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ROLE OF SEASONAL CHANGES IN AFLATOXIN B_1 CONTAMINATION IN COMPOUND CATTLE FEEDS IN KERALA, INDIA.

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Abstract

Aflatoxin B_1 (AFB_1) is the most toxic and primarily produced mycotoxin in Agricultural products around the globe, and its consumption causes life-threatening effects in humans and animals. Similarly, contaminated animal feeds will carry the toxin to animal products, like milk, eggs, and meat. This study discusses the role of seasonal changes in the contamination of Aflatoxin B_1 in three branded compound cattle feeds in Idukki, Kollam, and Palakkad districts in Kerala, India. The study revealed that the monsoon (South Western Monsoon (SWM) and North East Monsoon (NEM)) cause elevated Aflatoxin B_1 in the compound cattle feeds. On average, 65.28% of the total samples comply with the Bureau of Indian Standards (BIS) of AFB_1 (20 μgkg^{-1}) in compound cattle feeds, while the European Commission regulation (5 μgkg^{-1}) satisfies only 17.36%. Moreover, a significant correlation was observed between Water activity (aw) and AFB_1 in the compound cattle feed. The safe Water activity (a_w) of more than 95% of the samples confirmed that AFB_1 contamination occurred in the raw materials, not the compound cattle feeds. The theoretical extrapolation of Aflatoxin M_1 from Aflatoxin B_1 content in the tested samples pointed out that the cattle feed tested did not produce beyond the limit of FDA/Codex regulation for Aflatoxin M_1 (0.5 μgkg^{-1}) in the milk by the intake of compound cattle feeds maximum of 6 kg day^{-1} at the reported AFB1 contamination rate. This study will provide insights into AFB_1

contamination throughout various seasons in compound cattle feeds in three districts in Kerala and its subsequent carryover into milk.

Keywords: Aflatoxin, *Aspergillus*, Carcinogenicity, seasonal changes, compound cattle feeds, Water activity

1 Introduction

Aflatoxins (AFs) are one of the most potent mycotoxins, mainly found in crops. Chemically, they are polyketide derivative secondary metabolites with a difurocoumarin as their basic structure (Roze et al. 2013; Liew and Mohd-Redzwan 2018). The causative agents of AFs belong to the fungi *Aspergillus* section *flavi*, specifically *Aspergillus flavus* and *Aspergillus parasiticus*, which thrive in regions with warm temperatures and humidity. The emphasised polycyclic structure of AFs provided the compound with carcinogenicity and high thermal stability, which makes them a prime fungal food contaminant on Earth. More than 20 aflatoxins have been discovered so far, but only six of them, viz., Aflatoxin B_1 (AFB_1), Aflatoxin G_1 (AFG_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_2 (AFG_2), Aflatoxin M_1 (AFM_1), and Aflatoxin M_2 (AFM_2) represent the primary concern (Okoth et al. 2018). Aflatoxin B_1 (AFB_1) is one of the deadliest, most widely, and primarily found aflatoxins; thus, it receives the most global attention (Gizachew et al. 2019). The International Agency for Research on Cancer (IARC) has categorised and included natural AFs as class IA carcinogens (IARC 1993). In humans, AFs cause cancer, reduced immune response, retarded growth in children, etc. and recently, a synergy between Hepatitis B Virus (HBV) and AFs was reported in the emergence of Hepato Cellular Carcinoma (HCC). Similarly, in animals, AFs cause reduced milk production, deformities in the hepatic system, reduced growth, and some unspecified biological effects (Dhanasekaran et al. 2011).

AFs spread in agricultural products during favourable environmental conditions. In agricultural products, they are highly resistant to basic food processing procedures. Thus, AFs play a significant role in the health and economic sustainability of agriculture-based economies in middle and low-income countries (Udomkun et al. 2017). Moreover, during the era of international trade, the developed countries were also threatened by AFs contaminated foods.

From the health safety perspective of humans and animals, the prevalence of AFs happens through the consumption of fungal-contaminated crops or crop products and the consumption of contaminated animal by-products in the form of milk, meat and eggs (Iqbal et al. 2014). AFB_1 in animals causes various irregularities ranging from mild response to life-threatening. Similarly, AFB_1 reduces the quality and safety of animal products used for human consumption. AFM_1 is a hydroxylated metabolic by-product of AFB_1 found in the milk of mammals, especially in milch animals produced after consuming contaminated feeds (Eker et al. 2019).

FM_1 is produced by the hepatic detoxification system of mammals at a rate of 0.3-6.3% of the total AFB_1 ingested in the animals (Veldman et al. 1992; Diaz et al. 2004). Consequently, the ramification of consuming

contaminated feeds leads to health defects in livestock animals and produces contaminated animal products for human consumption (Abrunhosa et al. 2014). In short, the safety and quality of the feed for the animals should be addressed in the same magnitude as human food.

The highly dynamic climatic conditions severely affect the booming of fungal contaminants because of the favourable environmental conditions for their optimum growth (Yu et al. 2022; Valencia-Quintana et al. 2020). Specifically, some seasonal changes exacerbate the AFs contamination in animal feeds, which is reported from all over the globe (Minooeianhaghighi et al.2021; Becha and Devi 2013; Ismail et al. 2017). The warm and humid climate is the optimum condition for AFs production (Mutuli et al. 2021), and most of the AFs contamination is reported from tropical and subtropical regions (Acur et al. 2020). Similarly, Water activity (a_w) is another primary determinant for the thriving of causative fungal populations in food materials (Aharon. 2008).

This study looks for the seasonal influence in the contamination of AFB_1 in three branded compound cattle feeds in three prominent milk-producing districts of Kerala, India.

2 Materials and methods

2.1 Study area

The study was conducted in three districts in Kerala, India, during the period 2022-2023 (Fig.1). Kerala is an Indian state situated in the southernmost part, which experiences a typical tropical monsoon climate and distinct geography with a large stretch of the coastal area on the west side and a hilly area represented by Western Ghat on the east side (Kerala state action plan on climate change. 2014).

The three districts viz. Idukki, Kollam, and Palakkad are prominent milk-producing districts (NDDB India. 2016) in Kerala state; moreover, they represent distinct geography. The Idukki district represents high land with significant forest cover (Ramachandran 2016), while the Kollam district is a coastal area, and Palakkad majorly represents midland and plains. The presence of the Palakkad gap (the only opening in the Western ghats) (Raj and Azeez 2010) makes it the least rainy district in the state (Krishnakumar et al. 2009). Such unique geographical differences will affect the climate and may influence the fungal contamination in compound cattle feeds.

The seasonal changes are more evident in Kerala, with its warm climate and high humidity, which promotes the thriving of causative fungi (Cotty and Jaime 2007). In Kerala, there are four distinctive seasons with characteristic climates (Becha et al. 2013) they are winter (December-February), summer (March-May), southwest monsoon (June-September), and northeast

monsoon (October-November). So, the samples were collected each season, belonging to three major branded compound cattle feeds in the state.

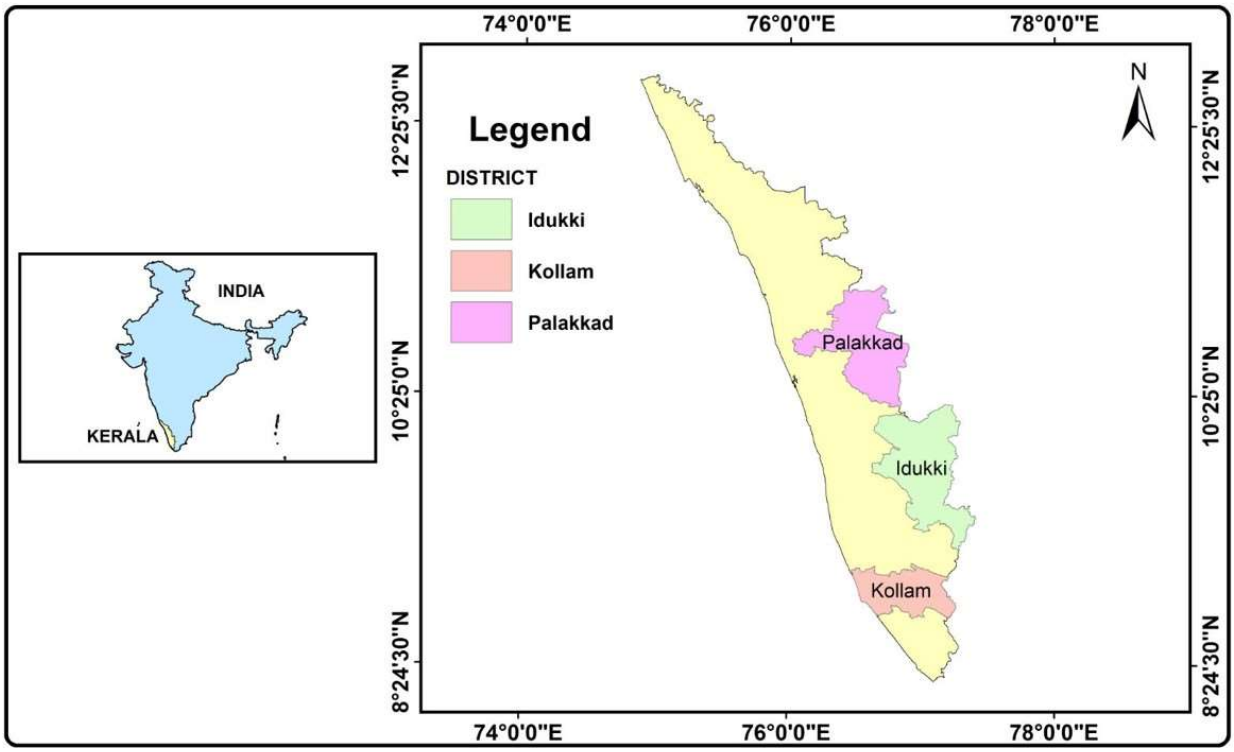

Fig.1 Study area with distinctive identification of three districts Palakkad, Idukki and Kollam belongs to the State of Kerala, India. Map prepared by Arc GIS

2.2 Collection of samples

A total of 144 samples of three branded compound cattle feeds were collected in a year during winter (December-February), summer (March-May), southwest monsoon (June-September), and northeast monsoon (October-November) seasons, randomly from cattle farms and houses in the study area. An aggregate of 500g of samples was collected from three positions (bottom, middle, and top) of the cattle feed sack based on the ISO 6497:2002 method. The sample was kept in a dry and air-tight black polythene bag and taken to the laboratory within a day; further, it was pulverised and held in a dark and dry place for analysis.

2.3 Water activity (a_w) of the samples

A water activity Meter (AQUALAB 4TEV, USA) with a dew point sensor (±0.0003a_w) was used to measure the a_w in the compound cattle feed sample. Powdered samples were filled in the half portion of the sample carrier (≈ 5gm), and the results were read out after 5 mins.

2.4 Quantification of Aflatoxin B_1 in the compound cattle feed and method validation

Aflatoxin quantification was performed by HPLC with a Fluorescence detector (FLD) with post-

column derivatisation with an electrochemical cell (Walter et al. 1988) by following modified AOAC Official Method 991.31. AFB_1 stock was prepared in 50 ml methanol by making up 1mg

of AFB_1(Sigma-Aldrich), and a working standard of 100 μgL^{-1} was prepared by methanol and 1% acetic acid in a 1:1 ratio.

HPLC-FLD method validation was carried out based on the method of linearity, recovery, precision, LOD (Limit of Detection), and LOQ (Limit of Quantification) as described by Fujita (2009). The linearity was evaluated using five different concentrations of AFB_1 within the range of 0.5-20 μgL^{-1} (0.5, 1, 2, 5, and 20). LOQ and LOD were determined using the formulas LOQ = 10 × σ/m and LOD = 3.3 × σ/m, where σ represents the residual standard deviation, and m stands for the slope of the calibration curve (Muscarella et al., 2009). AFB1-free compound cattle feed spiked with AFB_1 at different concentrations (5, 10, and 20 $\mu g\ kg^{-1}$) was used for the recovery study, and recovery was calculated as follows.

Recovery (%) = (AFB_1 quantity identified/ AFB_1 theoretical quantity) X 100.

Sample preparation and Immuno-Affinity column (IAC) cleanup

Before the HPLC analysis, the AFB_1 was extracted from the sample using the immunoaffinity column, and this procedure is termed sample cleanup. In the process, a mixture of 25g of sample and 5g of NaCl was blended with 100 ml of 80% HPLC grade methanol in distilled water, and the mix was filtered by fluted filter paper. Then, the 10 ml filtered mix was diluted with 40 ml distilled water. The diluted solution was filtered by glass microfiber paper. After that, 10 ml of the solution was passed through an Immunoaffinity column (Afla B, Vicam) at one drop per second and let pass entirely through it, followed by washing two times with 20 ml of double distilled water. Then, the Aflatoxin was eluted outed by 1 ml of HPLC grade methanol, and 20 µl of the eluate was used for HPLC analysis.

2.4.1 HPLC **analysis**

The HPLC system (Shimadzu, Tokyo, Japan) with a gradient pump (LC-20AT), a C18 column (250mmx 4.6mm, 5µm Shiseido, Japan), and a Fluorescent detector with post-column derivatisation Electrochemical cell (Kobra cell) were used for the analysis. An injection volume of 20 µl of the cleanup solution was used for AFB_1 detection. Meantime the mobile phase contains micro-filtered ethanol-water (40:60 v/v) with a trace amount of KBr and 4M HNO_3 (400ml HPLC methanol+600ml HPLC Water+119 mg KBr+ 350µl 4 M HNO_3) was used with a flow rate of 1.0 ml/ min. AFs detection is carried out by a Fluorescent detector in which Excitation occurs at 365 nm and Emission happens at 455 nm. In the meantime, the post-column derivatisation was carried out in the KOBRA® Cell in the presence of KBr and HNO3 before detection by the detector. The concentration of AFs present in the samples was calculated using the following formula.

$$\text{Conc. of } AFB_1 = \frac{\text{Std concentration of } AFB_1 \text{ X Sample area X Recovery X} 10^9}{\text{Std area X Sample concentration}}$$

2.5 Theoretical prognosis of AFM_1 and calculation of carry-over of AFB_1 into Milk.

Carry-over of AFB_1 into milk as AFM_1 is an inevitable metabolic reaction at a rate of 0.3-6.3% (Veldman et al. 1992; Diaz et al. 2004) carried out in the biological system as part of the detoxification of AFB_1. The carry-over occurs in the hepatic system of mammals and is excreted through Urine, Milk and Bile (HU et al. 1984). According to Veldman et al. (1922), a linear relationship has been proposed for the theoretical identification of AFM_1 from the consumed AFB_1 through the cattle feed per day and expressed as

AFM_1 (ng kg^{-1} in milk) = 1.19 × AFB_1 consumed (µg per cow per day) + 1.9

Moreover, for the extrapolation of the AFM_1, the mean of three-time replicate values corresponding to AFB_1 was used. In addition, the total quantity of compound cattle feeds was fixed at 3-6 kg day^{-1}, a range at which the compound cattle feeds were routinely given to the milch cattle observed while collecting the samples.

2.6 Statistical analysis

All the values analysed were the mean values obtained from three independent replications. Significance levels were assessed by ANOVA using the SPSS statistical tool. Similarly, Graphical representation was done by Origin 8.5 software.

3 Results and discussion

3.1 Validation of HPLC-FLD methods

The calibration curve formed from the data obtained from five different concentrations of AFB_1 was found to be linear with a coefficient of determination (R^2) value of 0.999 (Table 1). The LOD and LOQ values obtained from the calibration curve were 0. 65 and 1.98 µg kg^{-1}. Those values are below the Maximum permissible limit (MPL) for AFB_1 in compound cattle feed (5 µg kg^{-1}) by the European Union standards and found satisfactory. The data presented in Table 1 reported that the overall recovery for AFB_1 is 87.2 %, and the overall % RSD (Relative standard deviation) ranges between 0.19% and 0.54%. Since the recovery and the % RSD remain within the safe limit (recovery (70- 110%) & % RSD <20%), the method validity was identified as satisfactory (Muscarella et al. 2009). Similarly, no interfering peaks were observed in the chromatogram, and the retention time (RT) of AFB_1 was consistently observed at 16.39 minutes.

Table 1 AFB_1 spiked level in the toxin-free compound cattle feed for recovery and precision

Linearity and sensitivity						
Analyte	Range, µgkg^{-1}	slope	Intercept	R^2	LOD µgkg^{-1}	LOQ µgkg^{-1}
AFB_1	0.5-20	35907	3630.8	0.9999	0.655	1.98
Recovery						
Analyte	Spiking concentrations µgkg^{-1}	Recovery %		%RSD		
AFB_1	5	86.66		0.54		
	10	87.24		0.32		
	20	87.8		0.19		

*Results are the mean of three-time replicates

3.2 AFB_1 in the compound cattle feed samples

144 compound cattle feed samples of three different brands were analysed for AFB_1 and a_w during four seasons (winter, summer, southwest monsoon and northeast monsoon) in three districts (Idukki, Kollam and Palakkad) of Kerala state. In the current scenario, the highly unpredictably complex global climatic conditions lead to a myriad of fungal epidemics which cause catastrophic spreads of mycotoxins. That threatens the quality and safety of animal feeds in both raw and finished stages (Warnatzsch et al. 2020). In connection with that, the seasonal changes in Kerala are erratic and provide optimum conditions like temperature, humidity and moisture (Cotty et al. 2007) for the proliferation of AFs causative fungi. In total, an average of 17.23 ± 9.01 µg kg^{-1} of $AFB_1$with a minimum value of 2.16 µg kg^{-1} and a maximum value of 48.56 µg kg^{-1} were identified from the entire cattle feed samples. In India, the Bureau of Indian Standards (BIS) regulate AFB_1 in cattle feeds to 20 µg kg^{-1}. Similarly, the FDA (Food and Drug Administration) regulates the total AFs to 20 µg kg^{-1} limit. However, the European Commission regulates (ECR) AFB_1 in cattle feeds more stringently, and the maximum limit is 5 µg kg^{-1} (Bervis et al. 2021). This study concludes that an average of 63.89% of the total samples complied with the BIS standard. In comparison, the European Commission Regulation (ECR) for AFB_1 was satisfied by only 17.36% of the total samples. From the four seasons, the maximum aflatoxin contamination in the analysed samples was reported in the monsoon seasons, specifically in the NEM with a total average of
23.64 ± 5.13 µg kg^{-1} followed by SWM with 20.71± 8.11 µg kg^{-1} and comprehensively represented in Fig.2. This result is aligned with the findings of Becha and Devi (2013), they reported that the total AFs content in the animal feed was maximum during NEM. However, in the report of Ansari et al. (2018) and Akbar et al. (2019), the winter season was the most contaminating season. In Kerala, the NEM possesses a warm climate and high humidity, which are ideal for fungal growth; similarly, SWM has high humidity all over the state but comparatively low temperature (Report

on climate: Ministry of Environment & Forest, Govt. of India). Specifically, an average of 58.33% of samples collected during NEM failed to contain the statutory limit of BIS, and 100% of the sample failed to meet the EU standard for AFB_1; similarly, the SWM season was also found that an average of more than 97% of the total sample beyond the EU limit and represented in Table 2. However, the winter and summer seasons had a reduced contamination rate, which is supported

by the study of Becha and Devi (2013). The total average of AFB_1 content in the summer (SR) and winter (WR) seasons was identified as 12.11± 8.90 µg kg^{-1} and 12.44± 7.25 µg kg^{-1} respectively.

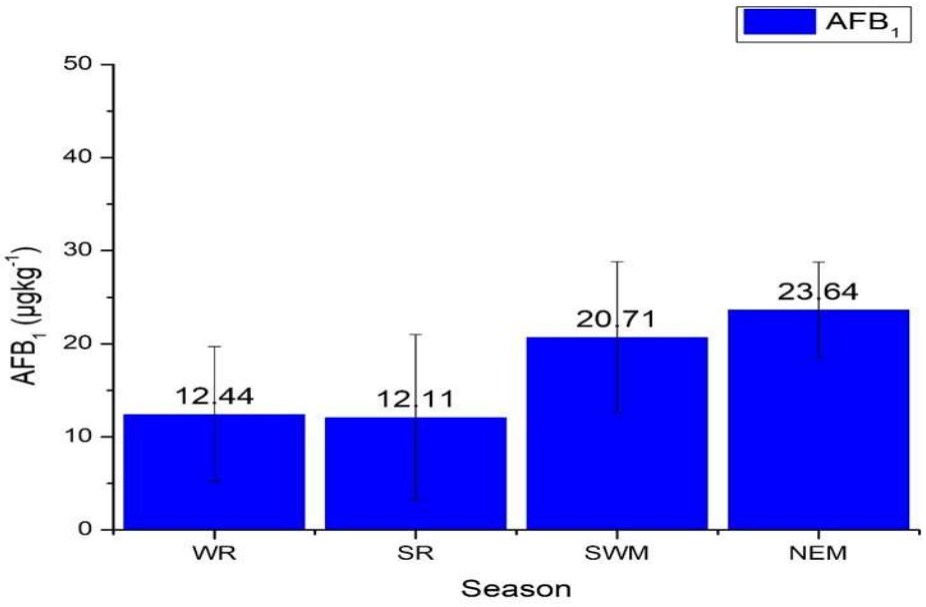

Fig.2 Total average of AFB_1 in the compound cattle feeds during four seasons in Kerala and the values represented here are mean of three-time replicated results with S.D in µg kg^{-1}

Table 2 Non-compliance of AFB_1 of compound cattle feeds from districts during each season

Seasons	Districts	Samples Non-comply with 20 ppb(BIS) of AFB_1 (%)	Samples Non-comply with 5 ppb(EU) of AFB_1(%)
	Idukki	8.33	75

Winter	Kollam	33.33	91.6
	Palakkad	8.33	50
Summer	Idukki	16.66	50
	Kollam	25.00	75
	Palakkad	8.33	50
SWM	Idukki	25.00	91.6
	Kollam	50.00	100
	Palakkad	41.66	100
NEM	Idukki	58.33	100
	Kollam	41.66	100
	Palakkad	75.00	100

BIS- Bureau of Indian Standards, EU- European Union, SWM - South Western Monsoon and NEM- North East Monsoon

Herein, the role of seasonal changes is reported in the contamination of AFB_1 in the compound cattle feeds, especially monsoon seasons (SWM and NEM) provide the most favourable condition, in which NEM showed the maximum contamination. During the time of winter and summer, moderate levels of humidity were reported in Kerala. However, it may change due to temperature and environmental factors like coastal areas, highlands, and forest cover (Report on climate: Ministry of Environment & Forest, Govt. of India). Only one sample from the two monsoon seasons was found below the ECR for AFB_1 in compound cattle feeds. Conversely, the summer and the winter seasons are safest for AFB_1 contamination in compound cattle feeds (Choudhary et al. 2020), specifically in the summer (Fig.3). Overall, the seasons in Kerala significantly ($(F(3,428)=65.841; P<0.01)$ affect the AFB_1 production in the compound cattle feeds during this study. Specifically, the Tukey test revealed that the NEM produces more significance, followed by the SWM.

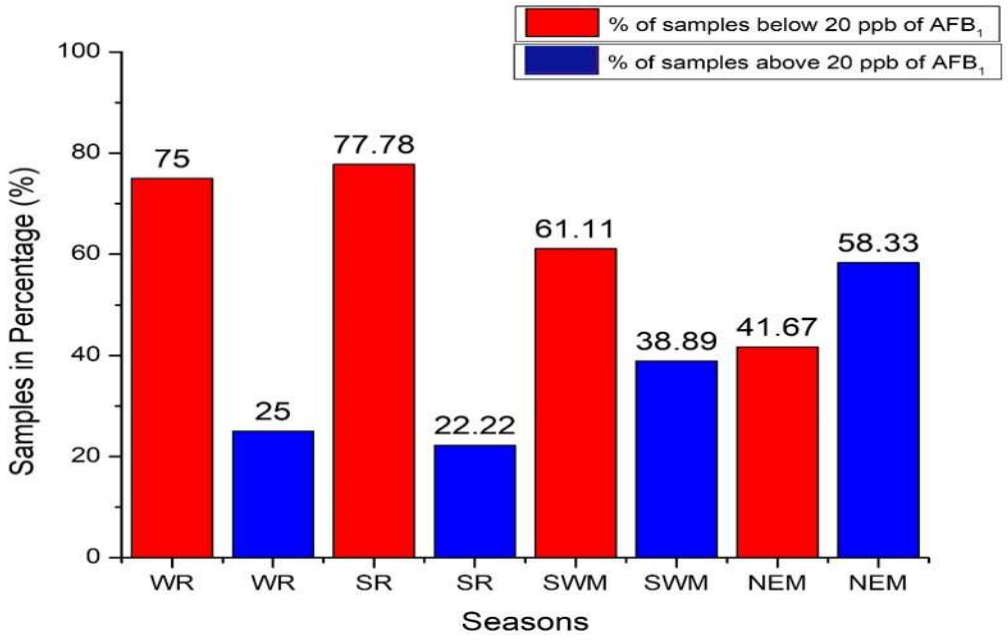

Fig.3 Seasonal changes in the AFB_1 contamination in the compound cattle feed and values represented here are the mean of three-time replicated results in percentage corresponding to 20 µg kg^{-1}statutory limit (BIS)

By taking the district-wise analysis, Idukki districts identified the least AFB_1 contaminated cattle feeds with a total average of 15.48 ± 8.98 µg kg^{-1}, followed by Palakkad having 16.30 ± 8.7 µg $kg^{-1,}$ and the most infected was Kollam district with 19.93± 8.6 µg kg^{-1} and a high degree of statistical significance was ($P<0.01$) observed. Specifically, feeds from the Kollam district produced more contaminated cattle feeds during the winter, summer, and SWM(Fig.4) seasons, and this variation can be justified by the presence of coastal area, which will increase the humidity and rainfall (Bergemann and Jakob 2016). In the meantime, Palakkad was identified as the moderately contaminated district; however, the least contaminated cattle feed in this study was observed here during the summertime, but in contrast, the same district also reported the maximum contaminated feeds during NEM. (Fig.4). Geographical specialities like the Palakkad gap and Palakkad Plain play a crucial role in climatic conditions, especially in temperature and humidity, which are essential for the proliferation and toxin production by causative fungi (Raj and Azeez 2010). Furthermore, Jothish and Nayar (2004) reported more than 50% of *Aspergillus* spores in indoor conditions of Palakkad; such a high concentration of causative fungal spores could make the food and feed items more vulnerable. While taking cattle feed brand, brand number B1 was identified as the most contaminated with a total average of 18.24 ± 8.50 µg kg^{-1}, followed by brands B2 and B3 with a total average of 16.99 ± 8.46 µg kg^{-1} and 16.44± 9.94 µg kg^{-1} respectively. Brand B3 produced the least AFB_1 contamination during summertime, but in contrast, the same brand is also responsible for the highest amount of AFB_1 in the NEM season (Fig.5).

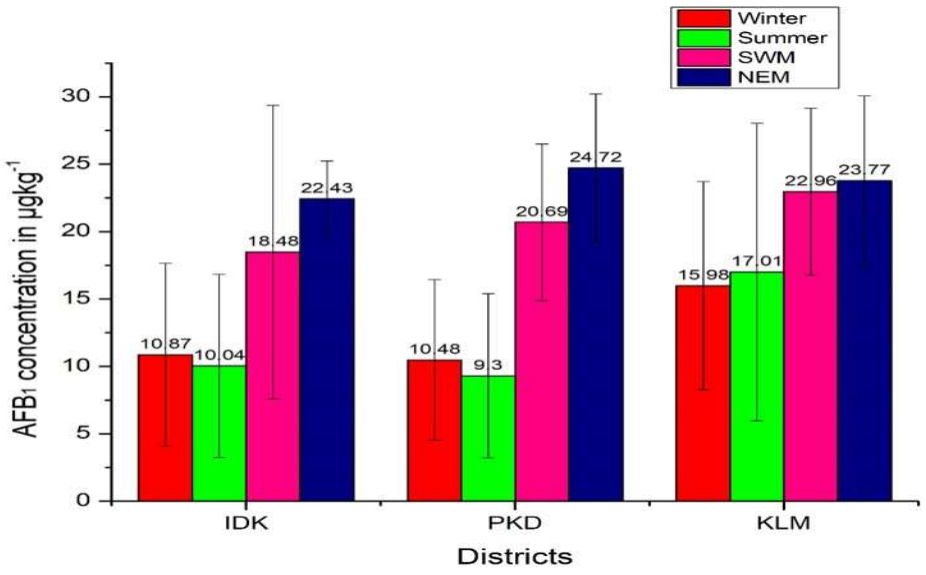

Fig.4 Total average of AFB_1 in compound cattle feed from three districts in four seasons and values represented here are mean of three-time replicated results with S.D in µg kg^{-1}

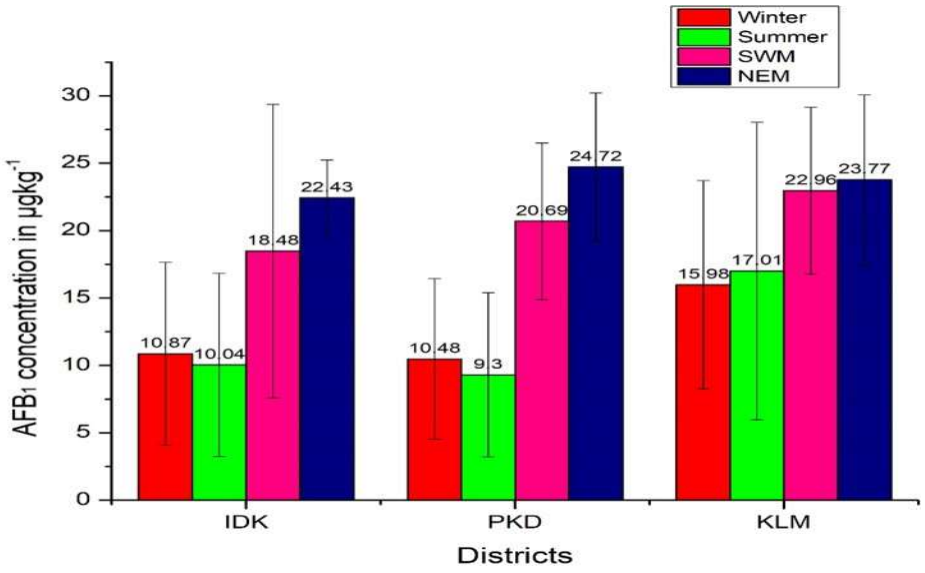

Fig.5 Total average of AFB_1 variations in compound cattle feed brands in four seasons and values represented here are mean of three-time replicated results with S.D in µg kg^{-1}

3.3 Water activity (a_w)

The a_w analysis of the sample found that the average of the total sample is 0.75± 0.05, which is considered to be the safe limit for foods, which debilitates the thriving of the food-spoiling fungal population and AFs production (Natarajan et al. 2022) (Fig.6). In analysis, the a_w was found to have a moderate level of significant positive correlation with AFB_1 ($r(432) = 0.591$; $P<0.01$) and showed statistical significance with seasons ($F(11,420) = 3.322$; $P<0.01$) and districts ($F(3,428) = 6.100$; $P<0.01$). Water activity a_w is a pivotal determinant for the flourishing of aflatoxin-producing fungi in the food and feeds (Tai et al. 2020). In causative fungi, a_w controls the proliferation and the regulation of the structural and functional gene clusters for Aflatoxin bio-synthesis (Schmidt et al. 2009). Notably, the mean of a_w in this study was 0.75± 0.05, with a maximum value of 0.96. The underlining factor found in this study is that the mean value reported belongs to the safe limit of a_W where none of the reports supports the production of AFs. Furthermore, Natarajan et al. (2021) reported that 0.87 a_w inhibits the production of AFB_1 and gene regulation; similarly, Northolt et al. (1976) supported 0.83 a_w as the minimum for AFB_1 synthesis, and Mousa et al. (2011) reported 0.82 a_w as the limiting point. With this in mind, it can be concluded that the AFB_1 synthesis may not occur at the stage of the finished product as compound cattle feeds but happened in the raw material stage either during the post-harvest phase or during the storage in the warehouse of the cattle feed producer. Using highly AFs contamination-prone crops like maize, cotton seeds and groundnuts (Wenndt et al. 2020; Almeida et al. 2019) in the feeds proves these findings. Meantime, 4.86% of the sample, which showed an average of 0.92 a_w proportionately produced high AFB_1 in the sample, and it may confirm the production of AFB_1 at the finished product as compound cattle feeds. It can be explained as the established optimum micro-environmental conditions due to the unhygienic storage facility or unsystematic handling of cattle feeds, which supports the proliferation of the causative fungi.

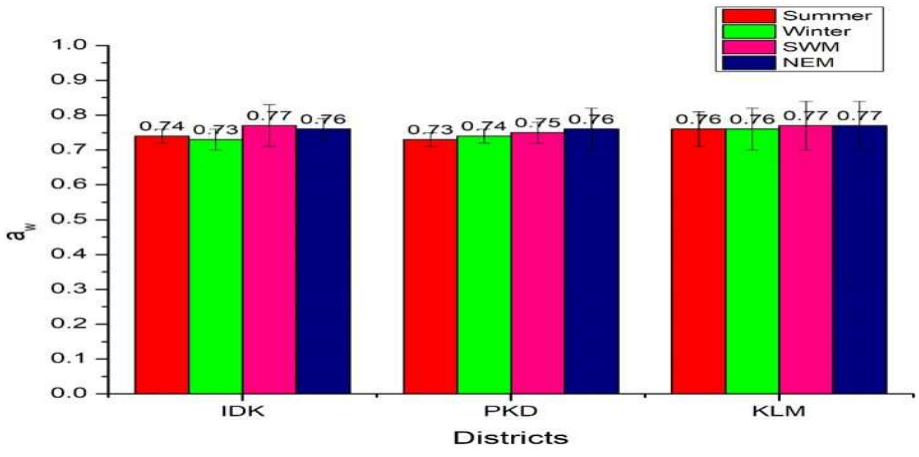

Fig.6 Total average of a_W in the compound cattle feeds from three districts during four seasons in Kerala and values represented here are mean of three-time replicated results with S.D

3.4 Theoretical prognosis of AFM_1 content in Milk at various quantities of compound cattle feeds per day

This study also shed light on the probability of AFB_1 carry-over into the milk as AFM_1, which is categorised as a Class 2B carcinogen by IARC (WHO, 1993). Theoretical extrapolation of AFM_1 from identified AFB_1 in the compound cattle feed was carried out based on the study by Veldman et al. (1922). From the results, an average of more than 50% of the total sample was safe for European Commission Regulation(ECR) for AFM_1 (0.05 µg kg^{-1}) at 3 and 4 kg day^{-1} quantity of compound cattle feed at the current level of AFB_1 contamination in the feeds during winter and summer (Table 3). Patyal et al. (2020), Akash et al. (2021), and Akbar et al. (2019) reported the presence of AFM_1 in dairy products in the Indian subcontinent at an alarming level. However, the theoretical prognosis of AFM_1 may not represent the pinpoint value as it is considered only the intake value of AFB1 and neither considering the total milk yield of the cattle. Similarly, cattle will consume other feeds besides compound cattle feeds, which can also be a source of AFB_1 (Patyal et al. 2021). In the meantime, the theoretical prognosis of AFM_1 concluded that the two seasons, summer and winter, would be the safest for the milk as per both the ECR regulation and FDA/Codex, as presented in Table 3. Moreover, all samples during NEM and more than 90% of the samples during SWM did not comply with the ECR regulation. However, compared with the FDA/Codex regulation on AFM_1 in milk (0.5µg kg^{-1}), all the extrapolated results were safe irrespective of quantity, season, brand, and location. After all, it can help to envisage the AFM_1 content in the milk. Interestingly, the theoretical prognosis of AFM_1 at the current contamination rate with up to the quantity of 6 kg day^{-1} of compound cattle feeds during the entire year fails to produce beyond the regulatory limit set by FDA/Codex Alimentarius (0.5 µg L^{-1})(CODEX STAN 193-1995). In connection with that, John and Manoj (2013) reported that an average of 65.62kg $Month^{-1}$ of compound cattle feeds had been consumed by 58% of total milch cattle in Kerala and also forecasted a rise in compound feed consumption by 5% shortly due to some particular circumstances. According to the report by John and Manoj (2013), there may be a chance for a further rise in AFs in the milk. In the meantime, the reports from the National Milk Safety and Quality Survey 2018 (NMQS-2018) conducted by the Food Safety Standard Authority of India (FSSAI. 2018) confirmed that 19.8% of the sample from Kerala was reported as non-compliant against AFM_1, which indirectly points out the safety concern of the cattle feeds. Despite that, the occurrence of AFM_1 in milk beyond MRL (Maximum residue limit) was reported in various parts of India (Sharma et al. 2019; Patyal et al. 2021).

Table 3 Extrapolated AFM_1 values carried over from AFB_1 during four seasons with 3-6 kg of compound cattle feeds

Seasons	Quantity of compound cattle feeds intake per day(kg day^{-1})	Range of predicted AFM_1 in ppb(µg L^{-1})	Mean of predicted AFM_1 in ppb (µg L^{-1})	Hypothetical percentage of AFM_1 that comply with EU standard (0.05 ppb (µg L^{-1})) in Milk	Hypothetical percentage of AFM_1 that comply with FDA/Codex standard (0.5 ppb (µg L^{-1})) in Milk
Winter	3	0.01- 0.11	0.04	55.56%	100%
	4	0.01- 0.15	0.06	47.25%	100%
	5	0.02-0.18	0.07	30.55%	100%
	6	0.02-0.23	0.09	27.78%	100%
Summer	3	0.01-0.07	0.04	61.11%	100%
	4	0.01-0.10	0.06	50.00%	100%
	5	0.02-0.12	0.07	44.44%	100%
	6	0.02-0.15	0.08	44.44%	100%
SWM	3	0.01-0.13	0.07	13.88%	100%
	4	0.02-0.18	0.08	8.33%	100%
	5	0.02-0.22	0.12	5.55%	100%
	6	0.03-0.26	0.14	5.55%	100%
NEM	3	0.06-0.14	0.08	Nil	100%
	4	0.08-0.19	0.11	Nil	100%
	5	0.10-0.24	0.13	Nil	100%
	6	0.12-0.29	0.16	Nil	100%

***EU- European Union, FDA- Food and Drug Administration, SWM - South Western Monsoon and NEM- North East Monsoon**

5 Conclusion

This study reveals the role of seasons in producing AFB_1 in the compound cattle feeds in three districts in Kerala. The significant number of non-compliant samples will be a threat to milk safety as well as cattle health. Moreover, the safe water activity (a_w) of more than 95% of the cattle feeds indicates the safety of the raw materials used for production. However, a compound cattle feed is a proportionate blend of grains, brans, deoiled cakes of various nuts and seeds, vitamins, and minerals; out of them, ingredients viz. maize, ground nut cake, and cotton seed cake are highly prone to aflatoxin contamination. So, procuring such sensitive raw materials requires utmost care and quality checks. Furthermore, their storage should be in climate-proof

warehouses with standardised SOPs (Standard operating procedures); otherwise, the erratic climate of Kerala will provide the optimum conditions for the proliferation of causative fungi. Similarly, a minute fraction of cattle feed (<5%) with an unsafe water activity (a_w) has proportionately produced a

high amount of AFB_1 in the compound cattle feed, revealing the need for proper awareness and training for the importance of its safety and storage. In conclusion, two monsoon seasons affect AFB_1 contamination; moreover, the safe management of the raw materials and awareness for the cattle farmers need to be improved for safe cattle feed, directly affecting milk safety and cattle health.

Declarations

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Author Contributions

Authors **Chammakalayil Sukumaran Arun** and **Pambayan Ulagan Mahalingam** contribute equally to the conceptualisation, sample collection, laboratory analysis, design, and critical review of important intellectual content, validation, and the first draft of the manuscript. **Haris Parengal** did the manuscript correction. All authors read and approved the final manuscript.

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