of 120 milk samples were collected and analyzed for the presence of AFM1. Out of the tested samples, 63.3% (76/120) of the samples exceeded the maximum permissible limit (MPL) of the European Union (EU) set by the European Commission (EC) (i.e. 0.05 μg/L), whereas, 10.83% (13/120) of samples were above the MPL set by Food Safety and Standards Authority of India (FSSAI) (i.e. 0.5 μg/L). On analysis, the overall mean concentration ± standard deviation (SD) of AFM1 concentration in milk samples was found to be 0.22 ± 0.36 μg/L.

**Correlation between AFM1 excretion in milk and indicators of rumen fermentation in bovines**

The Pearson correlation coefficient \( r \) was calculated between the AFM1 concentrations in milk of bovines and their respective rumen liquor parameters. The details of data on AFM1 in milk and rumen liquor parameters are presented in Supplementary Table 1.

Of the physico-chemical parameters analyzed, the data on colour, odour and consistency of the rumen fluid samples was found non suitable to deduct any significant association with AFM1 excretion in milk. The pH of the rumen liquor samples was found to be positively correlated with
AFM1 excretion in milk having the correlation coefficient (r) of 0.384 (category: fair correlation) with p-value of 0.000015 (statistically significant). The methylene blue reduction time of rumen liquor significantly increased (p-value of <0.00001) with increase in excretion of AFM1 in milk (r = −0.429; fair correlation). Another indicator of rumen microbial activity, SAT also significantly increased (p-value of 0.0005) with increase in excretion of AFM1 in milk (r = 0.312; fair correlation).

The analysis of rumen metabolites revealed TVFA's content of rumen liquor to be negatively correlated (fair correlation) while ammonia nitrogen content to be positively correlated (fair correlation) with correlation coefficient of −0.249 and 0.337 respectively. Both the associations were found to be statistically significant (p-value — TVFAs: 0.006; p-value — ammonia nitrogen: 0.00017).

For determining the effects of aflatoxins on rumen microflora, the bacterial and protozoal counts were taken into account. The analysis showed that as the AFM1 excretion in milk increased, both the TBC (r = −0.429) and TPC (r = −0.137) of rumen liquor decreased. The TBC was found to have fair correlation with the AFM1 excretion in milk with p-value of <0.00001 (statistically significant); however, the TPC of rumen liquor had a weak correlation which was statistically non-significant (p-value = 0.136). The correlation coefficient of various indicators of rumen fermentation is summarized in Table 1.

**Discussion**

The excretion of aflatoxin M1 in milk presents a continuous threat to food safety. The present study reported the AFM1 prevalence in milk from Punjab to be 10.83% with respect to the national standards, i.e. FSSAI. The epidemiological studies from other parts of India also showed the high presence of AFM1 in milk with 20.6% prevalence in Haryana (Sharma et al. 2020), 38% in Karnataka and 17.3% in Tamil Nadu (Siddappa et al. 2012), 75% in Goa (Kanungo and Bhand. 2014) and 09% in Uttar Pradesh (Rastogi et al. 2004).

The present study provides an insight on the possible alteration in rumen environment by the ingestion of aflatoxins by animals. The negative ‘fair correlation’ of −0.429 between the TBC of rumen liquor and AFM1 excretion in milk showed that the presence of aflatoxins has negative impact on the bacterial population in rumen. The results of the present study are in agreement with Mathur et al. (1976) who reported that AFB1 affected some rumen micro-organisms morphologically and physiologically and also inhibited their growth. Fink-Gremmels (2008) and Jiang et al. (2012) have also reported AFB1 of having negative impact on rumen microflora. However, no previous data was available on the effect aflatoxins have on rumon protozoal population.

The inhibitory effect of aflatoxins on the microbial growth and activity can be further supported by the increasing MBRT and SAT of rumen liquor with increasing AFM1 excretion in milk (Supplementary Table 1). The present study revealed that the TVFA’s content of rumen liquor and AFM1 excretion in milk were negatively correlated. It is in concordance with previous studies of Mathur et al. (1976), Jiang et al. (2012), Khodabandehloo et al. (2019) and Jiang et al. (2020), which also reported the decrease in TVFA’s concentration in rumen liquor on increasing AFB1 intake in diet, while Edrington et al. (1994) found no differences in ruminal VFA’s concentrations in growing lambs fed 2.5 mg AFB1 per kg diet, and Hellerich et al. (1986) also reported that AFB1 at 60–600 mg/kg did not influence the production of VFA in cattle. The decrease of VFA’s production in rumen can be attributed to impaired fermentation of rumen due to decrease in population and activity of rumen microflora as a result of high aflatoxin concentration in rumen (Fink-Gremmels 2008; Jiang et al. 2012).

In present study, the ammonia nitrogen concentration in rumen significantly increased with increase in excretion of AFM1 in milk. Previous studies by Khodabandehloo et al. (2019) and Jiang et al. (2020) also reported the increase in ammonia nitrogen concentration of rumen liquor with increase in AFB1 in diet of ruminants. On the contrary, Jiang et al. (2012) reported ammonia nitrogen concentration declined with the increase in AFB1 dosage. The increase in ammonia nitrogen concentration in rumen with

| Parameters          | Correlation coefficient (r) | p-value       |
|---------------------|-----------------------------|---------------|
| Physico-chemical parameters |                             |               |
| pH                  | 0.384 (positive fair correlation) | 0.000015*     |
| MBRT                | 0.429 (positive fair correlation) | 0.00001*      |
| SAT                 | 0.312 (positive fair correlation) | 0.0005*       |
| Rumen metabolites   |                             |               |
| TVFAs               | −0.249 (negative fair correlation) | 0.006*        |
| Ammonia nitrogen    | 0.337 (positive fair correlation) | 0.00017*      |
| Rumen microflora    |                             |               |
| TBC                 | −0.429 (negative fair correlation) | 0.00001*      |
| TPC                 | −0.137 (negative weak correlation) | 0.136        |

*Statistically significant (i.e. p-value < 0.05)
increase in AFM1 excretion through milk of bovines could be due to the negative impact aflatoxin have on growth and activity of rumen microflora as reported in previous (Fink-Gremmels 2008; Jiang et al. 2012) and in the present study which reduces the utilization of ammonia nitrogen by rumen microbes for bacterial protein synthesis in turn leading to rise in the ammonia nitrogen concentration of rumen. The increased ammonia nitrogen content raises the pH of rumen which further leads to increased denaturation of proteins and peptides into ammonia nitrogen.

Regarding the effect of aflatoxins on ruminal pH, the analysis in present study revealed pH of rumen to be increasing with increase in excretion of AFM1 in milk. The previous study by Khodabandehloo et al. (2019) also reported the increase in pH of rumen liquor of rams with increase in concentration of AFB1 in feed. The increase in pH of rumen with increasing AFM1 concentration in milk could be due to decrease in TVFA’s production and increasing ammonia nitrogen concentration as a result of negative impact on the growth of rumen microflora (Supplementary Table 1) and disruption of rumen fermentation (Fink-Gremmels 2008; Jiang et al. 2012) caused by increased aflatoxin concentration in rumen. Therefore, ingestion of aflatoxins certainly impacts ruminal digestion and in turn the overall health of the animal.

The present study has the limitation of not considering physiological, nutritional or other toxin effects on rumen flora of studied animals; therefore, we acknowledge that further in vitro and/or in vivo experimental studies need to be conducted in order to prove the causative effect(s) of aflatoxin on rumen microbes.

In conclusion, the present study reported that the excretion of AFM1 in milk of bovines is positively correlated with the pH, MBRT, SAT and ammonia nitrogen content of rumen liquor, while negatively correlated with TVFA’s content, TBC and TPC of rumen liquor. The above-mentioned results suggest that the presence of aflatoxins in rumen has negative impact on ruminal fermentation. However, further in vitro and in vivo studies are required to get detailed knowledge on which type of microbial population in rumen is inhibited by presence of aflatoxins and the associated inhibitory factors responsible for it.

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Author contribution PD and JSB conceived and designed research. HT and PD conducted sample collection and experiments. PD, JSB, RS and GS analyzed data and supervised the research. HT and PD wrote the manuscript. JSB, RS and GS reviewed and edited the manuscript. All authors read and approved the manuscript.

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Data availability All relevant data are within the paper and its supporting information files.

Code availability Not applicable.

Declarations

Statement of informed consent Farmer informed consent was asked during fieldwork. All applicable international, national and/or institutional guideline for the care and use of animals were followed.

Ethics approval The ethical permission for the collection of rumen liquor samples was taken from the Institutional Animal ethics Committee (IAEC) (IAEC/2019/188–221).

Consent to participate The consent of the dairy farmers for enrollment in the study was obtained and the samples were taken in presence of regional veterinary doctors.

Consent for publication All persons included as co-authors on this manuscript have provided their consent to be herein included.

Conflict of interest The authors declare no competing interests.

References

Barnett AJ, & Reid RL. (1957). Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. The volatile acid production from fresh grass. Journal of Agricultural Science, 48:315–21.

Benkerroum, N. (2020). Aflatoxins: Producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African countries. International Journal of Environmental Research and Public Health, 17(4), 1215.

Codex Alimentarius Commissions. General standard for contaminants and toxins in food and feed. CXS 193–1995, Adopted in 1995. Revised in 1997, 2006, 2008, 2009 amended in 2010, 2012, 2013, 2014, 2015, 2016, 2017. Food and Agriculture Organization of the United Nations; 2017.

Cohen, J. (2013). Statistical power analysis for the behavioral sciences. Academic press.

Conway EJ. (1957). Micro diffusion analysis and volumetric error;(4th Edn.) Crosby Lockwood and son. London. England.

Dirksen G. (1979). Digestive system-examination of rumen fluid. Clinical Examination of Cattle. Eds: G Rosenberger, G Dirksen, HD Grunder D Krause and M Stober. Verlag Paul. Berlin. 200–12.

EC (European Commission), European Commission Regulation (EC) No 165/2010; 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017. Food and Agriculture Organization of the United Nations; 2017.

Edrington, T. S., Harvey, R. B., & Kabena, L. F. (1994). Effect of aflatoxin in growing lambs fed ruminally degradable or escape protein sources. Journal of Animal Science, 72(5), 1274-1281.

Fink-Gremmels, J. (2008). The role of mycotoxins in the health and performance of dairy cows. The Veterinary Journal, 176(1), 84-92.
Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S. B., Nováková, A., Chen, A. J. & Houbraken, J. (2019). Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology, 93, 1-63.

FSSAI, Food Safety and Standards Authority of India (contaminants, toxins and residues) regulations. Notification No. 2–150 15/30/2010; 2011.

Gall, L. S., Burroughs, W., Gerlaugh, P., & Edgington, B. H. (1949). Special methods for rumen bacterial studies in the field. Journal of Animal Science, 8(3), 433-440.

Garry FB. (2002). Indigestion in ruminants. In: Smith B P (Ed) Large Animal Internal Medicine. 3rd Edn. pp. 731-41. WB Saunders Company Ltd.

Helferich, W. G., Baldwin, R. L., & Hsieh, D. P. H. (1986). [14C]-aflatoxin B1 metabolism in lactating goats and rats. Journal of Animal Science, 62(3), 697-705.

Intanoo, M., Kongkeitkajorn, M. B., Pattarajinda, V., Bernard, J. K., Callaway, T. R., Surisasyathaporn, W., & Phasuk, Y. (2018). Isolation and screening of aflatoxin-detoxifying yeast and bacteria from ruminal fluids to reduce aflatoxin B1 contamination in dairy cattle feed. Journal of Applied Microbiology, 125(6), 1603-1613.

Jiang, Y. H., Yang, H. J., & Lund, P. (2012). Effect of aflatoxin B1 on in vitro ruminal fermentation of rations high in alfalfa hay or ryegrass hay. Animal Feed Science and Technology, 175(1-2), 85-89.

Jiang, Y., Ogunade, I. M., Arriola, K. G., Pech-Cervantes, A. A., Kim, D. H., Li, X. & Adesogan, A. T. (2020). Effects of a physiologically relevant concentration of aflatoxin B1 with or without sequestering agents on in vitro rumen fermentation of a dairy cow diet. Journal of Dairy Science, 103(2), 1559-1565.

Kanungo, L., & Bhand, S. (2014). A survey of Aflatoxin M1 in some commercial milk samples and infant formula milk samples in Goa, India. Food and Agricultural Immunology, 25(4), 467-476.

Khodabandehloo, M., Malecky, M., Aliarabi, H., Saki, A. A., & Alipour, D. (2019). In vitro evaluation of aflatoxin B1 effect on gas production and ruminal fermentation parameters. Iranian Journal of Veterinary Research, 20(4), 263-269.

Lengemann, F. W., & Allen, N. N. (1955). The development of rumen function in the dairy calf I. Some characteristics of the rumen contents of cattle of various ages. Journal of Dairy Science, 38(6), 651–656.

Mathur, C. F., Smith, R. C., & Hawkins, G. E. (1976). Growth and morphology of Streptococcus bovis and of mixed rumen bacteria in the presence of aflatoxin B1, in vitro. Journal of Dairy Science, 59(3), 455-458.

McCullough, A. K., & Lloyd, R. S. (2019). Mechanisms underlying aflatoxin-associated mutagenesis—implications in carcinogenesis. DNA Repair, 77, 76-86.

Naga, M. A., & El-Shazly, K. (1969). Activities of rumen micro-organisms in water buffalo (Bos bubalus L.) and in Zebu cattle. Journal of Dairy Research, 36(1), 1-10.

Nguyen T, Flint S, & Palmer J. (2020). Control of aflatoxin M1 in milk by novel methods: A review. Food Chemistry, 311:125984.

Nidhina, N., Bhavya, M. L., Bhaskar, N., Muthukumar, S. P., & Murthy, P. S. (2017). Aflatoxin production by Aspergillus flavus in rumen liquor and its implications. Food Control, 71, 26-31.

Okoth, S., De Boevre, M., Vital, A., Diana Di Mavungu, J., Landsooht, S., Kyallo, M. & De Saeger, S. (2018). Genetic and toxicogenic variability within Aspergillus flavus population isolated from maize in two diverse environments in Kenya. Frontiers in Microbiology, 9, 57.

Rastogi, S., Dwivedi, P. D., Khanna, S. K., & Das, M. (2004). Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Control, 15(4), 287-290.

Sharma, H., Jadhav, V. J., & Garg, S. R. (2020). Aflatoxin M1 in milk in Hisar city, Haryana, India and risk assessment. Food Additives & Contaminants: Part B, 13(1), 59-63.

Siddappa, V., Nanjegowda, D. K., & Viswanath, P. (2012). Occurrence of aflatoxin M1 in some samples of UHT, raw & pasteurized milk from Indian states of Karnataka and Tamilnadu. Food and Chemical Toxicology, 50(11), 4158-4162.

Singh, H., Singh, G., & Nayyar, S. (2018). Effect of yea sacc1026 supplementation on feed intake and rumen fermentation in buffalo calves during summer season. Indian Journal of Animal Nutrition, 35(3), 305-312.

Upadhaya, S. D., Sung, H. G., Lee, C. H., Lee, S. Y., Kim, S. W., Cho, K. J., & Ha, J. K. (2009). Comparative study on the aflatoxin B1 degradation ability of rumen fluid from Holstein steers and Korean native goats. Journal of Veterinary Science, 10(1), 29-34.

Var, I., & Kabak, B. (2009). Detection of aflatoxin M1 in milk and dairy products consumed in Adana, Turkey. International Journal of Dairy Technology, 62(1), 15-18.

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