**Research Article**

# The Critical Control Point of *Aspergillus spp.* Aflatoxin Contamination in Smallholder Dairy Farms

**Authors:**
Sadam Suliman Mohamed Yousof[a,b], Ratmawati Malaka[c*], Sudirman Baco[c], Jamila Mustabi[d], Rizky Widiyanty Kadir[e]
[a]Department of Animal Production, Faculty of Veterinary Science, Gadarif University, Gadarif, 3221, Sudan
[b]Department of Animal Production, Doctoral Study Program, Faculty of Animal Science, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[c]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, 90245, South Sulawesi, Indonesia.
[d]Department of Nutrition and Animal Feed, Faculty of Animal Science, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia.
[e]Graduate Student of Animal Science, Faculty of Animal Science, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia.

Corresponding author: Prof. Dr. drh. Ratmawati Malaka, M.Sc
Postal address: 90245
University/organization email address: malaka@unhas.ac.id
ORCID ID: 0000-0003-1934-4949
Tel. number: +6281355727613



**Keywords**

AFB1, AFM1, *Aspergillus spp*, Food contamination, HACCP.

**Abstract**

Feed and food contamination by toxigenic fungi and their aflatoxins is one of the main threats to animal and human health worldwide and in the agricultural and industrial sectors. This study evaluated the contamination magnitude by *Aspergillus* species in dairy farms, aflatoxin AFB1 in cow feeds, and aflatoxin AFM1 in milk and local cheese (Dangke). One hundred twenty-two swabs from farms, 12 roughage feeds, 16 concentrated feeds, 39 fresh cow milk, and six cheese samples were analyzed for *Aspergillus* spp., AFB1, and AFM1 contamination. In isolation terms, *Aspergillus flavus* and *Aspergillus niger* were detected in 13.93% and 7.38% of the swab samples, respectively. The roughage feeds showed low levels of AFB1, detected in 8.33% with contamination of 7.32 µ/kg, while concentrated feed was detected in 37.5% with contamination levels of 27.8µg/kg. Aflatoxin AFM1 was detected in raw milk samples and represented approximately 69.2%, averaging 7.31µg/kg. All local cheese samples were free of AFM1. There were critical points regarding HACCP inside the farms, which play significant roles in contamination by fungi and aflatoxins. The fungi contamination

and aflatoxins pose dangerous public health problems to humans, especially infants and older people. However, monitoring programs for mycotoxin are critical in reducing contamination.

**Abbreviations**

| | |
|---|---|
| AFB1 | Aflatoxin B1 |
| AFM1 | Aflatoxin M1 |
| HACCP | Hazard Analysis Critical Control Points |
| AFG | Aflatoxin Green |
| PDA | Potato Dextrose Agar |
| ELISA | Enzyme Linked Immunosorbent Assay |
| SD | Standard Deviation |
| OR | Odd Ratio |
| LOD | Low Limits of Detection |
| EOD | Exceed Limits of Detection. |

## Introduction

Mycotoxins are poisonous materials formed by toxigenic fungi that attack agricultural products in the field or a storehouse in natural conditions, including bad storehouses, high moisture, high temperature, and insect infestation [1]. When set up in animal ration and feed ingredients, these contaminants might pose big trouble and risk to lactating cows when they become beyond normal levels. Initially, they had a mischievous effect on animal health, like decreased feeding efficiency, milk productivity, immunodeficiency, emaciation, laminitis, infertility, and abortion [2], [3]. Furtherly, they may have affected the food supply chain when they transferred from animal feed to milk and milk products [4], [5], [6], inferable from their resistance to increase in temperatures and tolerance to humidity, making milk production tasks deficient and insufficient for their total end during milk production and processing [7]. Subsequently, people are exposed to these poisons using contaminated animal products such as meat, milk, and dairy. People's exposure to mycotoxins can have several adverse health effects, some of them chronic and others acute diseases; in some cases, it isn't easy to recover from teratogenic, carcinogenic, and immunosuppressive. It might bring about death in critical cases like delayed chronic toxicity or high acute intoxication [8].

Their adverse consequences on all creatures and humans, mycotoxins cause huge monetary misfortunes for some countries, especially non-industrial nations, due to the expenses directed toward food safety [9]. The most common toxigenic growth fungi in agricultural products are species belonging to the *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* genera. *Fusarium*, *Aspergillus*, and *Penicillium* are considered considerable makers of mycotoxins in animal feed worldwide [10].

Additionally, fungal poisons like aflatoxins (AFB1, AFB2, AFG1, and AFG2) shaped by fungal species in the genera Aspergillus are critical mycotoxins found routinely in all dairy diets worldwide [11]. Mainly in tropical and subtropical areas worldwide, the issue is more articulated as it relates to humidity and high climate temperatures that lean toward the growth and multiplication of fungi. Grains and plant protein sources utilized in animal diets are the principal sources of fungal contamination and aflatoxins. *Aspergillus* multiplicates, preferably, on commodities at 15% or above moisture levels at 25 to 35 °C. Moisture level of more than 17.5% and temperature between 27 to 30°C are required for best aflatoxins production. Aflatoxins decrease the quality of ingredients by using the nutrients present in the ingredients for their digestion and spread [12].

There is developing proof to propose that seasonal and geological contrasts impact mycotoxins and aflatoxins formation in both food and feed [13]. Tragically, there is a need for

more research in Indonesia to elucidate this, especially in dairy farms and animal feed parts, despite the hot and wet conditions that characterize this country as tropical. Generally, previous studies conducted here elucidated the occurrence of aflatoxins in dairy feeds and milk contamination. The HACCP system has been presented worldwide for a considerable time to identify, assess, and control hazardous food safety factors. It is a coherent, fundamental, efficient food safety control system with a complicated construction intended to identify risks and critical circumstances and arrange to control them. From one perspective, this system ensures the safety of products on the way of the pecking order from maker to the shopper, empowers to recognize all the critical points that can influence the security and safety of the final product, takes out unsafe factors, and controls the total production process [14]. The research aims to isolate and identify the extent of *Aspergillus spp*. and its toxin production in smallholder dairy farms and to determine critical control points of contamination in the Environment, raw milk, and cheese processing units in Enrekang province, South Sulawesi, Indonesia.

## Results

### Socio-demographic of smallholder dairy farms in Enrekang regency, Indonesia

The socio-demographic and household characteristics showed that the milking cows ranged from 1 to 14 heads, with a mean (SD) of 5(0.78) per farm. Approximately 69% of the owners used elephant grass as roughage feed, 30.8% used rice bran as concentrated feed, and most had feed storage facilities. The owner had a chance of education, and most of them attended secondary school (about 84.61 %), of which a high percentage was male, as described below (Table 1). Our results were similar to those in the Ethiopia [16].

**Table 1.**

Socio-demographic features of smallholder dairy farms in Enrekang regency, Indonesia (N = 13).

| Variables | Characteristics | Value |
|---|---|---|
| Gender | Men | 11 |
| | Women | 2 |
| Education | University | 1 |
| | Secondary | 11 |
| | Primary | 1 |
| Age | Mean (SD) | 36(0.64) |

| | | |
|---|---|---|
| | 30-40 | 3 |
| | 50-60 | 8 |
| | 60-70 | 2 |
| Number of milking cow | Mean (SD) | 5(0.78) |
| Milk production /litter/ day | Mean (SD) | 5(0.86) |
| Type of roughage feeds | Elephant grass | 9 |
| | Elephant grass plus green corn | 4 |
| Type of concentrated feed | Rice bran | 4 |
| | Soya by products | 3 |
| | Rice bran plus commercial concentrate | 2 |
| | Rice bran plus soya by product | 3 |
| | Soya plus palm oil cake | 1 |
| Feed storage facilities | Yes | 10 |
| | No | 3 |

*SD: standard deviation.*

**Isolation of *Aspergillus spp.***

**Table 2.**

Isolation and distribution of *Aspergillus* species in different swab samples

| Source of samples | No of samples | *Aspergillus spp*. | | | | Total |
|---|---|---|---|---|---|---|
| | | *A. flavus* | *A.fumigatus* | *A. niger* | *A. terreus* | |
| Water | 12 | 3 | 0 | 1 | 1 | 5 |
| Cage floor | 12 | 4 | 0 | 2 | 2 | 8 |
| Cow udder | 38 | 5 | 3 | 3 | 1 | 12 |
| Milker hand | 12 | 2 | 1 | 1 | 1 | 5 |
| Milk cans | 12 | 2 | 1 | 0 | 1 | 4 |
| Cheese worker hand | 12 | 1 | 1 | 1 | 0 | 3 |
| Coconut mold | 12 | 0 | 0 | 0 | 1 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Banana leaf | 12 | 0 | 0 | 1 | 0 | 1 |
| Total | 122 | 17 (13.93%) | 6 (4.91%) | 9 (7.38%) | 7 (5.74%) | 39 (31.97 %) |

Among all collected samples, about 39 (31.97%) fungal were isolated and identified macroscopically as *Aspergillus* and segregated by colony color into subgenera and sections as Flavi (green colonies), Nigri (black colonies), Fumigate (blue colonies), and *Terrei* (brown colonies). Therefore, using macroscopic characters alone is insufficient and accurate for identification. Colony color has been examined microscopically to identify some micro features of isolated samples like conidiophore, vesicle, and conidia. Sometimes, molecular analysis is conducted to confirm the isolates and resolve the cultural limitations that have not been carried out in the current study (Figure 2). Mycological analysis in this study revealed that most of the samples were contaminated by diverse fungi, all identified as mycotoxigenic fungi. Yeasts were also present in the samples.

Figure 1. Collection of samples in farm sites

**Table 3.**

Levels of AFB1 contaminations in different animal feed types

| | Animal feed type | | | | |
|---|---|---|---|---|---|
| | Elephant grass | Rice bran | Soya by products | Commercial concentrate | Palm oil cake |
| AFs (µg/kg) | 7.32 | 32.81 | - | 44.08 | 6.3 |

| | | | | | |
|---|---|---|---|---|---|
| EU limits 20 (µg/kg) | LOD | EOD | LOD | EOD | LOD |
| SNI 200 (µg/kg) | LOD | LOD | LOD | LOD | LOD |

*LOD is low in terms of the detection limits. EOD exceeds the limits of detection.*

*Aspergillus flavus* was most frequent isolated among samples 17(13.93%), followed by *Aspergillus niger* 9(7.38%), *A. terreus* and *A. fumigatus*, which represented 6(4.91%), 7(5.74%) respectively, (Table 2). these results are similar to that reported domination of *Aspergillus flavus* fungi followed by *Aspergillus niger* in dairy animals and poultry feeds.

the current study showed that contamination of (31.97%) of samples by *Aspergillus* (Table 2), a high percentage was found in cows udder 12 (31.58%), followed by cage floor 8 (66.67%) and milker hands 5 (41.67). A low percentage was recorded in coconut mold 1 (8.33) and banana leaf 1 (8.33%) (work as natural packaging).

**Table 4.**

Logistic regression analysis of factors associated with Aflatoxins

| Factors | AFs | | OR | P – value |
|---|---|---|---|---|
| | ≤5µg/kg | ≥5µg/kg | | |
| **Level of education** | | | | |
| Primary | 1 | 0 | | |
| Secondary | 0 | 5 | 0.0 | 0.99 |
| University | 0 | 1 | | |
| **Gender** | | | | |
| Female | 0 | 2 | 0.0 | 0.99 |
| Male | 0 | 0 | | |
| **Type of roughage feed** | | | | |
| Elephant grass | 0 | 1 | 0.0 | 0.99 |
| **Type of concentrated feed** | | | | |
| Rice bran | 7 | 3 | | |
| Soya by products | 1 | 0 | | |
| Commercial concentrate | 0 | 2 | | |
| Palm oil cake | 0 | 1 | 69.95 | 0.99 |
| **Feed Storage** | | | | |

| | | | | |
|---|---|---|---|---|
| On floor | 1 | 2 | | |
| On Special place | 3 | 7 | 0.0 | 1.00 |

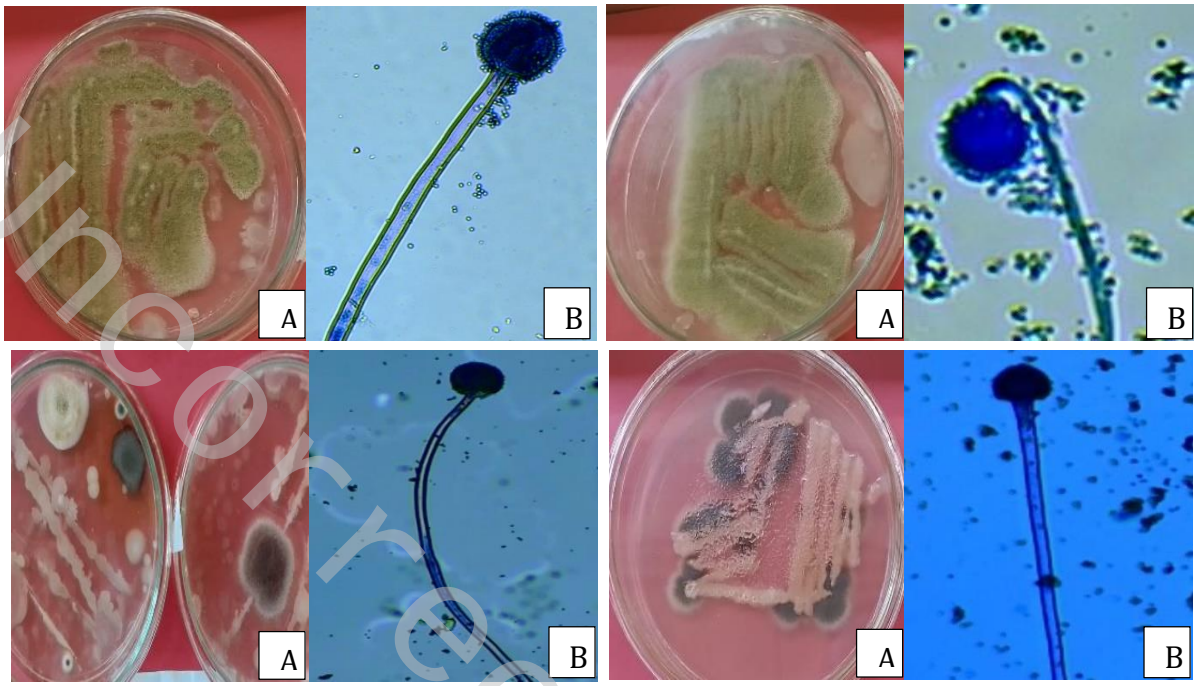


Figure 2. represented (A) macroscopic characters; and (B) microscopic characters of *Aspergillus sp.*

## Discussion

### Socio-demographic of smallholder dairy farms in Enrekang regency, Indonesia

The percentage of women in agricultural activity was less than that of men, sometimes she worked beside her husbands, and when they just worked, sometimes when she was widowed or divorced. The farmers needed more information about fungi and aflatoxins, and their knowledge about contamination was fragile. Fortunately, all of them agreed on the storage process. They stated that they stored their animal feeds in a specific place to avoid humidity and rain for less than one month. Thus, the growth of *Aspergillus* species will be less than expected when we compare their knowledge and feed practices inside the farms.

### Isolation of *Aspergillus spp.*

The main reason for the prevalence of these fungi is their ability to tolerate and live in a wide zone of temperatures. Much research has been conducted to investigate contamination by Aspergillus in animal feed. Most findings concluded that *Aspergillus flavus* is the most frequent fungi, followed by other species of *Aspergillus*. Unfortunately, till now, there has been no

published research about the prevalence of *Aspergillus spp*. in cage floors, cow udder, milker hands, milk cans, hands of cheese workers, and cheese mold.

From all isolated fungi, *Aspergillus flavus* was most dominant, followed by *A. niger, A. terreus,* and *A. fumigatus,* all of them known as mycotoxigenic fungi, udder of cows contained high contamination levels and then the cage floor these results explaining that the floor is not properly cleaning in addition to that the cows slumber on the floor and fungi moved to udder from cow to another, on the other hands the owners neglected the importance of hygiene and cleaning practices, it is essential to practice safety system to minimize contamination. We took samples from the milker's hands immediately after milking, which could suggest that the fungi spread from the cow's udder to the hands or vice versa. The problem is that these fungi will transmit contamination to milk and milk products directly or indirectly, and adverse health impacts on workers' and animals' health regularly inside farms. There were two ways of contamination of milk and milk-based foodstuff by aflatoxins [17]. In a study conducted, these findings disagreed with the present results. In another study carried out for the prevalence of aspergillus in well water in dairy farms, results revealed that contaminated water by *A. flavus, A. niger*, and *A. terreus* these results were similar to current results, and this similarity may be due to environmental conditions in the two countries.

The isolation of *Aspergillus* from animal feeds in Indonesia was carried out in another place in Bogor, West Java; the results revealed the presence of *A. flavus* in feed samples [18]. Another study was conducted on aspergillus in agricultural commodities in Indonesia, and their results revealed toxigenic aspergillus [19]. The natural weather conditions, including high humidity, rainfall, and moderate temperature in Indonesia, support the growth of fungi, especially *Aspergillus spp*. In South Sulawesi, there is no previous study about the isolation of fungi in a feed of dairy farms or farm environment; this made this study the first research in this area, which has considerable dairy farms. contamination levels and occurrence of AFB1 in animal feeds revealed that the absence of contaminations in the majority of roughage feed samples, only one sample 1(8.33%) was positive for AFB1 with a mean of 7.32µg/kg (Table 3), above legal limits set by (European Union) EU of 5 µg/kg of feed. Although it is less than the National Indonesian Standard SNI of 50µg/kg. the present results supported those obtained in Ethiopia, which reported that about 52% of feed samples were above EU limits. On the other hand, the findings here partially agreed with those obtained in Italy, showing a lower degree of AFB1 contamination [20].

However, the owner did not store roughage feed. They introduced it directly from the field to the animals, this explains that the fungi growth on feed when it was in the field, not during

storage and the roughage was contaminated; mold growth is suitable for humidity and temperature. To prevent mold growth and contamination, we should focus on the natural and chemical methods that decrease the multiplication of fungi in the field during harvest, transportation, and storage.

Concentrated feeds in this study revealed a low 6.3 µg/kg and high 44.08 µg/kg of AFB1 contamination levels. These incidents are different according to concentrated feed type. Palm oil cake showed low incidents, while rice bran and commercial concentrated feed showed high levels. High AFB1 in concentrate due to proven and scientific facts that concentrated feed has and holds high levels of fat, carbohydrate, and protein favourable for *Aspergillus* species multiplication and aflatoxin production [12]. These findings align with those of field (Omeiza et al. 2018), they detected AFB1 in animal feeds with concertation ranging from 10-20 µg/kg. Many factors can cause contamination of dairy cattle feed, such as type of feed, feed processing, storage and handling, geographic conditions, and owners' awareness of the risk of aflatoxin. Therefore, the owners in the present study area have no idea about aflatoxins; the lack of sufficient knowledge of aflatoxins might lead to their high occurrence in the feed of animals besides milk and milk products through animals [16].

Regarding the analysis of the factors associated with aflatoxins, many factors showed a strong relationship with aflatoxin; this study revealed that only concentrated feed positively impacts aflatoxin, and its increased aflatoxin is about six times more than roughage (Table 4). These results partially support those who reported concentrated feed has increased aflatoxin seven times more and disagree with them regarding education level, gender, feed storage, and type of roughages; these factors showed none of them have a positive effect on aflatoxin contents.

Aflatoxin AFM1 was found in 69.2% of raw milk samples with contamination levels from 6.14 to 10.02µg/kg with a mean of 7.31µg/kg. these results disagree with those recorded in Albania, which showed about 0.022 to 1µg/kg of AFM1 in milk [21]. A study in the Amazon region found AFM1 of about 0.06 µg/kg, less than these results [22]. These differences are related to feed, weather, and animal physiological status the concentration of AFM1 in milk was higher during the early period and decreased during the latter portion of lactation stage in dairy cows. All of the tested local cheese Dangke samples were free from aflatoxin AFM1, which might be due to the method of cheese processing that used the natural plant enzyme papain, which was extracted from the papaya tree as a coagulant. This finding disagrees with many researchers reporting the presence of AFM1 in different kinds of cheese fields [23, 24].

Regarding HACCP, these results identified many critical points and risks inside the farm that affect the quality of milk and, subsequently, the health of both animals and humans. Cows' udder contained high *Aspergillus spp.* followed by cage floor, milker hands, milk cans, and cheese worker hands. All of these are critical control points, and it is possible to control them to minimize the magnitude of biological and chemical hazard risks in the food supply chain and ensure food safety by implementing good management practices in dairy farms. The point is that milk quality starts from it until the final product.

Many studies have been conducted on implementing the HACCP program in dairy companies and dairy products [25]. In conclusion, the present results of this study elucidated that contamination of the farm environment by a high incidence of mycotoxigenic fungi, especially in cow's udder, cage floor, water, milker hands, milk cans, and cheese worker hands, which represented in *Aspergillus spp*. it was isolated and observed. Considerable levels of contamination by aflatoxins AFB1 and AFM1 in animal feeds and milk were recorded; concentrated feed type was the significant factor associated with the high prevalence of aflatoxin contamination levels. Moreover, all positive feed and milk samples are subject to Indonesian legislation. Still, it's essential to focus on implementing good practices for all feed production from the field during cultivation, harvest, transport, processing, storage, and feeding procedures.

The farmers should be trained and increase their awareness and knowledge about the health risks of aflatoxin for their animals and humans, and how to control and manage it by implanting the HACCP program, in addition to adopting hygiene and cleaning of milker hands, farm floor and cow udder and sanitation before and after milking to reduce contamination levels and produce clean milk. Therefore, further research and investigations are needed about aflatoxigenic fungi in dairy farms and their feed and produced milk to give more consideration to one health program strategy.

## Materials and Methods

### Study area

The study areas were intentionally chosen to serve the research aims. The site was located about 1300 m above sea level, with a day-to-day typical temperature of approximately 27.34°C. The climate of this area is a tropical rainstorm, described by the rainy season from November to June and the dry season from July- to November. Dairy cows are mostly kept through a zero-grazing system called 'stall feeding,' and dairy animals are supplemented with concentrated

feeds. The expected milk production in this area was estimated to be around 10-15 liters/head/day.

**Sampling**

Thirty-nine raw milk samples and six local cheese samples were collected from smallholder farms from August to September 2023. Simultaneously, different types of concentrated and roughage feeds were collected, from which the roughage (n=12), commercial concentrate (n=2), soya bean (n=4), palm oil meal (n=1), and rice bran (n=9).

At the same time, a total of 122 swabs from water, cage floor, milker hands, cow udders, milk cans, cheese maker hands, coconut mold, and banana leaf samples were collected (Figure 1). The samples were kept in a cool ice box at 4°C, transported to the laboratory, and stored until analysis. A structured questionnaire was used to assess farmers' knowledge, their practices of animal feeds, and farmers' experience with aflatoxin and fungi in feed, in addition to animal feed handling and storage. The samples were taken from all farms around the research area, and the results were represented and generalized to all communities there.

**Fungal analysis**

Equipment and selected media were correctly autoclaved before use, culturing and isolation of the swabs were completed in sanitized conditions, and laboratory windows and doors were kept shut. Two plates of Potato Dextrose Agar (PDA) were utilized for each swab sample; subsequently, the media was placed in (9 cm) plates and left to solidify at room temperature. Each swab sample was spread on the surface of the plates in duplicate, and each plate was continuously labelled with the code name of the farm from which the swab was taken and the swab name. After the culture process, the dishes were incubated at 30ºC for 1 to 4 days, and until the third day, changes were noted and recorded each day.

Controls were prepared using two sterile PDA dishes, which were used to test the general conditions and environment of the laboratory. *Aspergillus* species were identified based on their colonial morphology and colony color observed after incubation. As described, the isolates' microscopic character was examined using the lactophenol cotton blue staining technique [25]. One drop of the dye was placed on a prepared slide, and a small piece of the culture was taken and set in the decline of the dye using a mounting needle. The same needle was used to spread the culture. A cover slip was then delicately and gently put on the spread culture with delicate pressure to remove air bubbles. After that, the slide was then mounted and observed under the X40 objective lens. Identifying *Aspergillus spp*. depends on septate hyphae and rough and

colorless conidiophores that end in vesicles with the whole surface covered with either uni- or biseriate sterigmata.

**Determination of aflatoxins B1 and M1 from animal feed, milk, and cheese**

The samples were analysed for AFB1 and AFM1 in animal feeds, milk, and cheese by using a specific ELISA kit (Romer Labs, AgraQuant total Aflatoxin, Austria). Five mL from each raw milk sample were incubated for 30 min at four °C and centrifuged at 3000 g for ten min. After that, serum of milk under the fat layer was taken and then immediately assayed for AFM1 using a specific ELISA kit. Five g of ground samples of cheese and feeds (roughage and concentrated) were taken separately; in a clean pitcher, and 25 ml of 70% methanol extraction solution (extraction ratio of 1:5 of sample to extraction) were added. Raw milk samples were prepared as described above.

**Analysis of aflatoxin B1 and M1 in samples by competitive enzyme-linked immunosorbent assay**

All samples were analysed for AFB1 and AFM1 in animal feeds, milk, and cheese using a specific ELISA kit (Romer Labs, AgraQuant total Aflatoxin, Austria). The maximum and minimum amount was 4-40 ppb for AFM1 and AFB1 with high specificity and sensitivity. The kit materials were stored at 2-8°C; before the test started, the materials were incubated for one hour at room temperature. The kit test materials were used according to the manufacturer's instructions: About 200 µL of the conjugate solution was pipetted and moved into the dilution wells (supplied with the kit). Then the samples (100 µL) were pipetted into every dilution well (100 µL/well/sample). Standard samples were pipetted in duplicate (100 µL/well/standard). The solution was mixed well, 100 µL was moved from the dilution wells into antibody-coated wells, and the plate containing the samples was incubated at room temperature for 15 min. The unbound conjugate was removed with a washing solution five times (supplied with the kit) after a washing step. The washed wells were gently dried.

The aflatoxin substrate solution was added to the antibody-coated wells, and the plate was incubated again at room temperature for 5 min. The reaction was allowed to proceed in the dark, at the end of which a blue color developed. The reaction was stopped by adding 100 µL stop solution to the antibody-coated wells, and the color changed from blue to yellow. The absorbance was measured at 450 nm with a differential filter at 630 nm using an ELISA Plate Reader, and the absorption intensity was found to be inversely proportional to the aflatoxin concentration in samples. The aflatoxin soft worksheet program supplied with the kit was used to calculate the aflatoxin B1 and M1 concentrations in the samples.

**Statistical analysis**

Data were expressed as the mean ± standard deviation (SD) by descriptive statistics, and the feed samples were calculated as a percentage using software (version 26). In addition, logistic regression analysis was conducted, and an odd ratio (OR) with 95% confidence intervals was used to test the relationship between predictors and expected or outcome variables. Differences were considered statistically significant at $P< 0.05$.

## References


1. Aasa AO, Fru FF, Adelusi OA, Oyeyinka SA, Njobeh PB. A review of toxigenic fungi and mycotoxins in feeds and food commodities in West Africa. World Mycotoxin J. 2023;16(1):33–47. DOI: 10.3920/WMJ2021.2766.
2. Janik E, Niemcewicz M, Ceremuga M, Stela M, Saluk-bijak J, Siadkowski A, Bijak M. Int J Mol Sci. 2020;8187:1–21. DOI: 10.3390/ijms21218187.
3. Yang C, Song G, Lim W. Effects of mycotoxin-contaminated feed on farm animals. J Hazard Mater. 2020;389(October 2019):122087. DOI: 10.1016/j.jhazmat.2020.122087.
4. Claudious G, Sheperd M, Charles MT, Prosper J, Jerikias M. Isolation of AspergillusFlavus from Dairy Cattle Feed and Assessment of Aflatoxin M1 in Milk from Small Dairy Farms around Harare, Zimbabwe. HSOA Advances in Microbiology Research. 2019;3(009):2–7. DOI: 10.24966/AMR-694X/100009.
5. Izzo L, Mikušová P, Lombardi S, Sulyok M, Ritieni A. Analysis of Mycotoxin and Secondary Metabolites in Commercial and Traditional Slovak Cheese Samples. Toxins. 2022;14(2):1–12. DOI: 10.3390/toxins14020134.
6. Pereira CS, Cunha SC, Fernandes JO. Prevalent mycotoxins in animal feed: Occurrence and analytical methods. Toxins. 2019;11(5). DOI: 10.3390/toxins11050290.
7. Djekic I, Petrovic J, Jovetic M, Redzepovic-Djordjevic A, Stulic M, Lorenzo JM, Iammarino M, Tomasevic I. Aflatoxins in milk and dairy products: Occurrence and exposure assessment for the serbian population. Applied Sciences. 2020;10(21):1–17. DOI: 10.3390/app10217420.
8. Omotayo OP, Omotayo AO, Babalola OO, Mwanza M. Comparative study of aflatoxin contamination of winter and summer ginger from the North West Province of South Africa. Toxicol Rep. 2019;6(December 2018):489–95. DOI: 10.1016/j.toxrep.2019.05.011.
9. Ochieng PE, Scippo ML, Kemboi DC, Croubels S, Okoth S, Kang'ethe EK, et al. Mycotoxins in poultry feed and feed ingredients from sub-saharan africa and their impact on the production of broiler and layer chickens: A review. Toxins. 2021;13(9). DOI: 10.3390/toxins13090633.
10. Javadikasgari H, Soltesz EG, Gillinov AM. Surgery for Atrial Fibrillation: Atlas of Cardiac Surgical Techniques. 2nd ed. Philadelphia, USA: Elsevier; 2018. DOI: 10.1016/B978-0-323-46294-5.00028-5.

11. Rodríguez-Blanco M, Ramos AJ, Sanchis V, Marín S. Mycotoxins occurrence and fungal populations in different types of silages for dairy cows in Spain. Fungal Biol. 2021;125(2):103–14. DOI: 10.1016/j.funbio.2019.08.006.
12. Motbaynor A, Kassaye D, Keffale M, Wasihun P. Magnitude of Aflatoxigenic Aspergillus Species, Level of Aflatoxin B1, and Associated Factors in Stored Feed at Poultry Farms in Dire Dawa, Ethiopia. Vet Med Int. 2021;2021:1–11. DOI: 10.1155/2021/6638083.
13. Adekoya I, Njobeh P, Obadina A, Landschoot S, Audenaert K, Okoth S, Boevre MD, Saeger SD. Investigation of the metabolic profile and toxigenic variability of fungal species occurring in fermented foods and beverage from nigeria and South Africa using UPLC-MS/MS. Toxins. 2019;11(2). DOI: 10.3390/toxins11020085.
14. Parkinson G. Modern Science. Surrealism. Cambridge, UK: Cambridge University Press 2021. DOI: 10.1017/9781108862639.005.
15. Tadele F, Demissie B, Amsalu A, Demelash H, Mengist Z, Ambelu A, Yenew C. Aflatoxin contamination of animal feeds and its predictors among dairy farms in Northwest Ethiopia: One Health approach implications. Frontiers in veterinary sciene. 2023;1–8. DOI: 10.3389/fvets.2023.1123573.
16. Sadam SMY, Malaka R, Baco S, Mustabi J. Aflatoxin M1 in Milk: Occurrence and Its Risk Association: A Review. Hasanuddin Journal of Animal Science (HAJAS). 2022;4(2):68–81. DOI: 10.20956/hajas.v4i2.22097.
17. Sukmawati D, Setyaningsih A, Handayanik T, Rahayu S, Rustam Y, Moersilah M, et al. Isolation and characterization of aflatoxigenic Aspergillus spp. from maize of livestock feed from Bogor. IOP Conference Series: Materials Science and Engineering. Institute of Physics Publishing. 2018;434:1–8. DOI: 10.1088/1757-899X/434/1/012105
18. Anidah, Rahayu WP, Nurjanah S, Retnowati I. Molecular characterization of aspergillus flavus toxigenicity in agricultural commodities in Indonesia. Biotropia. 2023;30(2):195–205. DOI: 10.11598/btb.2023.30.2.1842.
19. Ceniti C, Costanzo N, Spina AA, Rodolfi M, Tilocca B, Piras C, Britti D, Morittu VM. Fungal Contamination and Aflatoxin B1 Detected in Hay for Dairy Cows in South Italy. Front Nutr. 2021;8:704976. DOI: 10.3389/fnut.2021.704976.
20. Topi D, Spahiu J, Rexhepi A, Marku N. Two-year survey of aflatoxin M1 in milk marketed in Albania, and human exposure assessment. Food Control. 2022 Jun 1;136.

21. Diogenes LCG, Kluczkovski AM, Goes AFF, Pinto S de CB, Araujo CF, Kluczkovski-Junior A. Occurrence of Aflatoxin M1 and Risk Assessment in Bovine Milk from the Amazon Region. J Food Res. 2023;12(2):23. DOI: 10.1016/j.foodcont.2015.01.025.
22. Sarmast E, Fallah AA, Jafari T, Mousavi Khaneghah A. Impacts of unit operation of cheese manufacturing on the aflatoxin M1 level: A global systematic review and meta-analysis. Lwt. 2021;148:111772. DOI: 10.1016/j.lwt.2021.111772.
23. Mousavi Khaneghah A, Moosavi M, Omar SS, Oliveira CAF, Karimi-Dehkordi M, Fakhri Y, Huseyn E, Nematollahi A, Faharani M, Sant'Ana AS. The prevalence and concentration of aflatoxin M1 among different types of cheeses: A global systematic review, meta-analysis, and meta-regression. Food Control. 2021;125:107960. DOI: 10.1016/j.foodcont.2021.107960.
24. Oktavianty O, Hamdala I, Putro WW. Risk Analysis of Environmentally Friendly Milk Production Process with GMP and HACCP. International Conference on Innovation and Technology. 2021:93–101. DOI: 10.2991/aer.k.211221.012.
25. Omeiza GK, Kabir J, Kwaga JKP, Kwanashie CN, Mwanza M, Ngoma L. A risk assessment study of the occurrence and distribution of aflatoxigenic Aspergillus flavus and aflatoxin B1 in dairy cattle feeds in a central northern state, Nigeria. Toxicol Rep. 2018;5:846–856. DOI: 10.1016/j.toxrep.2018.08.011.