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Title:	**Microbiological Evaluation of Different Types of Branded and Non-branded Ready-to-Eat Snacks Sold in Elementary Schools of District Peshawar, Pakistan**
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Determination and Detoxification of Aflatoxins in Cattle Feed Samples Collected from Local Areas of Lahore, Pakistan

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ABSTRACT

Aflatoxins are naturally occurring toxic metabolites produced by fungi in various food crops. The current study aims to determine the level of aflatoxins by using thin-layer chromatography in cattle feed samples and evaluates different methods including physical, chemical, and biological methods used for detoxification. A total of 80 samples including 40 fresh feed samples (20 wanda and 20 makai) and 40 dry feed samples (10 samples of dana, 10 samples of chokhar, 10 samples of toori, 3 samples of Khal, and 7 samples of makai dana) were collected from 22 areas of Lahore, Pakistan. Physical methods used for detoxification included washing and boiling, while chemical methods included detoxification with 10% citric acid, 10% acetic acid, 2% sodium hydroxide, and 2% hydrochloric acid. Finally, biological methods included detoxification with 0.15% mustard oil and 0.15% black seed oil. Among the samples, 9 wanda, 7 makai, 3 khal, 2 dana, and 2 chokhar samples were found to be contaminated. Only 6.25% of the samples exceeded the permissible limit of 20 parts per billion (ppb) set by the USFDA. A chemical method applied to khal samples achieved the highest reduction percentage at 63.64%. Meanwhile, physical and biological methods resulted in reduction percentages of 23.91% and 35.72%, respectively. These findings demonstrate the efficacy of various approaches, particularly highlighting the significant reduction achieved with the chemical method in khal samples. Moreover, these findings contribute to the understanding of effective strategies for mitigating aflatoxin contamination in cattle feed.

Keywords: aflatoxins, cattle feed, contamination, detection, detoxification

1. INTRODUCTION

Aflatoxins are metabolites produced by food polluting fungi and they are secondary in nature [1]. Aflatoxin, originating from *Aspergillus flavus*, was identified in 1960 as the mold accountable for generating toxins. This toxic substance can induce a range of adverse health effects in human beings spanning from acute to chronic outcomes, such as liver cancer, chronic hepatitis, jaundice, hepatomegaly, and cirrhosis [2].

Aspergillus flavus and *Aspergillus parasiticus* are two species of fungi commonly found in hot and humid climates, with *Aspergillus flavus* particularly favoring the aerial parts of plants [3]. Among various aflatoxins, namely B1, B2, G1, and G2, aflatoxin B1 stands out as the most potent and perilous naturally occurring carcinogenic toxin for both human beings and animals [4, 5]

Aflatoxin B1 is detoxified by the humen when they take in these aflatoxins, however, they undergo some metabolic processes and form metabolites that are

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secondary in nature, in their liver [6, 7]. Aflatoxins have various health influences, such as genetic mutation [8], nervous system damage [9], epigenetic effects [10], reproductive abnormalities [11], and retarded growth [12]. Through careful examination, it was confirmed that aflatoxin B1 undergoes conversion into Aflatoxin M1, a milk toxin found in dissolved form. This discovery raised concerns regarding the potential contamination of the food chain through the amplification of this aflatoxin in meat, eggs, milk, and milk products [13–15].

Aflatoxin contamination in cattle feed is a significant concern, both at the national and international levels. In Pakistan, studies have highlighted the presence of aflatoxins in cattle feed, posing risks to animal health and productivity. A study found aflatoxin contamination in the feed and milk of dairy animals, emphasizing the need for effective control measures. In response to this issue, national regulatory authorities in Pakistan have been actively engaged in implementing strategies to mitigate aflatoxin contamination, including monitoring programs, awareness campaigns, and enforcement of quality standards [16]. Internationally, organizations such as the Food and Agriculture Organization (FAO) of the United Nations (UNO) have been working to address aflatoxin contamination in livestock feed and food products. They have developed guidelines, standards, and initiatives to promote safe practices and to ensure the quality and safety of feed and dairy products, worldwide [17].

The hot and moist climate acts as a controlling element because it stimulates the production and proliferation of molds and toxins [18]. Another way to attain the maximum production of milk and milk products is the selection of a good breed along with regular feed [19]. The current research aims to determine the presence of aflatoxins, analyze the efficacy of the methods of detoxification for aflatoxins in feed samples, and perform a comparative analysis of fresh and dry feed cattle.

2. MATERIALS AND METHODS

Samples were collected from different areas of Lahore including Sitara Colony, Shanghai Road, Muhammadi Road, Gul Colony, Javed Colony, Nishtar Town, Chungi Amir Sidhu, Nurpur Pullarwan village, Jahaman, Ashiana Road, Sitara Colony 2, Pul Bandian Wala, Muft pura, Hudiara, Bedian, Kasur, Kahna Nau, Gaga, Harbanspura, Barki, Shahdara and Gulshan Yaseen Colony during the period June-August, 2021. The samples were analyzed at the Aflatoxin Laboratory, Food and Biotechnology Research Centre (FBRC), Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore. Different chemicals and reagents used in this study were 200ml chloroform, 0.05g celite, sodium sulfate, 40% anhydrous ether, and 5ml acetone.

2.1. Sample Collection

A total of 80 cattle feed samples (both fresh and dry) of commonly known forage grasses (*Sorghum* spp), chokhar (*Triticum aestivum L.)*, toori (*Triticum* spp), khal (*Gossypium* spp), and makai dana (*Zea mays*) found in Pakistan (Table 1) were collected from places including Sitara Colony, Shanghai Road, Muhammadi Road, Gul Colony, Javed Colony, Nishtar Town, Chungi Amir Sidhu, Nurpur Pullarwan village, Jahaman, Ashiana Road, Sitara Colony 2, Pul Bandian Wala, Muft pura, Hudiara, Bedian, Kasur, Kahna Nau, Gaga, Harbanspura, Barki, Shahdara and Gulshan Yaseen Colony in Lahore to determine and detoxify aflatoxins. All the samples, each weighing 50g, were collected

and stored in sealed polyethylene bags for both detection and detoxification purposes. Fresh samples were stored at -4°C in the refrigerator until further processing.

Table 1. Aflatoxin Concentration (ppb) in Fresh Feed Samples of Cattle Collected from Different Areas of Lahore

Sr. No	Sample Id	Aflatoxin Concentration (ppb)
Fresh Wanda		
1	W_A	0
2	W_B	20.73
3	W_C	7.70
4	W_D	0
5	W_E	0
6	W_F	0
7	W_G	11.89
8	W_H	5.70
9	W_I	3.026
10	W_J	0
11	W_K	0
12	W_L	6.52
13	W_M	0
14	W_N	0
15	W_O	7.71
16	W_P	0
17	W_Q	0
18	WE	4.30
19	W_S	0
20	W_T	3.82
Makai		
21	M_A	20.12
22	M_B	0
23	M_C	0
24	M_D	0
25	M_E	7.32
26	M_F	4.20
27	M_G	0
28	M_H	4.27
29	M_I	0
30	M_J	0
31	M_K	0
32	M_L	4.60
33	M_M	0
34	M_N	0
35	M_O	0
36	M_P	0
37	M_Q	3.12
38	M_R	3.83
39	M_S	0
40	M_T	0

2.2. Determination of Aflatoxins

Feed samples were properly shaken to obtain a uniformly mixed sample. Subsequently, 50g of each feed sample was added to separate conical flasks and marked accordingly. To each flask, 25ml of distilled water was added and shaken by hand. Afterwards, 200ml of chloroform and 0.05g of celite were added to each sample. The flasks were carefully covered with aluminum foil and securely fixed on a wrist action shaker for 30 minutes. After the shaking, the flasks were removed cautiously and filtration was performed using three-fold Whatman filter paper placed on the top of a beaker. Additionally, 0.05g of sodium sulfate was added on top of the filter paper. Then, 50ml of the filtrate from each sample was transferred into separate beakers and placed on a hot plate until the filtrate evaporated in a steam bath.

2.3. Spotting on Thin Layer Chromatography (TLC) Plate

Initially, 0.5ml of chloroform was added to each dried sample to create dilutions using a pipette. Subsequently, immediate spotting was performed by using a capillary tube on the TLC (Thin-Layer Chromatography) plate using this solution. Furthermore, a 5µl standard was also applied to the TLC plate. Before spotting, the plate was prepared by labeling it and drawing a line measuring 1cm above the

lower edge using a lead pencil. To prevent contamination during spotting, the capillary tube was washed with chloroform after each spot. Then, the prepared TLC plates were placed inside the chromatographic tanks [20].

2.3.1. First Mobile Phase. The chromatographic tank was filled with 50ml of 40% anhydrous ether, serving as the initial mobile phase. The TLC plate was placed with caution at the center of the tank and allowed to develop until reaching a point that was 1cm below the labeling. Subsequently, the developed plate was carefully removed from the tank and dried on a hot plate for approximately 4-5 seconds.

2.3.2. Second Mobile Phase. For the subsequent development of the TLC plate to detect aflatoxin contamination, a second mobile phase consisting of 5ml acetone and 45ml chloroform (1:9 v/v) was added. Following the initial development in the first phase, the plate was redeveloped during the second phase. Subsequently, the plate was dried once again and taken for the confirmation of aflatoxin detection.

2.3.3. Confirmation To confirm the presence of aflatoxins, two types of analyses were conducted, namely qualitative and quantitative analyses. In qualitative analysis, the focus was on detecting the presence of aflatoxins in the samples using a UV light scanner that emitted UV light at a wavelength of 365nm. On the other hand, quantitative analysis aimed to determine the quantity of aflatoxins present in all the samples by utilizing various standards. Since aflatoxin B1 is commonly found in food and feed samples, a standard concentration of 2.07 parts per billion (ppb) was used specifically for its detection.

2.3.4. Qualitative Analysis The TLC plate, after going through both mobile phases, was sent to the main Aflatoxin Laboratory PCSIR for confirmation. Confirmation was done under the UV light with a wavelength of 363nm using a UV spectrophotometer. The samples with aflatoxin contamination appeared with blue spots throughout the TLC plate, while the samples without aflatoxin contamination showed orange-red spots. Hence, aflatoxin contamination was detected [20].

2.3.5. Quantitative Analysis. To determine the quantity of aflatoxin present in the samples, quantitative analysis was performed by using various standards. Aflatoxin concentration was determined using the following formula:

Aflatoxin (ppb) = S×Y×V/W×Z

S= Values in ml of AF standard of equivalent intensity to Z

Y= Concentration of AF Standard in mg/ml

Z= Volume in ml of sample extract required to give fluorine intensity compared to that of S = ml of AF standard

V= Volume in ml of solvent required to dilute final extract

W= Weight in grams of the original sample contained in the final extract [20]

2.4. Detoxification of Aflatoxins

Two most contaminated samples, that is, khal (25.39 ppb) and makai (20.12 ppb) were separated and treated with certain physical and chemical methods for detoxification. Physical methods adopted for detoxification included washing, heating, and boiling. Feed samples were washed with simple water (10-15 min), hot water, and then boiled again with excess water for some time (5-10 min). The solutions of different chemicals including 10% citric acid, 10% acetic acid, 2%

sodium hydroxide, and 2% hydrochloric acid were added in conical flasks separately and placed on a wrist action shaker for 2 hours. After shaking, re-quantification was done for this method of detoxification. Furthermore, 0.15% mustard oil and 0.15% black seed oil were used as the biological methods for detoxification. The contaminated samples were mixed with these oils separately in separate conical flasks and placed on a wrist action shaker for 30 minutes. Then, re-quantification was done by using the same TLC method, observed under a UV light scanner, and calculated by using the same formula [21].

2.5. Statistical Analysis

The statistical tools used were ANOVA and *t*-test.

3. RESULTS

The results indicated that 23 samples (28.75%), out of a total of 80, were found to be contaminated, while 71.25% of samples were non-contaminated. Out of these 23 contaminated samples, 5 were found beyond the permissible range, while 18 samples were found within the permissible range. Contaminated samples are not good for human consumption. Their consumption can cause serious gastrointestinal issues, cancer, reproductive abnormalities, and retarded growth. According to the US Food and Drug Administration (USFDA) guidelines for aflatoxin levels, the permissible level of aflatoxin in dairy animal feed is 20 parts per billion. In all these 23 contaminated samples, aflatoxin B1 was detected.

Out of the 40 fresh feed samples (20 fresh wanda and 20 makai), 16 were contaminated with aflatoxin B1 including 9 samples of wanda and 7 of makai. Furthermore, 1 sample of wanda was beyond the permissible range (20.73 ppb) and 8 samples were within the permissible range (11.89, 7.70, 5.70, 6.52, 7.71, 4.30, 3.82, and 3.026 ppb). While, 6 samples of makai were within the permissible range (7.32, 4.20, 4.27, 4.60, 3.12 and 3.83 ppb) and 1 sample of makai was beyond the permissible range (20.12 ppb). The concentration of aflatoxins (ppb) in 40 fresh samples of cattle feed is shown in Figure 1.

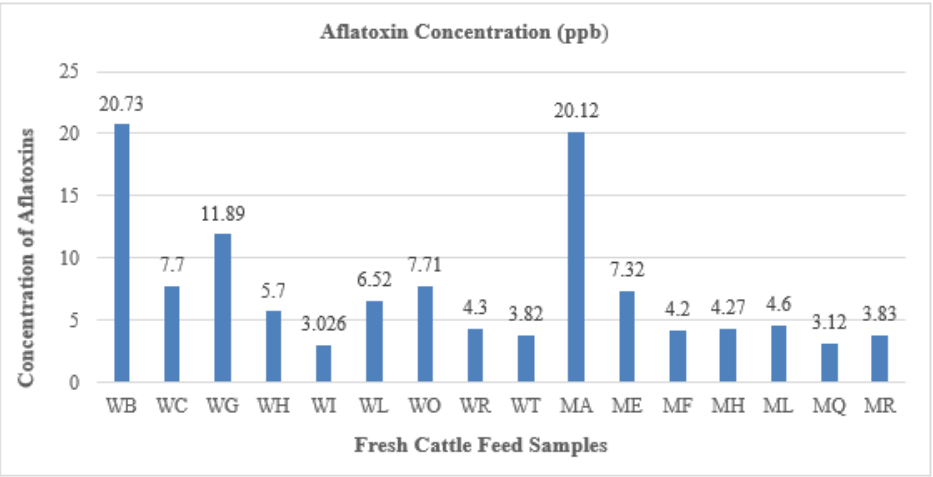

Figure 1. Aflatoxins in Fresh Feed Samples

Figure 1 Shows aflatoxin concentration (ppb) in fresh feed samples of cattle. Out of 40 fresh samples of cattle feed, 2 samples were found beyond the permissible level while 14 samples were detected within the permissible level.

Out of the 40 dry feed samples (10 samples of dana, 10 samples of chokhar, 10 samples of toori, 3 samples of khal, and 7 samples of makai dana), 7 were contaminated with aflatoxin B1 including 3 samples of khal, 2 samples of dana, and 2 samples of chokhar. All the contaminated samples of dana (1.77 and 3.96 ppb) and chokhar (1.44 and 3.99 ppb) were within the permissible range, while all the three samples of khal were above the permissible range (20.73, 20.12, and 25.39 ppb). The concentration of aflatoxins (ppb) in 40 dry samples of cattle feed is shown in Figure 2.

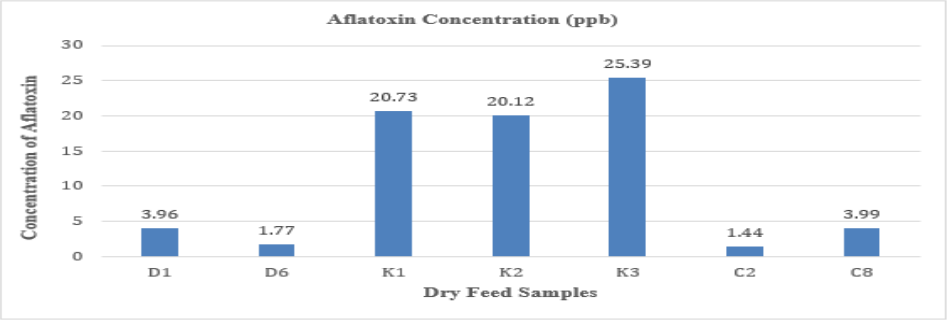

Figure 2. Aflatoxins in Dry Feed Samples

Figure 2 Shows aflatoxin concentration (ppb) in dry feed samples of cattle. Out of 40 dry feed samples (10 Dana, 10 Chokhar, 10 Toori, 3 Khal, and 7 Makai Dana samples), 7 samples were contaminated with aflatoxin B1. Out of these 7 samples, 3 samples of Khal, 2 samples of Dana, and 2 samples of Chokhar were found contaminated.

A total of 80 samples were collected and categorized into wanda, makai, chokhar, dana, khal, makai dana, and toori. Out of the 80 samples, 5 (6.25%) were beyond the permissible range and 18 (22.5%) were within the permissible range. While, 71.25% samples were uncontaminated. The detail of the total cattle feed samples is shown in Figure 3.

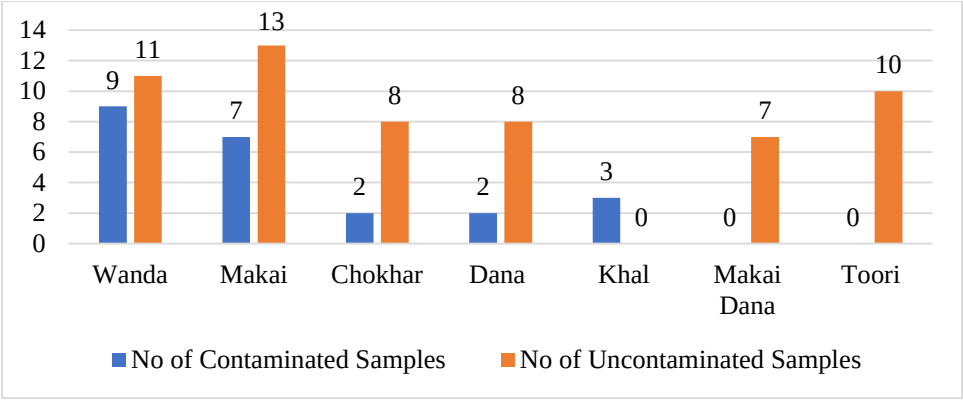

Figure 3. Details of Aflatoxin Concentration

Figure 3 shows feed-wise details of aflatoxin concentration. It shows contamination in different feed samples. Blue bars show the number of contaminated samples while orange bars show the number of uncontaminated samples

Khal showed the highest percentage of reduction after washing with simple water that is, 12.09%. After washing with hot water, makai showed the highest percentage of reduction, that is, 18.78. While, after boiling for 15 minutes with excess water, khal was found to have the highest percentage of reduction, that is, 23.91% (Table 2). Khal also showed the highest percentage of reduction with 10% citric acid, that is, 63.64%, while the lowest percentage found was 23.91% with 2% sodium hydroxide (Table 2).

Different oils are used for the detoxification of aflatoxins in cattle feed samples. In this research, the oils used for this purpose were mustard oil and black seed oil. The highest percentage of reduction was found in khal with mustard oil, that is, 35.72%. While, the lowest percentage found was 23.76% with black seed oil in makai (Table 2). It was found that the contamination of aflatoxin B1 in both groups remains significant as the *p*-value is less than 0.05, that is, $p \leq 0.049$.

Table 2. Detoxification of Aflatoxins by Different Methods

Sr. No.	The method used for detoxification	Contaminated Sample	Initial Concentration (ppb)	Concentration After Detoxification (ppb)	Reduction (%)
		Physical methods			
1.	Washing (distilled water)	Makai	20.12	18.64	7.35
2.	Washing (distilled water)	Khal	25.39	22.32	12.09
3.	Washing (hot water)	Makai	20.12	16.34	18.78
4.	Washing (hot water)	Khal	25.39	21.32	16.03
5.	Boiling with water	Makai	20.12	15.45	23.21
6.	Boiling with water	Khal	25.39	19.32	23.91
		Chemical methods			
7.	10% Citric Acid	Makai	20.12	9.63	52.13
8.	10% Citric Acid	Khal	25.39	9.23	63.64
9.	10% Acetic Acid	Makai	20.12	10.52	47.70
10.	10% Acetic Acid	Khal	25.39	12.50	50.76
11.	2% Sodium Hydroxide	Makai	20.12	12.34	38.66
12.	2% Sodium Hydroxide	Khal	25.39	19.32	23.91
13.	2% Hydrochloric acid	Makai	20.12	10.60	47.32
14.	2% Hydrochloric acid	Khal	25.39	10.23	59.71

Sr. No.	The method used for detoxification	Contaminated Sample	Initial Concentration (ppb)	Concentration After Detoxification (ppb)	Reduction (%)
			Biological methods		
15.	Mustard Oil	Makai	20.12	14.45	28.18
16.	Mustard Oil	Khal	25.39	16.32	35.72
17.	Black Seed Oil	Makai	20.12	15.34	23.76
18.	Black Seed Oil	Khal	25.39	17.32	31.78

4. DISCUSSION

The analysis of aflatoxin B1 indicated the presence of the maximum level of aflatoxins in corn components. The outcomes disclosed that only 1% of the studied cases exceeded the threshold level of toxins in milk. Contamination was increased when contaminated feed components were mixed in the preparation (corn that was contaminated), while repetitions that surpassed the threshold were found up to 28.5%. It was concluded that nourishing regimes including the configuration of rudimentary fiber and feeding roughages of cattle should be checked and measured with great care regarding their potential for aflatoxin enclosure [22].

A similar method was adopted to evaluate the chances of aflatoxin B1 contamination and its limitation in compound feed for dairy cows [23]. Improved feed ingredients were recommended after obtaining the results, including a decrease of sunflower seed (23 to 1.5g per 100g), citrus pulp (10 to 0g per 100g), and soybean (10 to 5.1g per 100g), along with the greater use of corn components (20.5 to 29.4g per 100g), wheat (2 to 30g per 100g), and palm kernel (16 to 22.5g per 100g) regarding normal preparations. The recommended diet is wheat which is comparatively cheap but may be unavailable during seasonal variations or unapproachable in some topographical areas. It is claimed that 98.8% of the replicated diet would display values less than the permissible threshold, as opposed to 75.6% of cases expected using a normal preparation.

In another investigation, cattle feed and milk samples were collected and mycotoxins concentration was examined in them by using the enzymatic immunoassay (ELISA) method. The average amount of aflatoxin B1, zearalenone, and deoxynivalenol present in the feed was 3.01ppb, 467ppb, and 218.5ppb, respectively [24]. Food with aflatoxin contamination if consumed regularly may cause dangerous problems. Another study estimated aflatoxin B1 in cattle feed and rice. According to this investigation, 12 rice samples out of 50 exhibited the presence of aflatoxins (1.5 - 20ppb). Cattle feed (that includes grains, maize, corn, hay, and silage) is susceptible to aflatoxin contamination, since 25 samples of cattle feed out of 60 showed the presence of aflatoxins (1.9 - 28.5ppb). Although the production of aflatoxins decreases the growth of fungi, special steps must be taken during transportation, loading, drying, cutting, and collection [25].

Despite favorable weather conditions in Nigeria, there are still chances of high contamination in feed because of *Aspergillus flavus*. This results in high risks to health because of the intake of dairy products. High-performance liquid

chromatography (HPLC) and an immunoaffinity column were employed to assess the levels of aflatoxin B1 as well as the presence of *Aspergillus flavus* (a fungus known to produce aflatoxins) in feed samples, while maintaining the standards set by the European Union and the United States Food and Drug Administration (USFDA). The analysis identified 55.8% of the isolated *Aspergillus flavus* strains. Among them, 25% were confirmed to be aflatoxigenic, capable of producing aflatoxins. A shocking number of feeds tested positive for aflatoxin B1 and the concentrations found were between 5 to ≥20ppb [26].

In another research, the presence of aflatoxin B1 was investigated in grains and dairy cattle feed. Samples including wheat, maize, grain mixture, barley, and dairy cattle feed were collected and examined through ELISA. Only 16.4% aflatoxin B1 was observed in all the samples. The sample of maize was the most contaminated. Ratios higher than the allowed levels observed were 17.9% in the mixture of grains and 12.3% in cattle feed. It was revealed that the most dangerous factors for the formation of molds and aflatoxin B1 are climate conditions in the case of cattle feed, while grain mixture, harvesting, and region of cultivation are the factors in the case of maize [27].

A study conducted on animal feed and feedstuff ingredients to detect different forms of mycotoxin contamination indicated that there is a possibility of some types of mycotoxins existing in animal feed, other than aflatoxin B1. According to the results, 9.26% aflatoxin B1 in feed ingredients and 5.71% aflatoxin B1 in mixed feed were detected, while other types of mycotoxins were also detected in the feed. Aflatoxin A was found in 24.07% ratio in feedstuffs and 22.86% in mixed feed. Persistent consumption of these mycotoxins can lead to major health problems in animals [28].

HPLC method with a silica gel 60G F254-based stationary phase and acetone: chloroform (1: 9) based mobile phase was used for the analysis of 59 samples of feed. The detection limit reported was 0.5ppb [29]. A similar method was used to find out aflatoxin B1 in 97 feed samples of dairy cattle. In this method, the mixture used for elution consisted of chloroform (28), acetone (4), and water (6) [30]. Aflatoxins were exposed by using a wavelength of 366nm.

Aflatoxin B1 levels in the ingredients of dairy cow feed and total mixed rations were studied at two farms. Twenty-four hours after feeding, feed milk was collected from the particular group of cows. The levels of aflatoxin B1 in feed and aflatoxin M1 in milk were detected by using enzyme-linked immunosorbent assay (ELISA). Both types of aflatoxins were detected in 100% of feed and milk samples. Aflatoxin B1 range in the feed constituents was 1.6-104.7ppb and in TMRs its was 11.0-56.0ppb. The results also revealed that the presence of aflatoxin B1 in feed varied with the management of feed farms [31]. Another study checked the levels of aflatoxins in dairy cow feed and raw milk. A total of 193 feed samples and 375 milk samples were collected from different regions of Spain. Out of the total feed samples, 34.7% were detected positive with total aflatoxins. The range detected was 0.05 to 6.45ppb, while 12.4% of samples were detected positive with aflatoxin B1. The range of aflatoxin M1 in milk samples was 0.009-1.36ppb and the percentage detected was 18.9%. No single sample of feed exceeded the permissible limit, that is, 5ppb set by the European Union, while milk samples (Aflatoxin M1) exceeded the

permissible limit, that is, 50ppb [32].

Citric acid is involved in the process of preservation. It is added to different sorts of foods and drinks. Also, it has exclusive, acidic, and sour flavors. Approximately 5% of citric acid is present in lemon juice. Aflatoxin B1 is detoxified in rice by aqueous citric acid. In this investigation, samples contaminated with aflatoxin B1 were dipped for detoxification in lemon juice. The results revealed that citric acid was 63.59% to 90% efficient against aflatoxin B1. In recent investigations, citric acid (lemon) and pistachio were heated and over 70% of aflatoxin B1 was degraded [33-34].

In an investigation, a degrading strain of microbes for aflatoxin B1 *Stenotrophomonas acidoaminiphila* CW117 and its ability to detoxify the aflatoxin were investigated. At 45 mg/l, CW117 degraded the aflatoxin B1 substrate in 24 hours, while 4.1mg/l aflatoxin B1 took 48 hours for degradation. Effective degradation of aflatoxin B1 by this strain shows its importance in the development of detoxification in different feeds and foodstuffs. The degrading active components were found in the supernatant (cell-free). The rate of degradation was directly proportional to the rising temperature for incubation (0 to 90°C) and 90°C was determined as the most stable temperature. The optimum pH for degradation was 6 to 7 and it was inhibited only through proteinase K, metal chelators (EDTA and EGTA), and sodium dodecyl sulfate (a denaturant of protein). Further, 29.3% of aflatoxin B1 was degraded by 0.5mg/ml of the recombinant laccase (rLC1) in 24 hours from CW117, while 76.7% of aflatoxin B1 was degraded by the cell-free supernatant at the same time but with the a few components of proteins. The results revealed that the CW117 strain can degrade aflatoxin B1 using the mixture of enzymes and oxides of the micromolecule [35].

To avoid the contamination of aflatoxin B1, samples (contaminated) were treated with the oil of black seed. This oil was found to be tremendously effective and decreased contamination up to 100%. *Nigella sativa* oils are famous because of their antifungal activity for all kinds of tested fungi. An oil of Nigella sativa was found to be efficient at 0.15%, for example, causing complete inhibition of *A. alternata* and *F. moniliforme* at 0.1% and 0.15% concentrations [36–38].

4.1. Conclusion

This study investigated the presence of aflatoxin in cow feed in Pakistan and explored the efficacy of various detoxification methods (physical, chemical, and biological) for aflatoxin B1. Additionally, a comparative analysis was conducted between fresh and dry cattle feed samples. The findings revealed the presence of aflatoxins in the tested feed samples, emphasizing the need for effective detoxification methods. Chemical methods showed promising efficacy in reducing aflatoxin levels. Furthermore, comparative analysis highlighted differences in aflatoxin levels between fresh and dry feed, suggesting the potential influence of storage conditions. These findings contribute to the current understanding of aflatoxin contamination in cow feed and provide insights for developing strategies to mitigate its harmful effects on livestock health.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. No funding was provided.

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