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RESEARCH

Aflatoxin B_1 contamination and fungal diversity in oilseed cakes and formulated feeds from livestock feed markets in Addis Ababa and surrounding areas

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Abstract

This study was conducted in Addis Ababa, surrounding peri-urban and urban feed markets to assess the prevalence of aflatoxin B_1 (AFB_1) and identify fungal diversity in livestock feeds. Two hundred and ninety samples of oil seed cakes and formulated feeds were collected from the randomly selected feed vendors. The samples were analysed for aflatoxin B_1 using a Helica® Aflatoxin B_1 (low matrix) Enzyme-linked immunoassay (ELISA) kit. To validate the ELISA results, a subset of randomly selected samples was further analysed using High-performance liquid chromatography-fluorescence detection (HPLC-FLD). The results indicated that 92% of poultry ration, 72% of dairy ration, 66% of linseed cake, 64% of noug seed cake, 61% of cotton seed cake, and 36% of soybean cake were contaminated with AFB_1 exceeding the permissible level (20 µg/kg) in Ethiopia. To evaluate fungal diversity, genomic DNA was extracted, pooled by feed type, and sequenced. The findings revealed variations in the diversity indices of fungal phyla, families, genera and species. The Metabarcoding results revealed *Ascomycota* as the dominant phylum in all feeds, with *Aspergillaceae*, *Nectriaceae*, *Botyosphaeriaceae*, and *Cladosporiaceae* being the dominant families. *Aspergillaceae* accounts for 53%, 43%, 16%, 33%, 11%, and 28% of the total fungal contamination detected in cotton seed cake (S1), noug seed cake (S2), soybean cake (S3), linseed cake (S4), poultry ration (S5), and dairy ration (S6), respectively. The synergistic presence of multiple fungal species, some of which are known to be harmful, was identified in the feed samples. Among the species, the proportion of *Aspergillus flavus* found was 1.8%, 3.8%, 0%, 0.4%, 1.2%, and 1.2% in the feeds S1–S6, respectively. The findings highlight the critical need for enhanced feed management practices, improved education and training for feed handlers on feed safety, stricter regulatory measures, and more research on mycotoxins mitigation.

Keywords Aspergillus flavus · Concentrate · Feed safety · Fungal contamination · Mycotoxin

Introduction

Ethiopia is the second most populous country in Africa, with approximately 22.17% of its population living in urban areas (UN 2024). The demand for livestock products has been increasing due to population growth and increased urbanization (Menale 2019), leading to a corresponding rise in urban and peri-urban livestock production and a subsequent high demand for feed. Feed is a crucial commodity and a fundamental pillar in achieving the economic, social, and environmental goals of livestock production (Wendimu 2021). The quantity and quality of nutrients, as well as advances in feeding science, significantly impact

Michael Blummel has passed away since the completion of the research.

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the economic viability of animal husbandry (Bediye et al. 2018). For animals to achieve their genetically selected production potential, nutrients must be made available and partitioned towards productive functions.

Feed resources traded in local Ethiopian markets include cereals, agro-industry by-products, crop residues, hay, and green fodder (Yosef et al. 2022). Utilizing agro-industry by-products in livestock feed formulation is a common practice. Oilseed cakes, among these by-products, serve as crucial ingredients as protein sources for the growth and development of livestock (Melesse et al. 2023). Oilseeds cultivated in Ethiopia include noug seed, sesame, soybean, linseed and cottonseed (Alemaw and Gurmu 2023). Although Ethiopia has the largest livestock population in Africa (CSA 2020a), livestock productivity is still limited by feed shortages, poor feed quality (FAO 2019), and high feed costs (Yosef et al. 2022). Concentrate feed prices set by the market often disregard feed quality considerations (Melesse et al. 2023). The production, processing, handling, storage, and market-related factors significantly influence the quality and safety of feed (Negash 2020).

Fungal contamination is one of the critical factors affecting feed quality and safety. The diversity of fungi in crops is influenced by pre- and post-harvest conditions, geographical location and weather patterns (Orina et al. 2020). Filamentous fungi pose significant challenges to food security and safety because they produce mycotoxins (Avery et al. 2019). Xu et al. (2022) reported that mycotoxins in animal feeds and food products can cause mycotoxicosis in both humans and animals. Even at low doses, they can disrupt nutrient digestion, absorption, metabolism and physiology. Aflatoxins are considered among the most significant mycotoxins affecting both human and animal health globally (Marshall et al. 2020). These toxic compounds, known for their hepatotoxicity and immunosuppressive properties (IARC 2015), can severely reduce feed intake and animal productivity (Xu et al. 2022). Aflatoxins are produced primarily by *Aspergillus* species, which thrive in warm, humid environments and can contaminate a wide range of crops. When livestock consume aflatoxin B1 (AFB_1) contaminated feed, toxic residues may accumulate in animal products such as milk or meat, posing potential public health risks through human consumption (Tolosa et al. 2021).

Nutritional quality of oil seed by-products has been evaluated previously in Ethiopia (Walelegne 2023; Tsehay 2021; and Abdelghani 2008). These analyses provide crucial insights into the composition and suitability of oilseed cakes and other by-products for livestock feed formulations. Concurrently, aflatoxin contamination in these by-products has been assessed (Rehrahie et al. 2018; Gizachew et al. 2016). Despite these investigations, a substantial gap persists in understanding the fungal diversity within livestock feed. Metagenomic studies, focusing specifically on fungal populations in oilseed cakes and formulated feeds, have not yet been conducted. Such research is crucial, as it can reveal the diverse array of fungal species present, including aflatoxin-producing fungi such as *Aspergillus flavus*, and elucidate their interactions with other fungal communities.

This study aims to provide information on the prevalence of AFB_1 and aflatoxin producing fungal species, as well as their co-existence with other mycotoxin producing fungal populations, in selected oilseed cakes and formulated feeds used in Addis Ababa and surrounding districts. Understanding the diversity and co-existence of mycotoxin producing fungi in feeds supports targeted monitoring and regulatory measures to reduce fungal toxin risks in Ethiopia's feed and food system.

Materials and methods

Study area

A cross-sectional study was conducted in Addis Ababa and surrounding districts (Ada', Mojo, Adama, Addaberga, Wolmera, Sheno, Sebeta, and Sululta) (Fig. 1; Table 1) in 2019. These districts are known for small-scale commercial livestock farming in urban and peri-urban areas. Livestock feed markets, primarily located along main roads for easy transportation access, were also present in the districts. The feed markets were identified and randomly selected for this study.

Sample collection and preparation

Information on the number of feed vendors in each selected district was obtained from the respective administrative focal persons. 50% of feed vendors in each districts markets were randomly selected based on their accessibility. The availability of feed types varied from one vendor to another; this led to unequal sample numbers across feed vendors and districts (Table 1). Therefore, all available feeds of interest were sampled from the feed vendors. As a result, the overall level of aflatoxin contamination was estimated from all feed samples collected across the districts. The final number of samples (290) was therefore informed by vendor distribution, feed availability, and practical field conditions. A kilogram of representative sample of similar feeds from each feed shop was thoroughly mixed according to the procedure (ISO 6497:2002) and put in a double layered kaki paper bag. Each bag was labelled with the type of feed sample, date of collection, vendor code and district of collection and sample number to allow traceability. The

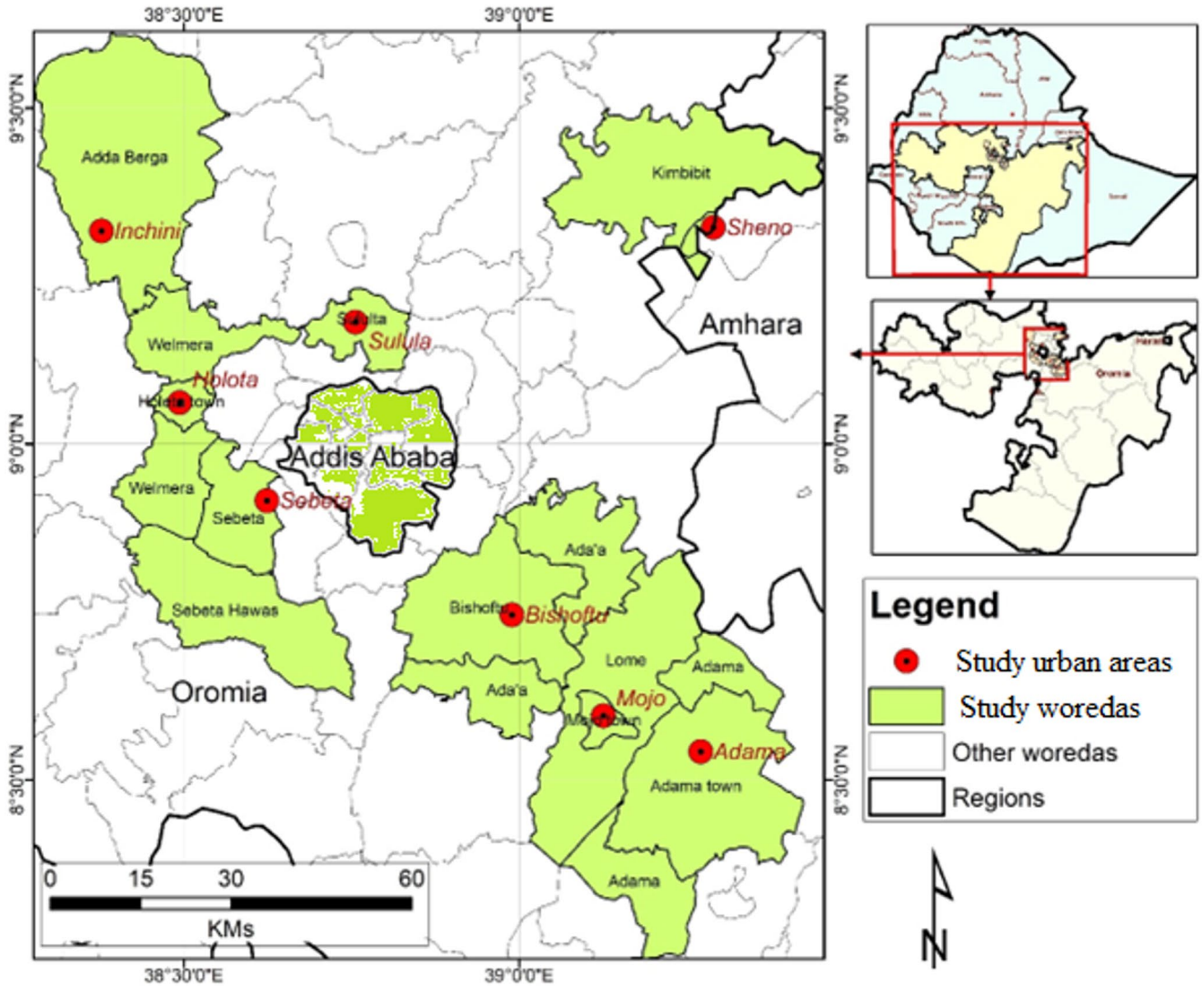

Fig. 1 Livestock feed collection market locationsSource: Study area mapped by Climate, Geospatial & Biometrics Research Directorate, Ethiopian Institute of Agricultural Research (EIAR)

Table 1 Location coordinates and number of feed samples collected for aflatoxin B_1 (AFB_1) analysis

			Feed types						
Area name	Latitude	Longitude	Cotton seed cake	Linseed cake	Noug seed cake	Soybean cake	Dairy ration	Poultry ration	Total
Ada'	8°45′21″N	39°10′09″E	1	4	5	4	14	9	37
Addaberga	9°24′53″N	38°25′10″E	0	0	2	0	3	0	5
Adama	8°32'30"N	39°16'13"E	14	14	3	1	27	7	66
Addis Ababa	9°01'28"N	38°44'14"E	1	0	3	5	6	11	26
Mojo	8°35'19"N	39°7'38''E	21	18	6	1	20	7	73
Sebeta	8°54′14″N	38°34′50″E	0	2	8	2	6	0	18
Sheno	9°19′21″N	39°17′21″E	1	11	10	0	7	4	33
Sululta	9°10'52"N	38°45'20"E	0	1	6	0	16	0	23
Welmera	9°3'41"N	38°29'35"E	0	3	4	0	2	0	9
Total			38	53	47	13	101	38	290

samples were transported to the International Livestock Research Institute (ILRI) molecular biology laboratory on the collection days and stored in a cold room (4 °C).

Samples were ground in the ILRI Addis Ababa Feed Nutritional Analysis Laboratory, using a CHRISTY (Christy and Norris limited process engineers Chelmsford England)

mill and passed through a 1 mm sieve. To avoid cross-contamination between milling, the mill was cleaned between each milling. To remove remaining feed from corners and gaps, a vacuum cleaner (compact, Moulinex 1200 Electronic) was used and then lint-free wipes moistened with 70% ethanol were used to wipe the inside surface. Each ground sample was well mixed and representative sub samples were then taken for AFB_1 assay and DNA extraction.

Aflatoxin analysis

The Helica ® Aflatoxin B_1 (low matrix) Enzyme-linked immunoassay (ELISA) for quantitative detection of AFB_1 was used to determine aflatoxin B_1 concentrations in the samples. The AFB_1 extraction and ELISA procedure specifically for animal feed was executed according to the manufacturer's instructions (Helica Biosystems Inc, Low Matrix ELISA Kit).

AFB_1 was extracted following the procedure described by Gizachew et al. (2016); a representative subsample (0.1g) of each ground feed sample was added to 10 ml of 80% acetonitrile (High performance liquid chromatography (HPLC) grade acetonitrile, Fisher Scientific, USA). The mixture was shaken in a sealed 15 ml falcon tube for 10 min, centrifuged (2,058 x g) for 5 min, then the supernatant was collected for analysis, an aliquot was diluted 1:10 in reconstituted wash buffer (Phosphate buffered saline (PBS) with 0.05% Tween 20), and a final dilution of 1:1000 was used for the calculation. To validate efficiency of extraction of this method, 30 randomly selected samples were analysed for total aflatoxin using High Performance Liquid Chromatography-Fluorescence (HPLC-FLD) detection.

AFB_1 was analysed according to the manufacturer's instructions. 100 µl of each standard and the prepared samples, were mixed with 200 µl of sample assay diluent making 300 µl in the mixing well. Then, 100 µl from each mixing well was transferred to a monoclonal antibody (low matrix specific for AFB_1 antibody) coated microtiter plate well and incubated at room temperature for 30 min. The liquid was then discarded and the wells drained by tapping on absorbent paper, before the well was washed three times with wash buffer and drained on absorbent paper. Then 100 µl of horseradish peroxidase (HRP), aflatoxin enzyme conjugate, was added to the wells. After incubating for 30 min at room temperature, the liquid was decanted and the well was washed three times with any residual wash buffer drained. 100 µl of substrate solution was added to each well and incubated for 10 min in the dark at room temperature. Finally, 100 µl of stop solution was added to stop the reaction. The optical density of each standard and the samples was measured at 450 nm using a microplate plate reader ELx 800 (BioTek Instrumentals, Inc., USA).

The standard concentrations were plotted along the x-axis on a log scale. The corresponding %B/Bo values were plotted along the y-axis. Reader Fit software (Gen5 a Software Version 2.01.14) was used to fit the standard curve ($R^2 > 0.99$). A Data Analysis Worksheet for the Low Matrix ELISA Kit was used to record the AFB_1 concentration.

To confirm the validity of the AFB_1 results analysed with ELISA, 30 samples were randomly selected (among which the highest detected samples were included) and sent to Biosciences Eastern and Central Africa (BecA-ILRI Hub) laboratory in Nairobi for HPLC-FLD analysis. HPLC was validated using a low-level standard solution in six replicates. The limits of detection and quantification were determined as 0.27 ppb and 0.91 ppb, respectively; relative standard deviation was 4.3%, and uncertainty 8.7%. Spike recoveries were 86.6–88.5% (mean 87.6 ± 0.98%) which is in the acceptable range (70–120%). The correlation of the results run by HPLC-FLD and ELISA methods was done with linear regression analysis; Coefficient of determination (R^2), the slope and intercept were calculated using Excel scatter plots.

DNA extraction and sequencing

Fungal genomic DNA was extracted from 50 mg of ground feed samples using DNeasy® Plant Mini Kit Cat. No. 69,104 and 69,106 (QIAGEN, Venlo, The Netherlands), following the manufacturer's protocol. Each sample was lysed by adding 500 µL Buffer AP1 with 4 µL RNase A; then the mixture was incubated in a Clifton water bath at 65 °C for 20 min with mixing by inverting the tubes three times. Following lysis 130 µL of Buffer P3 was added, the lysate was cooled in ice for 5 min, then centrifuged (eppendorf centrifuge 5424 R) at 20,000 X g for 5 min. The supernatant was put through a QIAshredder spin column placed in a 2 ml collection tube by centrifuging for 2 min at 20,000 X g. The flowthrough was transferred into a 2 ml eppendorf tube and mixed by pipetting with 1.5 ml of AW1 buffer. The mixture was then loaded onto a DNeasy Mini spin column in 750 µL aliquots and centrifuged at ≥8000 rpm for 1 min. The column was washed by adding 500 µL of Buffer AW2 and centrifuging for 1 min at ≥8,000 rpm, followed by a second wash with 500 µL Buffer AW2 and centrifugation at 20,000 × g for 2 min. To elute the DNA, the spin column was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL of Buffer AE was added directly to the membrane. After a 5-minute incubation at room temperature, the column was centrifuged at 8,000 × g for 1 min. A second elution using another 100 µL of Buffer AE was performed to maximize DNA yield. Eluted DNA was stored at −20 °C until further analysis.

The extracted genomic DNA was sent to Genohub, Admera Health, USA for library preparation and

sequencing. First, the genomic DNA was quantified using a Qubit 2.0 DNA HS Assay (ThermoFisher, Massachusetts, USA) and the quality assessed using a Tapestation genomic DNA Assay (Agilent Technologies, California, USA). Fifty nanograms of the isolated genomic DNA was used for Polymerase Chain Reaction (PCR) amplification using proprietary primers (Admera Health, LLC, South Plainfield, NJ) covering the hypervariable regions, ITS1 and ITS2. Primer selection and design were undertaken to achieve a comprehensive taxonomic coverage, and spike-in controls were used to generate maximal data. The quantity of the final libraries was assessed using a Qubit 2.0 and quality was assessed by TapeStation D1000 ScreenTape. Illumina® 8-ntdual-indices adaptors were used followed by sequencing on an Illumina® Miseq (Illumina, California, USA) with a read length of 250 paired-end reads per sample (500 K in each direction). The fungal diversity of the top 50 taxonomies at family, genus, and species levels were identified using the QIIME2 platform integrated with a pre-trained Naïve Bayes taxonomic classifier. However, phylum level taxonomic labelling was done using the taxonomy identifier from NCBI using the species as a starting point. Rarefaction measurement was run on the basis of each species (species richness) versus operational taxonomic unit (OTU) abundance.

Statistical analysis

Descriptive statistics were used in Microsoft Excel 2013 (Microsoft Office Professional Plus 2013, Version 15.0) to present the mean aflatoxin quantity in the feed samples. Data were summarized as mean ± standard error (SE), range and median. To compare the significance of AFB_1 contamination level between the different feed types, Wilcoxon Rank Sum (Mann-Whitney U), R version 4.5.0 was used. For metagenomic data, Qiime2 (version 2021.2) was used to filter the raw data. The final non-chimeric reads were summarized, and Dada2 was used to cluster the OTUs. The taxonomy was classified based on the file: unite-ver8-99-classifier-04.02.2020.qza. The abundance-based coverage estimator (Ace) and Chao1 index (Chao1) were used to provide an estimate of the species richness, while the Shannon, Simpson and InvSimpson indices were used to provide an indication of the species diversity. Beta-diversity was calculated and compared among the six DNA extracts. Finally, bar plots were developed at family, genus, and species levels using the R package "metacoder".

Results

Aflatoxin B_1 (AFB_1) contamination of feed samples

AFB_1 was detected in 92% of the poultry ration, 72% of the dairy ration, 66% of the linseed cake, 64% of the noug seed cake, 61% of the cotton seed cake and 36% of the soybean cake (Fig. 2). The detected concentrations exceeded the maximum allowable level set by European Union (EU) and Ethiopian standard authority (ES ISO14718 Aflatoxin B_1 µg/kg Max. 20.0). Samples holding AFB_1 below the threshold of 0.1 µg/kg are below detection limit (BDL) of ELISA assay.

The average contamination level for each feed type along the standard error indicated the variability of the contamination in each feed type (Table 2). The results showed that there was a wide range of contamination level in each feed type. The highest median concentration (245.62 µg/kg) was observed in linseed cake showing that most of the samples had the highest AFB_1 contamination level, followed by poultry ration and noug seed cake.

AFB_1 contamination in feed samples was not evenly distributed. However, comparison using the Wilcoxon rank sum test with continuity correction (Table S1) showed differences in AFB_1 contamination level among samples

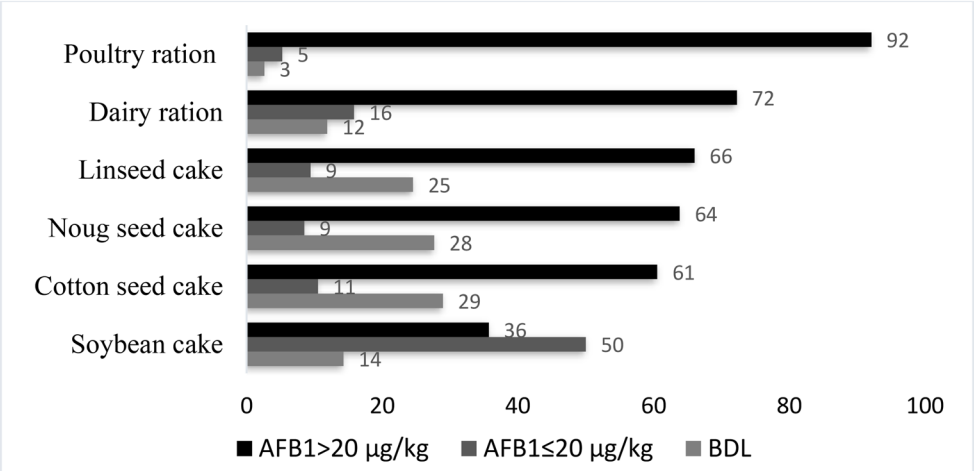

Fig. 2 Percentage occurrence of AFB_1 in feed samples

Table 2 Aflatoxin B1 (AFB_1) concentration (µg/kg) in oil seed cakes and formulated feeds

Feed type	Mean ± SE	Range (Min-Max)	Median
Dairy ration	129.4 ± 11.98	BDL − 442	86.47
Poultry ration	162.3 ± 14.34	BDL − 293	192.29
Cotton seed cake	108.8 ± 31.87	BDL − 793	33.54
Linseed cake	202.1 ± 24.54	BDL − 556	245.62
Noug seed cake	162.1 ± 24.79	BDL − 518	104.58
Soybean cake	18.9 ± 4.30	BDL − 49	14.78

with significance values ranging from $P<0.001$ up to not significant.

To validate the findings, HPLC (gold standard) analysis was conducted on 30 randomly selected ELISA-tested samples revealing a strong correlation between the two methods ($r(27)=0.96$, $P<0.01$). This correlation was further supported by a linear regression equation ($y=0.7817x+15.215$, $R^2 = 0.9223$) (Fig. 3), confirming the reliability of ELISA for AFB_1 quantification. However, two noug seed cake samples showed very high concentration of AFB_1 (1708.44 µg/kg and 1549.09 µg/kg). The HPLC conformation test results for these samples were 1232.71 µg/kg and 4954.16 µg/kg, respectively. Due to their deviation from the mean result, they were not included in the summary statistics (mean, range, standard error) shown above.

Fungal distribution in the selected feeds

The DNA sequence results showed that *Ascomycota* was the dominant phylum in all feeds, while the phyla *Basidiomycota* and *Mucoromycota* were found in smaller numbers in all the feeds except in feed S4 (Figure S1). The dominant fungal families in the feed samples included *Aspergillaceae*, *Nectriaceae*, *Botyosphaeriaceae* and *Cladosporiaceae*. *Aspergillaceae* were represented in 53%, 43%, 16%, 33% 10.6% and 28% of the samples S1-S6, respectively, (Figure S2). The second dominant family was *Nectriaceae* observed in 23%, 7%, 21%, 3%, 16.6% and 13.6% of samples from the S1-S6 feed types, respectively. The third most represented family was *Botryosphaeriaceae* which occurred in 4%, 20%, 1.5%, 7.6%, 4.6% and 5.8% of the samples from S1-S6. *Cladosporiaceae* was the fourth dominating family with up to 2.7%, 11%, 1.9%, 3%, 5% and 4.8% representation in the S1-S6 feeds.

The sequence results revealed that the genus *Aspergillus* was found in all feed types (Fig. 4; Table 3). This genus was detected in cotton seed cake (S1), noug cake (S2), linseed cake (S4), dairy ration (S6), soybean cake (S3) and poultry ration (S5) in 57%, 41%, 27%, 25%, 15%, and 8% of the samples respectively, (Table 3). Figure 6 shows the bar plot constructed from the abundance of different fungal genera.

The DNA sequence analysis showed that *Aspergillus glaucus* and *Fusarium lacertarum* were the most represented fungal species in the feed samples (Figure S3). *A. glaucus* accounted for 38%, 12.6%, 21%, 20%, 5.7%, and 18% of the fungi species detected in the studied feeds S1-S6, respectively. *F. lacertarum* was the next most dominant fungal species in the feeds S1-S6 representing 38%, 8%, and 3% 2% 4% and 4%, respectively, of the fungal species detected. The representation of *A. flavus* was 1.8%, 3.8%, 0%, 0.4%, 1.2%, and 1.2% of fungal species in the feeds S1–S6, respectively.

Evaluation of diversity of fungi in feeds

A principal component analysis (PCA) using unifrac was calculated to analyse and measure the differences or dissimilarity of the types of fungal species present across the six feed types. Dissimilarity in fungal diversity across the six feed samples show component 1 and component 2 explain 30.4% and 26.1% of the variance respectively (Figure S4). Soybean cake (S3) showed the highest level of dissimilarity with a diversity index of 0.4, indicating its difference from the other five feed types. Cotton seed cake (S1) also indicated much more dissimilarity. Linseed cake (S4) showed a moderate level of dissimilarity, not as far away as S3. Other feeds, poultry ration (S5), and dairy cake (S4) showed a moderate level of dissimilarity, not as far away as S3. Other feeds, poultry ration (S5), and dairy ration (S6) indicated they contained fungal communities that are more similar than S3 and S4.

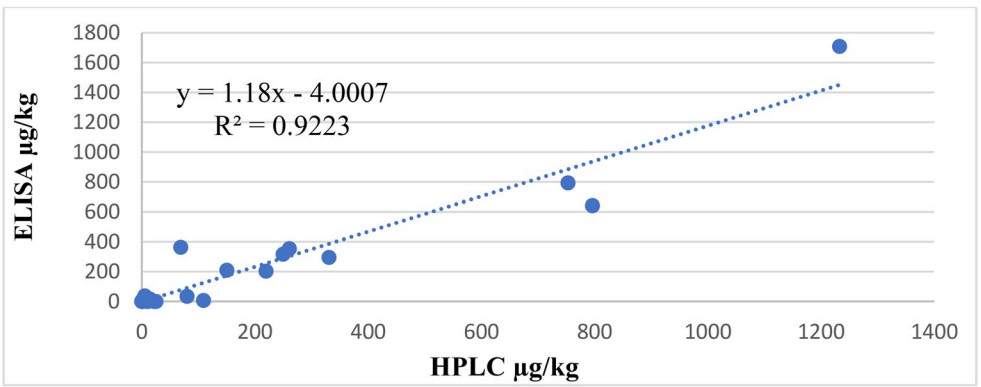

Fig. 3 Correlation between HPLC and ELISA results of feed samples for aflatoxin B_1 (µg/kg)

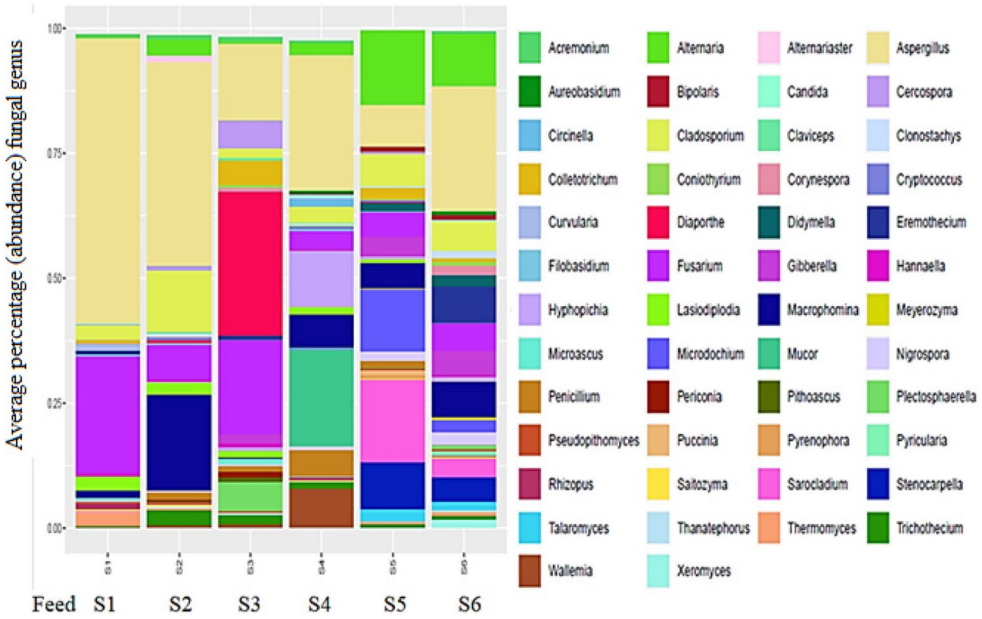

Fig. 4 Bar plot showing the average percentage or abundance of each fungal genus in each feed samples in cotton seed cake (S1), noug seed cake (S2), soybean cake (S3), linseed cake (S4), poultry ration (S5) and dairy ration (S6)

Table 3 Fungi genera ranking one to seven in the selected feed samples

Cotton seed cake (S1)	%	Noug seed cake(S2)	%	Soybean cake(S3)	%	Linseed cake%	%	Poultry ration	%	Dairy ration S6	%
Aspergillus	57	*Aspergillus*	41	*Diaporthe*	29	*Aspergillus*	27	*Sarocladium*	17	*Aspergillus*	25
Fusarium	24	*Macrophomina*	19	*Fusarium*	19	*Mucor*	20	*Alternaria*	15	*Alternaria*	11
Cladosporium	3	*Cladosporium*	12	*Aspergillus*	15	*Hyphopichia*	11	*Microdochium*	13	*Macrophomina*	7
Lasiodiplodia	3	*Fusarium*	7	*Plectosphaerella*	6	*Wallemia*	8	*Stenocarpella*	9	*Eremothecium*	7
Thermomyces	3	*Alternaria*	3	*Cercospora*	5	*Macrophomina*	7	*Aspergillus*	8	*Cladosporium*	6
Macrophomina	2	*Trichothecium*	3	*Colletotrichum*	5	*Penicillium*	5	*Cladosporium*	6	*Fusarium*	6
Rhizopus	1	*Lasiodiplodia*	2	*Cladosporium*	2	*Fusarium*	4	*Fusarium*	5	*Gibberella*	5

Rarefaction measurement

The rarefaction curve indicated that the sequencing depth was sufficient to include all fungal species, including those present in low numbers. It was run on the basis of each species (species richness) versus Operational Taxonomic Unit (OTU) abundance. Each of the samples contained a different species richness. The feed S1 had the highest species richness followed by S4. Feed Samples S3 and S6 showed medium richness. Feed types S2 and S5 had the lowest species richness (Figure S5).

Evaluation of richness and evenness of fungal dynamics in the different feeds

Different measures of alpha diversity matrix (Cao1, ACE, Shannon, Simpson and Invsimpson) were applied to the Operational Taxonomic Unit (OTU) abundance data of the feeds (Fig. 5).

Alpha diversity indices such as Choa 1 and ACE (species richness), Shannon, Simpson and InvSimpson (species evenness) quantified species richness in descending order. According to the results, Choa 1 indicated that S4, S3, and S5 represented a high species richness; when tested with the ACE S4, S3, and S5 demonstrated richness in the same higher to lower manner. The evenness indices Shannon, Simpson, and InvSimpson revealed that S6, S5, and S4 had the most even fungal dispersion in descending order.

Discussion

Aflatoxin B_1 contamination of feeds and feed ingredients

Concerns about agricultural commodities due to fungal incidence and mycotoxin contamination have been widely recognized (Bhat and Reddy 2017). Among these mycotoxins, AFB_1 has significant negative effects on both animal and human health (Anelich 2014). In Ethiopia, agro-industrial by-products are commonly used as feed ingredients for dairy and poultry production, including oilseed by-products

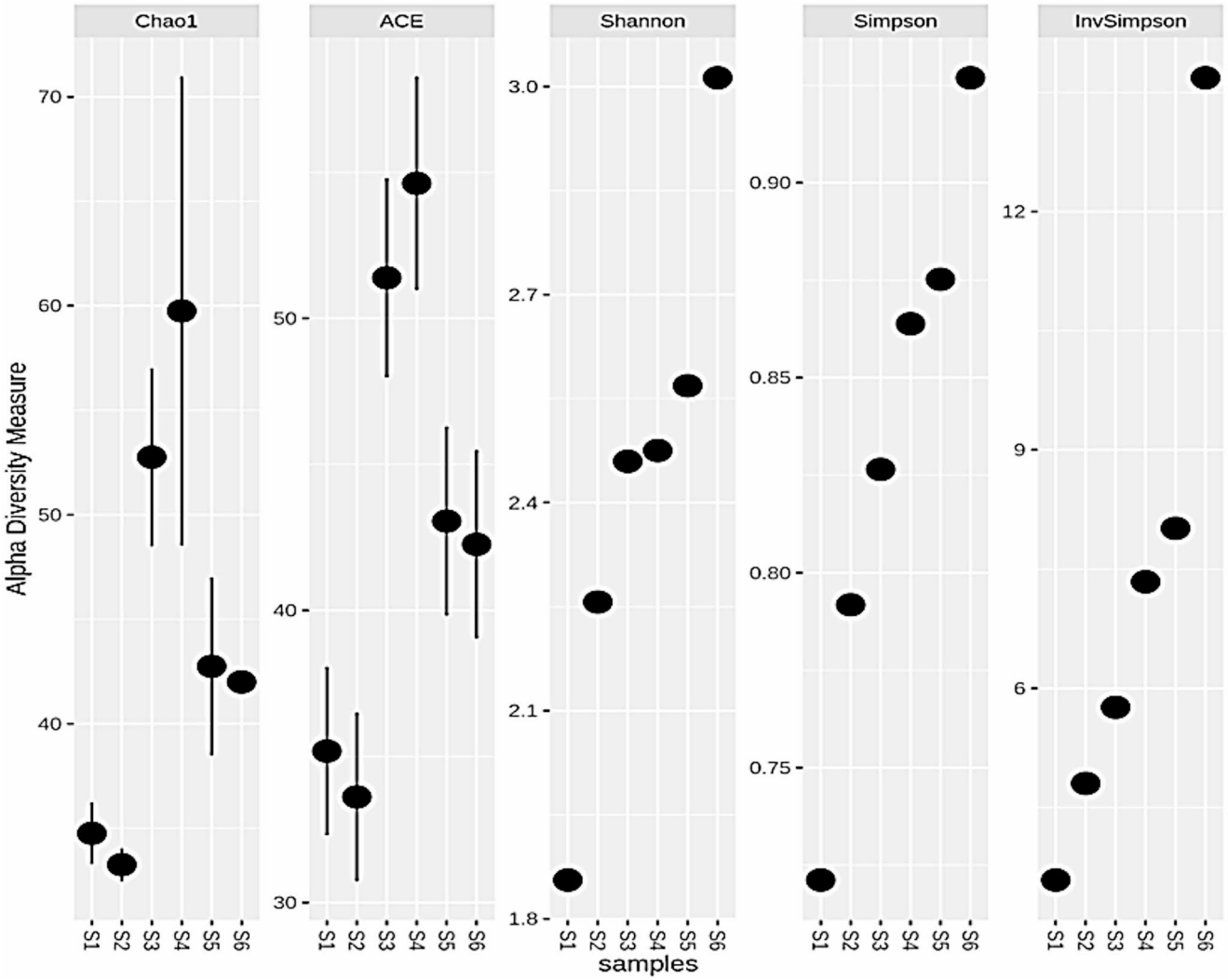

Fig. 5 Alpha diversity Measures (Cao1, ACE, Shannon, Simpson and Invsimpson) for Cotton seed cake (S1), Noug seed cake (S2), Soybean cake (S3), Linseed cake (S4), poultry ration (S5) and dairy ration (S6)

(oilseed cakes) from both modern and traditional processing units (FAO 2019). Oilseed cakes are vital components in the formulation of dairy and poultry feeds due to their high protein content (Gizachew et al. 2016; Mengistu et al. 2017; Menale 2019; Demissie 2022). However, despite their nutritional benefits, oilseed cakes have also been shown to contain aflatoxin B_1, posing potential risks to both animal and human health. Consumption of contaminated dairy feed can convert aflatoxin B_1into aflatoxin M_1 in milk (Pettersson 2012; Campagnollo et al. 2016), and poultry products can also be affected (Oliveira et al. 2000; Tatfo et al. 2022).

In this study, the majority of animal feed samples collected from feed markets were found to contain aflatoxin B_1. In most cases, the concentration of aflatoxin B_1 exceeded the maximum limit set by the Ethiopian Standards Authority (ESA 2019; ES ISO 14718: Aflatoxin B_1µg/kg Max. 20.0), as well as the limits established by the European Union (EU 2002) and Food and Drug Administration (FDA 2019). For dairy animals, the Food and Drug Administration (FDA) sets a maximum AFB_1 limit of 20 µg/kg, as AFB_1 present in animal feed can be metabolised into aflatoxin M_1 (AFM_1), which can subsequently be passed into milk (Sect. 683.100 Action Levels for Aflatoxins in Animal food).

The study showed that 76% of dairy ration samples were contaminated with aflatoxin B_1 above the permissible limit, with levels up to 442 µg/kg. This finding aligns with a similar study by Gizachew et al. (2016), who reported contamination levels of up to 419 µg/kg using a similar ELISA procedure. Tadele et al. (2023) found contamination levels as high as 306.9 µg/kg, and Mengesha et al. (2024) reported AFB_1contamination in dairy feeds up to 370.51 µg/kg. This study also revealed that 92% of the poultry ration samples were contaminated with AFB_1 above the permissible limit, with concentrations reaching up to 293 µg/kg. Similarly,

Mengesha et al. (2024), using a HPLC method, found layer and broiler feeds contaminated with aflatoxin B_1 at concentrations of up to 139 µg/kg and 148.68 µg/kg, respectively. The findings by Kassaw et al. (2022) reported an average AFB_1 concentration in poultry feed of 70.11 µg/kg, with a maximum of 633.94 µg/kg. These results supported the findings of this study, indicating that there could be significant risk to poultry health and food safety.

Compared to other feed ingredients in the study, soybean cake exhibited the lowest levels of AFB_1 contamination, with only 36% of samples showing amounts above 20 µg/kg. The average concentration was 18.9 ± 4.30 µg/kg, ranging from undetectable levels to 49 µg/kg. This result aligns with the findings by Motbaynor et al. (2021) where the mean AFB_1 range recorded was from 5 to 53 µg/kg. Soybean cake showed the lowest risk of AFB_1 contamination; in support of this, Mengesha et al. (2024) reported aflatoxin B_1 in soybean cake at levels below 5 µg/kg. Additionally, aflatoxin concentrations in soybean meal from two regions in Ghana ranged from non-detectable to 3.27 µg/kg, and mostly below the Ghana Standard Agency's limit of 20 µg/kg for feed ingredients (Nsiah et al. 2023). Gupta and Venkitasubramanian (1975) suggested that the low level of aflatoxin in soybean meal may be due to the small amount of zinc (0.01 mg/g) bound to phytic acid in soybeans. Li et al. (2023) also suggested that phytic acid could act as a plant-derived antifungal agent. In contrast, 66% of the cottonseed cake, 64% of linseed cake, and 61% of noug seed cake exhibited significantly higher contamination rates that often exceeded the permissible limits of AFB_1. The average AFB_1 concentration levels detected in the three ingredients were 108.8 ± 31.87, 202.1 ± 24.54 and 162.1 ± 24.79 µg/kg and ranging up to 793. 556, and 518 µg/kg of AFB_1, respectively. Poultry ration and linseed cake had highest median value and were statistically not different ($P = 0.29$) and both are significantly more contaminated than dairy ration ($P < 0.06$). Dairy ration is more contaminated than cotton seed cake ($P = 1.015$).

Concerning noug seed cake, the result aligns with findings by Gizachew et al. (2016), who reported contamination levels between 290 and 397 µg/kg. Furthermore, Gizachew et al. (2019) demonstrated the impact of water activity and temperature on the growth of *A. flavus* and subsequent production of AFB_1, with levels ranging from 203 to 282 µg/kg. More concerning and outlying, AFB_1 concentration in two noug seed cake samples were also recorded with the levels 1,708.44 µg/kg and 1,549.09 µg/kg, with subsequent HPLC analysis detecting levels of 1,232.71 µg/kg and 4,954.16 µg/kg, respectively. These findings highlight severe contamination risks in the tested samples. In linseed cakes, Ting et al. (2020) reported similarly high levels of AFB_1 in ground linseed, reaching concentrations of up to 3,644 µg/kg. Cracked, crushed, or ground oilseeds are more susceptible to fungal growth and aflatoxin production due to the removal of the protective seed coat allowing fungi access to readily available nutrients (Ting et al. 2020). While limited studies on cottonseed cake in Ethiopia exist, the wide variability in levels in this study aligns with studies conducted in Pakistan by Shar et al. (2020) and Yunus et al. (2020), who reported concentration levels of 89 µg/kg and 595.9 µg/kg, respectively.

Although samples can be traced to feed vendors, a key limitation of this study is that information was not collected on the pre- and post-harvest practices. Market-based sampling, while reflective of actual exposure risk, does not provide information on the origin or handling history of feed samples. Future studies should prioritize traceable sampling along the supply chain to identify critical control points to realize targeted interventions. The findings in this study serves as a baseline for further assessments and policy development to implement improved record-keeping and supply chain management in Ethiopian feed markets.

Evaluation of fungal diversity in feeds

The fungal DNA sequence showed that *Ascomycota* constituted the most abundant phylum in all samples. *Basidiomycota* and *Mucoromycota* were the next most abundant, but in significantly smaller quantities in all feed samples except in linseed cake where they appeared in a relatively higher abundance. *Basidiomycota* and *Mucoromycota* phyla can pose significant risks to both livestock and human health (Petrikkos et al. 2012); in this study, mould species such as *Rhizopus arrhizus* and *Mucor circinelloides* were detected under the phylum *Mucoromycota*. Although, we could not find any evidence that shows the production of mycotoxin, these fungi are known to cause a rare but serious disease, mucormycosis, which is caused by inhaling fungal spores or consuming contaminated feed, resulting in respiratory, digestive, or systemic infections (Petrikkos et al. 2012). Among the species in phylum *Basidiomycota*, the pathogens *Wallemia sebi* and *Wallemia muriae*, which are reported as a human health problem, were identified in this study. The species *W. sebi* and *W. muriae* cause the condition related with lung issues commonly referred to as "farmer's lung" (Zajc and Gunde-Cimerman 2018); and the same authors reported fatal livestock toxicosis associated with contaminated hay in the UK. The impact of *Ascomycota*; however, is particularly concerning due to its association with mycotoxin-producing species, which have direct implications in food safety. The fungal families identified in this study were predominantly from the *Ascomycota* phylum. Within this group, important genera such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* were detected (Fig. 4). These

genera include species known to produce mycotoxins, which can cause acute and chronic toxicity in both humans and animals (Navale et al. 2021).

The variation in fungal species found in the study may be attributed to different crop cultivation environments (Beccari et al. 2020) and storage conditions (Cao et al. 2022), and differences in nutrient content of the samples, which can influence fungal growth (Gizachew et al. 2019). Other pre-harvest and post-harvest conditions, and feed storage conditions (Zeng et al. 2022; Cao et al. 2022), could also contribute to the observed differences, particularly in *A. flavus* abundance. Although aflatoxin B_1 was detected in soybean cake at a low level, *A. flavus* was not detected in the DNA sample. This could be due to the low abundance of the fungus, making it difficult to detect at the sequencing depth used despite the positive rarefaction result (Sanchez-Cid et al. 2022). Other significant mycotoxin-producing fungi detected in other studies include *Fusarium* species, such as *F. proliferatum* (Alshannaq 2017), F. *oxysporum*, and *F. poae* (Shabeer et al. 2021), as well as *Alternaria alternata*, which is known to produce mycotoxins harmful to animals (EFSA 2011). The saprophytic fungi *Alternaria* and *Mucor spp.*, such as *A. glaucus*, *Mucor circinelloides*, and *M. racemosus* were identified in the feed samples. These fungi have been reported to have the ability to deplete and breakdown organic material, causing loss of nutritional value and feed deterioration (Thomma 2003; Buckle 1983).

In this study, although AFB_1 was detected in high amounts, *Aspergillus flavus* was not the dominant fungus (0% −3.8%) found in the studied feed fungal community. This mismatch can be explained by several factors; one reason could be antagonism or competition for nutrients by other species of fungi identified, for instance like *Fusarium. proliferatum* and *A. flavus* (Phan et al. 2021); and bacteria which are naturally found on livestock feeds (Palumbo et al. 2006; Watkinson 2016), although we did not assess the bacterial community. On the other hand, this big variation in abundance could be as a result of raw material handling (Zeng et al. 2022; Cao et al. 2022), nutrient content, storage conditions (Cao et al. 2022) and cultivation area of raw materials (Beccari et al. 2020). Khan et al. (2018) noted that the favourable environmental conditions, especially optimal temperature and humidity can favour the growth of fungi and of the synthesis of their toxic metabolites. As a result, even the small population of aflatoxin producing fungi can build up aflatoxin. Due to their chemical nature, aflatoxins exhibit high stability, (Tahir et al. 2018). Therefore, once formed these toxins persist due to their chemically stable structure, remaining intact even after the fungi themselves have been eliminated. In addition, in the sequencing experiment there could be draw backs of the experiment such as primer bias in targeting ITS regions and also library preparations that cause low coverage of certain species during the sequencing pipeline (Ross et al. 2013; Schirmer et al. 2015). Database annotation issues like fungal ITS databases (e.g., UNITE, GenBank) may have limited or inconsistent annotations for *Aspergillus flavus* as has been seen generally for *Aspergillus* species (Steenwyk et al. 2024).

This study demonstrated the possible risks associated with AFB_1 contamination in oilseed cakes used in Ethiopian livestock feeds, which pose potential health risks to animal and human populations. The fungal contamination observed in the six feed samples aligns with the global trends where genera such as *Aspergillus* and *Fusarium* are common contaminants. *A. flavus*, a powerful pathogen, was identified in all feed samples except soybean cake. AFB_1 was detected in all samples in the study, with the exception of soybean cake, mostly at higher than the internationally allowable level of 20 µg/kg. Considering the co-existence of different filamentous mycotoxin producing fungi, there is a high probability that the feeds were contaminated with different mycotoxins. Saprophytic fungal *Botryosphaeriaceae* species which cause feed quality deterioration were also identified in the samples. Therefore, to minimise contamination and toxicity, strict feed management measures at every step of the livestock feed value chain must be enforced. Continual study and observation are vital to understand the dynamics and nature of the contaminating fungi in livestock feed. The findings highlight the critical need for enhanced feed management practices, improved education and training for feed handlers, and stricter regulatory measures to mitigate aflatoxin contamination in Ethiopia.

This study provides insightful information that can serve as a foundation for future research on mycotoxins, feed/food safety studies that could benefit human and animal health. The DNA-based profiling used in the study offers a broad view of the fungal community present in the samples, and the concentration of aflatoxin B1 was also assessed. However, this approach didn't allow the researchers to establish the direct correlation of the presence or amount of *Aspergillus* species (viable fungi) to the actual AFB_1 being produced in the feed. To better understand how *Aspergillus flavus* behaves in relation to the other existing fungi (especially with dominant fungi species) in the same feed or environment, further research is needed to explore their population dynamics. Adding culture-based methods in future studies could help fill this gap by identifying which fungi are actively producing toxins, and could lead to discovering effective biological control options to reduce aflatoxin contamination.

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Author contributions Adey Melesse as first author led the research and prepared the initial draft of the manuscript. Alemayehu Teresa supervised the research and contributed to drafting the paper. Tesfaye Alemu supervised the PhD project, alongside Jean Hanson, who also provided guidance on the manuscript's structure and conceptual framework. Michael Blummel offered initial advice on structuring the project and continued supervising until his passing in 2020. Nebiat Negusse contributed statistical expertise. Yilikal Assefa, Yonas Asmare, and Prasad KVSV provided expertise on Aflatoxin evaluation. Chris S. Jones supported the PhD project and contributed to the final editing of the manuscript. All authors contributed to and approved the final version of the manuscript.

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Data availability The data supporting the conclusions of this article will be made available by the authors, when needed.

Declarations

Competing interests The authors declare no competing interests.

References

Abdelghani N, Angustias ML, Fernández-Martínez JM, Velasco L (2008) Novel seed oil types of Ethiopian mustard with high levels of polyunsaturated fatty acids. Ind Crops Prod 27(3). https://doi.org/10.1016/j.indcrop.2007.12.004

Alemaw G, Gurmu F (2023) Towards edible oil self-sufficiency in ethiopia: lessons and prospects. Cogent Food Agric 9(1). https://doi.org/10.1080/23311932.2023.2198742

Alshannaq A, Yu JH (2017) Occurrence, toxicity, and analysis of major Mycotoxins in food. Int J Environ Res Public Health 14(6):E632. https://doi.org/10.3390/ijerph14060632

Anelich L (2014) Foodborne diseases: Prevalence of foodborne diseases in Africa. In: Motarjemi Y (ed) Encyclopedia of food safety. Academic Press, pp 262–275

Avery SV, Singleton I, Magan N, Goldman GH (2019) The fungal threat to global food security. Fungal Biol 123(8):555–557. https://doi.org/10.1016/j.funbio.2019.03.006

Beccari G, Prodi A, Senatore MT, Balmas V, Tini F, Onofri A, Pedini L, Sulyok M, Brocca L, Covarelli L (2020) Cultivation area affects the presence of fungal communities and secondary metabolites in Italian durum wheat grains. Toxins 12(2):97. https://doi.org/10.3390/toxins12020097

Bediye S, Nemi G, Makkar HPS (2018) Ethiopian feed industry: current status, challenges and opportunities. Broadening Horizons

Bhat R, Reddy KR (2017) Challenges and issues concerning Mycotoxin contamination in oil seeds and their edible oils: updates from last decade. Food Chem 215:425–437. https://doi.org/10.1016/j.foodchem.2016.07.161

Buckle AE (1983) The occurrence of Mycotoxins in cereals and animal feed-stuffs. Vet Res Commun 7(1):171–186. https://doi.org/10.1007/BF02228614

Campagnollo FB, Ganev KC, Khaneghah AM, Portela JB, Cruz AG, Granato D, Corassin CH, Oliveira CAF, Sant'Ana AS (2016) The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review. Food Control 68:310–329. https://doi.org/10.1016/j.foodcont.2016.04.007

Cao D, Lou Y, Jiang X, Zhang D, Liu J (2022) Fungal diversity in barley under different storage conditions. Front Microbiol 13:895975. https://doi.org/10.3389/fmicb.2022.895975

Central Statistical Agency (CSA) (2020) Agricultural Sample Survey. Report on Livestock and Livestock Characteristics. Volume II. Statistical Bulletin 587, Addis Ababa, pp 9–11

Demissie N (2022) Study on compound animal feed demand and animal products, supply, price and marketing in Ethiopia. Biomed J Sci Tech Res 41(3):1–10. https://doi.org/10.26717/BJSTR.2022.41.006619

Ethiopian Standards Authority (ESA) (2019) Animal feeding stuffs: determination of aflatoxin B1 content of mixed feeding stuffs; method using high-performance liquid chromatography (ES ISO 14718:1998). Addis Ababa, Ethiopia

European Commission (EU) (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Off J Eur Communities L140:10–21

European Food Safety Authority (EFSA) (2011) Scientific opinion on the risks for animal and public health related to the presence of alternaria toxins in feed and food. EFSA J 9(10):2407. https://doi.org/10.2903/j.efsa.2011.2407

Food and Drug Administration (FDA) (2019) CPG Sect. 683.100: Action levels for aflatoxins in animal feeds. Silver Spring, MD

Food and Agriculture Organization (FAO) (2019) Ethiopia: Availability and utilization of agro-industrial by-products as animal feed 2018. Rome

Gizachew D, Szonyi B, Tegegne A, Hanson J, Grace D (2016) Aflatoxin contamination of milk and dairy feeds in the greater addis Ababa milk shed, Ethiopia. Food Control 59:773–779. https://doi.org/10.1016/j.foodcont.2015.06.060

Gizachew D, Chang CH, Szonyi B, De La Torre S, Ting WE (2019) Aflatoxin B1 (AFB1) production by Aspergillus flavus and Aspergillus parasiticus on ground Nyjer seeds: the effect of water activity and temperature. Int J Food Microbiol 296:8–13. https://doi.org/10.1016/j.ijfoodmicro.2019.02.017

Gupta SK, Venkitasubramanian TA (1975) Production of aflatoxin on soybeans. Appl Microbiol 29(6):834–836. https://doi.org/10.1128/am.29.6.834-836.1975

International Agency for Research on Cancer (IARC) (2015) Mycotoxin control in low- and middle-income countries. IARC Working Group Report No. 9, Lyon, France

Kassaw TS, Megerssa YC, Woldemariyam FT (2022) Occurrence of aflatoxins in poultry feed in selected chicken rearing villages of Bishoftu, Ethiopia. Vet Med Res Rep 13:277–286. https://doi.org/10.2147/VMRR.S384148

Khan MF et al (2018) Aflatoxin B1 production by Aspergillus flavus and its detection in contaminated food samples. Mycotoxin Res 34(2):85–91

Li N, Wu YX, Zhang YD, Wang SR, Zhang GC, Yang J (2023) Phytic acid is a new substitutable plant-derived antifungal agent for the seedling blight of Pinus sylvestris var. Mongolica caused by fusarium oxysporum. Pestic Biochem Physiol 191:105341. https://doi.org/10.1016/j.pestbp.2023.105341

Marshall H, Meneely JP, Quinn B, Zhao Y, Bourke P, Gilmore BF, Zhang G, Elliott CT (2020) Novel decontamination approaches and their potential application for post-harvest aflatoxin control. Trends Food Sci Technol 106:489–496. https://doi.org/10.1016/j.tifs.2020.11.001

Melesse A, Bezabih M, Adie A, Asmare Y, Prasad KVSV, Devulapalli R, Duncan AJ (2023) Price-quality relationships for the main livestock feed types in the Ethiopian feed market. Front Anim Sci 4:1194974. https://doi.org/10.3389/fanim.2023.1194974

Menale M (2019) Urban and peri-urban dairy cattle production in ethiopia: a review. Online J Anim Feed Res 9:173–177

Mengesha G, Bekele T, Ashagrie H, Woldegiorgis AZ (2024) Level of aflatoxins in dairy feeds, poultry feeds, and feed ingredients produced by feed factories in addis Ababa, Ethiopia. Mycotoxin Res 40(2):309–318. https://doi.org/10.1007/s12550-024-00531-8

Mengistu A, Kebede G, Feyissa F, Assefa G (2017) Review on major feed resources in ethiopia: conditions, challenges and opportunities. Acad Res J Agric Sci Res 5(3):176–185

Motbaynor A, Ambachew K, Kassaye D, Dawit K, Keffale M, Migbaru W, Pawlos P (2021) 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 2021:6638083. https://doi.org/10.1155/2021/6638083

Navale V, Vamkudoth KR, Ajmera S, Dhuri V (2021) Aspergillus derived Mycotoxins in food and the environment: prevalence, detection, and toxicity. Toxicol Rep 8:1008–1030. https://doi.org/10.1016/j.toxrep.2021.04.013

Negash D (2020) Animal feed safety: cases and approaches to identify the contaminants and toxins. Adv Nutr Food Sci 5(2):1–10

Nsiah B, Ofori H, Oduro-Yeboah C, Kyereh E, Johnson PT (2023) Incidences of aflatoxin contaminations in ingredients, feed and products of poultry from two regions in Ghana. Heliyon 9(12):e22567. https://doi.org/10.1016/j.heliyon.2023.e22567

Oliveira CAF, Kobashigawa E, Reis TA, Mestieri L, Albuquerque R, Correa LMB (2000) Aflatoxin B1 residues in eggs of laying hens fed a diet containing different levels of the Mycotoxin. Food Addit Contam 17(6):459–462. https://doi.org/10.1080/02652030050034037

Orina A, Gavrilova OP, Gagkaeva T, Burkin A, Kononenko G (2020) The contamination of fabaceae plants with fungi and Mycotoxins. Agric Food Sci 29(3):265–275. https://doi.org/10.23986/afsci.89171

Palumbo JD, Baker JL, Mahoney NE (2006) Isolation of bacterial antagonists of *Aspergillus flavus* from almonds. Microb Ecol 52:45–52. https://doi.org/10.1007/s00248-006-9096-y

Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP (2012) Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis 54(Suppl 1):S23–S34. https://doi.org/10.1093/cid/cir866

Pettersson H (2012) Mycotoxin contamination of animal feed. In: Fink-Gremmels J, editor. Animal feed contamination. Woodhead Publishing, pp 233–285. https://doi.org/10.1533/9780857093615.3.233

Phan LTK, Tran TM, Audenaert K, Jacxsens L, Eeckhout M (2021) Contamination of Fusarium proliferatum and Aspergillus flavus in the Rice Chain Linked to Crop Seasons, Cultivation Regions, and Traditional Agricultural Practices in Mekong Delta, Vietnam. Foods, 10(9), 2064. https://doi.org/10.3390/foods10092064

Rehrahie M, Getnet A, Fassil A (2018) Determination of aflatoxin in dairy feeds and milk in some selected areas of Ethiopia. Food Environ Saf 17(3):286–299

Ross MG, Russ C, Costello M, Hollinger A, Lennon NJ, Hegarty R, Nusbaum C, Jaffe DB (2013) Characterizing and measuring bias in sequence data. Genome Biol 14:R51

Sanchez-Cid C, Luna R, Saiz-Jimenez C (2022) Characterization of airborne fungi in indoor environments: a case study. Aerobiologia 18(3):207–213. https://doi.org/10.1007/s10453-022-09745-8

Schirmer M, Ijaz UZ, D'Amore R, Hall N, Sloan WT, Quince C (2015) Insight into biases and sequencing errors for amplicon sequencing with the illumina miseq platform. Nucleic Acids Res 43(6):e37. https://doi.org/10.1093/nar/gku1341

Shabeer S, Tahira R, Jamal A (2021) Fusarium spp. Mycotoxin production, diseases, and their management: an overview. Pakistan J Agric Res 34(2):278–293. https://doi.org/10.17582/journal.pjar/2021/34.2.278.293

Shar ZH, Pirkash O, Shar HH, Sherazi STH, Mahesar SA (2020) Aflatoxins in cotton seeds and cotton seed cake from Pakistan. Food Addit Contam Part B 13(1):72–76. https://doi.org/10.1080/19393210.2019.1698661

Steenwyk JL, Balamurugan C, Raja HA, Gonçalves C, Li N, Martin F, Berman J, Oberlies NH, Gibbons JG, Goldman GH, Geiser DM, Houbraken J, Hibbett DS, Rokas A (2024) Phylogenomics reveals extensive misidentification of fungal strains from the genus Aspergillus. Microbiol Spectr 12:e03980–e03923. https://doi.org/10.1128/spectrum.03980-23

Tadele F, Demissie B, Amsalu A, Demelash H, Mengist Z, Ambelu A, Yenew C (2023) Aflatoxin contamination of animal feeds and its predictors among dairy farms in Northwest ethiopia: one health approach implications. Front Vet Sci 10:1123573. https://doi.org/10.3389/fvets.2023.1123573

Tahir NI, Hussain S, Javed M, Rehman H, Shahzady TG, Parveen B, Ali KG (2018) Nature of aflatoxins: their extraction, analysis, and control. J Food Saf 38(6):e12561. https://doi.org/10.1111/jfs.12561

Tatfo Keutchatang FP, Tchuenchieu AK, Nguegwouo E, Mouafo HT, Bouelet Ntsama IS, Kansci G, Medoua GN (2022) Occurrence of total aflatoxins, aflatoxin B1, and Ochratoxin A in chicken and eggs in some Cameroon urban areas and population dietary exposure. J Environ Public Health 2022:5541049. https://doi.org/10.1155/2022/5541049

Thomma BP (2003) Alternaria spp: from general saprophyte to specific parasite. Mol Plant Pathol 4(4):225–236. https://doi.org/10.1046/j.1364-3703.2003.00173.x

Ting WTE, Chang CH, Szonyi B, Gizachew D (2020) Growth and aflatoxin B1, B2, G1, and G2 production by Aspergillus flavus and Aspergillus parasiticus on ground flax seeds (Linum usitatissimum). J Food Prot 83(6):975–983. https://doi.org/10.4315/JFP-19-539

Tolosa J, Rodríguez-Carrasco Y, Ruiz MJ, Vila-Donat P (2021) Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: A review. Food Chem Toxicol 158:112661. https://doi.org/10.1016/j.fct.2021.112661

Tsehay S, Ortiz R, Geleta M, Bekele E, Tesfaye K, Johansson E (2021) Nutritional profile of the Ethiopian oilseed crop Noug (Guizotia abyssinica Cass): opportunities for its improvement as a source for human nutrition. Foods 10(8):1778. https://doi.org/10.3390/foods10081778

United Nations Department of Economic and Social Affairs (UN 2024 Revision) - Population Division. World Population. https://www.worldometers.info/world-population/ethiopia-population/

Walelegne M, Meheret F, Derseh MB, Dejene M, Asmare YT, Prasad KVSV, Duncan AJ (2023) Near-infrared reflectance spectroscopy using a portable instrument to measure the nutritive value of oilseed meals as livestock feed. Front Anim Sci 4:1203449. https://doi.org/10.3389/fanim.2023.1203449

Watkinson SC, Boddy L, Money NP (eds) (2016) Interactions between fungi and other microbes. The fungi, 3rd edn. Academic, London, pp 337–360. https://doi.org/10.1016/B978-0-12-382034-1.00010-4

Wendimu GY (2021) The challenges and prospects of Ethiopian agriculture. Cogent Food Agric 7(1):1923619. https://doi.org/10.1080/23311932.2021.1923619

Xu R, Kiarie EG, Yiannikouris A et al (2022) Nutritional impact of Mycotoxins in food animal production and strategies for mitigation. J Anim Sci Biotechnol 13(1):69. https://doi.org/10.1186/s40104-022-00714-2

Yosef T, Demise N, Tadesse T, Daniel T (2022) Research Article study on the animal feed ingredients and livestock product supply, price and market-related constraints in Ethiopia. Int J Agric Res 17(3):102–115. https://doi.org/10.3923/ijar.2022.102.115

Yunus AW, Ullah A, Lindahl JF, Anwar Z, Ullah A, Saif S, Ibrahim MNM (2020) Aflatoxin contamination of milk produced in peri-urban farms of pakistan: prevalence and contributory factors. Front Microbiol 11:159. https://doi.org/10.3389/fmicb.2020.00159

Zajc J, Gunde-Cimerman N (2018) The genus wallemia: from contamination of food to health threat. Microorganisms 6(2):46. https://doi.org/10.3390/microorganisms6020046

Zeng Q, Man X, Lebreton A, Dai Y, Martin FM (2022) The bacterial and fungal microbiomes of ectomycorrhizal roots from stone Oaks and Yunnan Pines in the subtropical forests of the Ailao mountains of Yunnan. Front Microbiol. https://doi.org/10.3389/fmicb.2022.916337