Prevalence and Characterization of Moulds Associated with Fish Feeds Sold in Kisii County, Kenya

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Authors’ contributions
This work was carried out in collaboration among all authors. Author ISN designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Authors EM, ROO and JK managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT
There is an increase in aquaculture in Kenya due to increased demand for fish as a source of white meat and increased population growth. Most fish farmers use plant-based ingredients such as peanuts, cottonseed, soybeans, maize bran and wheat as sources of protein for the fish feeds. These ingredients are very susceptible to attack by aflatoxigenic fungi. In humid climatic conditions like those found in Kisii County, growth of such fungi on fish feeds is accelerated due to absorption of moisture from the environment as a result of poor storage and sometimes improper drying. This study was conducted to determine the moulds associated with fish feeds sold in Kisii. Commercial fish feeds from five main outlets in Kisii County were sampled and analysed. Home-made fish feeds were obtained from three groups. Fungi were isolated using various media and percentage isolation
determined. The results show that fifteen fungal species were associated with fish feeds sold in Kisii County. They include *Mucor* spp, *Penicillium glabrum*, *Fusarium oxysporium*, *Aspergillus oryzae*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Alternaria* spp, *Penicillium citrinum*, *Stachybotrys* spp, *Cladosporium* spp, *Aureobasidium* spp, *Eurotium* spp, *Aspergillus versicolor*, *Aspergillus fumigatus* and *Aspergillus niger*. The aflatoxigenic fungi comprising of *A. flavus*, *A. parasiticus* and *A. niger* were most prevalent in fish feeds obtained from Egetuki outlet (29 %) and least prevalent in Dombett (16.6 %). The mean differences of fungal species were statistically significant ($P<0.05$) in four outlets. This shows that fish feeds sold in Kisii county are contaminated with aflatoxigenic fungi.

**Keywords:** Mould; fish feeds; prevalence; aflatoxigenic fungi; home-made.

### 1. INTRODUCTION

Fish represent an important source of food for human and animal consumption. This has resulted in fast development of aquaculture [1]. In order to provide quality fish from aquaculture, fish nutrition is very important. The fish must be fed on a nutritionally balanced feed free from mycotoxigenic fungi. This will ensure proper growth, good reproductive performance, quality flesh and healthy fish. The common ingredients of commercial fish and home-made feeds commonly used in Kenya are shown in Table 1. Some of the ingredients used in fish feed production such as maize gluten, groundnut oilcake, sunflower oilcake and soy bean meal are substrates for fungal growth [2]. Under favourable conditions toxigenic fungi grow, multiply and produce mycotoxins during post-harvest storage of fish ingredients or during storage of compounded fish feed [3].

**Table 1. Common ingredients of commercial and home-made fish feeds in Kenya**

| Commercial fish feed ingredients | Home-made fish feed ingredients |
|---------------------------------|---------------------------------|
| Wheat bran                      | Cassava                         |
| Omena fish meal                 | Spinach                         |
| Nile perch fish meal            | Shrimp                          |
| Groundnut oil cake              | Omensa                          |
| Maize gluten meal               | Peas                            |
| Rice bran                       | Carrots                         |
| Soybean oil cake                | Garlic                          |
| Molasses                        | Vitamin mix                     |
| Sunflower seed oil cake         | Waste blood                     |
| Bone meal                       | Rumen contents                  |
| Meat and bone meal              | Kales                           |
| Cotton seed cake                | Groundnuts                      |
| Brewers dried yeast             | Soybeans                        |
| Gelatin                         | Maize                           |

*Source: [4] and [5]*

Studies conducted in some countries have confirmed the presence of moulds in fish feeds, some of which are toxigenic. They include [6] who isolated *Fusarium oxysporium* and *Mucor* species from fish feeds in Nigeria, [7] isolated toxigenic *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* species and *Aspergillus niger* from finished fish feeds from farms in Rio de Janeiro in Brazil. In Egypt, [8] isolated *Aspergillus* species, *Penicillium* species, *Fusarium* species and *Alternaria* species from fish feeds, [9] isolated *Fusarium* species, *Mucor* species, *Alternaria* species and nine different species of *Aspergillus* from fish feeds in Iran, [10] isolated *Cladosporium* species, *Eurotium* and four different species of *Penicillium* from rainbow trout feeds in Argentina while [11] isolated *Aspergillus flavus* and *Fusarium* species from tilapia feeds in Mexico. A number of moulds were isolated from finished fish feeds and fish feed ingredients from small holder farms in East Africa [12] while [13] isolated *Aspergillus* species, *Mucor* species, *Rhizopus* species, *Saprolegnia* species and *Penicillium* species from formulated and commercial feeds in three fish farms in humid tropical environments of Kenya.

The presence of aflatoxigenic fungi such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus niger* in fish feeds is an indication that fish that consume such feed will accumulate the aflatoxins which will be hazardous to them and humans who consume them [14]. It is therefore important to monitor and do surveillance of such fungi in fish feeds in order to avert negative health effects that could arise from consumption of such contaminated fish feeds. This study was conducted to determine the prevalence and type of moulds associated with fish feeds sold in Kisii County, Kenya.

### 2. MATERIALS AND METHODS

#### 2.1 Study Area

The study was conducted in Kenya in Kisii County which lies between latitude 0º 30’ and 10º South and longitude 34º 38’ and 35º 0’ East. It is located to the South East of Lake Victoria and
bordered by six counties with Narok to the South, Migori to the West, Homabay to the North West, Kisumu to the North, Bomet to the South East and Nyamira to the East. It has a population of 1,266,860 according to Kenya National Census of 2019 [15]. The county has over 3000 fish farmers, operating 3,129 fish ponds, courtesy of the economic stimulus program [16]. It has humidity of 61-76% due to heavy rainfall (over 1,500 mm per annum) in September to December and March to June. The month of May has the highest humidity of 76% while February has the lowest humidity of 61%. The temperature of Kisii County ranges from 18.5-28 °C [17] and [18]. It has the lowest humidity of 61% while February has the highest humidity of 76%. The temperature of Kisii County ranges from 18.5-28 °C [17] and [18].

2.3 Methods

2.2 Materials

The materials that were used during this study include; sterile bags, labels, blender, petri-dishes, Czapek Dox agar, Aspergillus flavus and parasiticus agar, microscope, microscope slides, microscope slide cover slips, glass rods, droppers, lactophenol cotton blue dye, camera and fish feed samples.

2.3 Methods

2.3.1 Sample collection

Four five-kilogram (Kg) bags of commercial fish feeds were purchased from each of the five main fish feed outlets in Kisii County namely Dombetty enterprises, Egetuki aquashop, Zamo enterprises, Enochem enterprises and Jumbo enterprises. Another set comprising of home-made fish feed samples were obtained from Obomo, JuaKali and Mwanyagetinge self-help groups which are the main producers of home-made fish feeds in the county. Samples of half kilogram were obtained from the top, middle and bottom layers of each bag of commercial and home-made fish feeds, mixed well in separate sterile bags and labelled. These formed composite samples of 6 Kg from each outlet and home-made producer. The samples were transported to the Pathology Laboratory in Kenya Agricultural and Livestock Research Organization (KALRO) Kisii for mycological assay.

2.3.2 Enumeration, isolation and identification of fungal isolates

Mycological assays that were used include; enumeration of fungal colony forming units (CFUs), isolation and identification which largely involved microscopy. Enumeration was done according to the protocol by [19]. Fish feed samples were ground in a blender and 10 grams of each sample was homogenized in 90 ml of distilled water. Serial dilutions of $10^2$ to $10^3$ were made and 0.1ml aliquots spread plated in triplicates onto Sabourand Dextrose Agar (SDA) for quantifying and detecting moulds in the ground fish feed samples. The plates were incubated at 25 °C for 5 to 7 days. Those with colonies between 30 and 300 were enumerated in form of colony forming units (CFU) [20]. The abundance of fungi was determined in form of the CFU. The colonies were counted and the CFU per gram of sample determined through calculations by taking into account the dilution factor.

From the fungal growth on primary cultures, fungal isolates were sub-cultured using a plug that was taken from the periphery of fungal cultures and placed onto fresh media of SDA to get distinct colonies. The obtained pure isolates were then stored in the dark at 25 °C until fruiting structures formed for further identification [21]. Czapek Dox and Aspergillus flavus and Parasiticus agar (AFPA) were used to differentiate the colonies of Aspergillus flavus and Aspergillus parasiticus. Taxonomic identification of the various moulds was carried out according to macro and microscopic characteristics of the colonies using identification keys by [22,19,23,24]. Morphological features of Aspergillus cultures were studied, the major and remarkable macroscopic features in species identification were the colony diameter, colour (conidia and reverse), exudates and colony texture. Slide culture method for microscopic study of most of the isolates was also carried out. When the mould sporulated, the cover slip was carefully withdrawn from the agar and mounted in a drop of lactophenol cotton blue dye on a microscope slide. Microscopic characteristics used for identification were conidial heads, stipes, colour, vesicles shape and seriation, metula covering, conidia shape and roughness.

2.4 Data Analysis

Percentage isolation of various fungi was calculated and T-test performed.
3. RESULTS AND DISCUSSION

A total of three hundred and thirty-two isolates were obtained from samples purchased from five main fish feed outlets in Kisii County. These comprised of fifteen fungal species namely; Mucor spp, Penicillium glabrum, Fusarium oxysporium, Aspergillus oryzae, Aspergillus flavus, Aspergillus parasiticus, Alternaria spp, Penicillium citrinum, Stachybotrys spp, Cladosporium spp, Aureobasidium spp, Eurotium spp, Aspergillus versicolor, Aspergillus fumigatus and Aspergillus niger. From home-made fish feeds a total of one hundred and twenty-eight fungal isolates comprising of eight different fungal species were obtained. In total four hundred and sixty isolates were obtained from both commercial and home-made fish feeds. Moisture content of commercial fish feeds ranged between 10.2-22.0 % while that of home-made feeds ranged between 14.0-28.6 %.

One hundred and twenty-two isolates were obtained from samples purchased from Egetuki aquashop. These isolates comprised of thirteen fungal species which were Mucor spp 12 (9.8 %), Fusarium oxysporium 14 (11.5 %), Aspergillus oryzae 4 (3.3 %), Aspergillus flavus 16 (13.1 %), Aspergillus parasiticus 6 (4.9 %), Alternaria spp 10 (8.2 %), Penicillium citrinum 16 (13.1 %), Cladosporium spp 2 (1.6 %), Aureobasidium spp 6 (4.9 %), Eurotium spp 8 (6.6 %), Aspergillus versicolor 10 (8.2 %), Aspergillus fumigatus 6 (4.9 %) and Aspergillus niger 14 (11.5 %). Penicillium citrinum and Aspergillus flavus (13.1 %) were the most frequently isolated fungi while Aspergillus oryzae (3.3 %) was the least isolated (Fig. 1).

Sixty isolates were obtained from samples purchased from Dombetty enterprises. The samples comprised of nine fungal species which were Mucor spp 10 (16.7 %), Penicillium glabrum 6 (10 %), Fusarium oxysporium 4 (6.7 %), Aspergillus flavus 8 (13.3 %), Aspergillus parasiticus 2 (3.3 %), Alternaria spp 10 (16.7 %), Penicillium citrinum 8 (13.3 %), Stachybotrys spp 4 (6.7 %) and Aspergillus versicolor 8 (13.3 %). Mucor spp and Alternaria were the most frequently isolated from this outlet while Aspergillus parasiticus was the least isolated (Fig. 2).

The isolates obtained from Enochem enterprises were fifty-six in number. They comprised of eleven fungal species which were Mucor spp 6 (10.7 %), Fusarium oxysporium 4 (7.1 %), Aspergillus oryzae 4 (7.1 %), Aspergillus flavus 6 (10.7 %), Alternaria spp 4 (7.1 %), Penicillium citrinum 2 (3.6 %), Stachybotrys spp 4 (7.1 %), Cladosporium spp 6 (10.7 %), Aspergillus versicolor 4 (7.1 %), Aspergillus fumigatus 8 (14.3 %) and Aspergillus niger 8 (14.3 %). Aspergillus fumigatus and Aspergillus niger were the most frequently isolated from sample purchased from Enochem enterprises. Penicillium citrinum was the least frequently isolated followed by Fusarium oxysporium, Aspergillus oryzae, Alternaria spp, Stachybotrys and Aspergillus versicolor all of which represented 7.1 % of the total isolates obtained from this outlet (Fig. 3).

Fifty isolates were obtained from samples purchased from Jumbo enterprises. These isolates comprised of eleven fungal species which were Mucor 2 (4.0 %), Penicillium glabrum 6 (12.0 %), Fusarium oxysporium 8 (16.0 %), Aspergillus flavus 4 (8.0 %), Aspergillus parasiticus 2 (4.0 %), Penicillium citrinum 6 (12.0 %), Cladosporium spp 4 (8.0 %), Eurotium spp 2 (4.0 %), Aspergillus versicolor 8 (16.0 %), Aspergillus fumigatus 4 (8.0 %) and Aspergillus niger 4 (8.0 %). The isolates of Fusarium oxysporium and Penicillium versicolor were the most frequently isolated while Mucor spp, Eurotium spp and Aspergillus parasiticus were the least frequently isolated from sample obtained from Jumbo enterprises. Penicillium glabrum and Penicillium citrinum were the second most frequently isolated (Table 2).

Table 2. Isolation frequency of fungal species obtained from Jumbo enterprises

| Fungi                  | No. of isolates | % isolation frequency |
|-----------------------|-----------------|-----------------------|
| Mucor spp             | 2               | 4                     |
| Penicillium glabrum   | 6               | 12                    |
| Fusarium oxysporium   | 8               | 16                    |
| Aspergillus flavus    | 4               | 8                     |
| Aspergillus parasiticus| 2              | 4                     |
| Penicillium citrinum  | 6               | 12                    |
| Cladosporium spp      | 4               | 8                     |
| Eurotium spp          | 2               | 4                     |
| Aspergillus versicolor| 8               | 16                    |
| Aspergillus fumigatus | 4               | 8                     |
| Aspergillus niger     | 4               | 8                     |
| Total isolates        | 50              | 100                   |

Fish feed samples purchased from Zamo enterprises had the least number of fungal isolates which was forty-four. The isolates comprised of eight fungal species which were
Mucor spp 6 (13.6 %), Fusarium oxysporium 6 (13.6 %), Aspergillus oryzae 2 (4.5 %), Aspergillus flavus 8 (18.2 %), Penicillium citrinum 12 (27.4 %), Aureobasidium spp 4 (9.1 %), Aspergillus fumigatus 2 (4.5 %) and Aspergillus niger 4 (9.1 %). Penicillium citrinum (27.4 %) was the most frequently isolated fungus from this outlet while Aspergillus oryzae and Aspergillus fumigatus (4.5 %) were the least frequently isolated (Fig. 4).

From the five main fish feed outlets Penicillium citrinum had the highest percentage isolation frequency of 13.3 % followed by Aspergillus flavus with 12.7 %, then Mucor spp and Fusarium oxysporium which had an isolation frequency of 10.8 % each. Mucor spp, Fusarium oxysporium, Penicillium citrinum and Aspergillus flavus were isolated from samples purchased from all the outlets as shown in Table 3. Most fungi were isolated from samples that were purchased from Egetuki aquashop. Thirteen different fungal species out of the fifteen different fungal species isolated from all fish feeds purchased from the five outlets were isolated from Egetuki aquashop samples. Samples from Zamo enterprises recorded the least numbers of both fungal species and fungal isolates (Table 3).
The aflatoxigenic fungi comprising of *A. flavus*, *A. parasiticus* and *A. niger* were most prevalent in fish feeds obtained from Egetuki outlet (29.5 %) followed by Zamo (27.3 %), Enochem (25.0 %), Jumbo (20.0 %) while Dombetty had the least prevalence (16.6 %) (Table 4). The mean differences of fungal species were statistically significant in all the outlets except those from Egetuki aquashop (*P*=0.42). The *P*-values for the other outlets were Dombetty enterprise (*P*=0.02), Zamo enterprises (*P*=0.04), Enochem Enterprises (*P*=0.003) and Jumbo (*P*=0.007).

A total of one hundred and twenty-eight fungal isolates were obtained from samples of home-made fish feeds. The samples were obtained from three main home-made fish feed producers (Obomo, JuaKali and Mwanyagetinge self-help group) in Kisii county. The fungal isolates obtained were from eight different fungal species namely, *Trichoderma* species 10 (7.8 %), *Rhizopus* species 30 (23.4 %), *Penicillium* species 16 (12.5 %), *Fusarium* species 8 (6.3 %), *Aspergillus niger* 8 (6.3 %), *Aspergillus flavus* 20 (15.6 %), *Cladosporium* species 20 (15.6 %) and *Aureobasidium* spp 16 (12.5 %) (Table 5).

The aflatoxigenic fungi isolated from the home-made feeds were *A. flavus* and *A. niger* while *Fusarium* and *Penicillium* species produce...
mycotoxins. All these species represented 50 % of all fungal species isolated from home-made feeds. There was a significant difference between the means of the different fungal species isolated from the home-made feeds (P= 0.008). However, there was no significant difference between the percentage isolation frequencies of fungi in home-made and commercial fish feeds (P=0.46).

Fifteen fungal species were isolated from both commercial and home-made fish feeds. The most frequently isolated fungus from the commercial fish feeds was *Penicillium citrinum* (13.3 %), followed by *Aspergillus flavus* which had a frequency of 12.7 %. The least isolated were *Stachybotrys* spp and *Aspergillus parasiticus*. The most isolated fungus from home-made feeds was *Rhizopus* spp (23.4 %) while the least isolated were *Fusarium* spp and *Aspergillus niger* (6.3 %). *Rhizopus* spp was most frequently isolated probably due to their ubiquitous and saprophytic nature. They are commonly found in soil, rotting vegetation and excrement of animals. Some species are plant pathogens and agents of diseases in humans and animals as observed by [25] and [26]. This finding is not in agreement with that reported by

### Table 3. Isolation frequency of fungal species from commercial fish feeds

| Fungi                  | Egetuki | Dombetty | Zamo | Enochem | Jumbo | % frequency |
|------------------------|---------|----------|------|---------|-------|-------------|
| *Mucor* spp            | 12      | 10       | 6    | 6       | 2     | 10.8        |
| *P. glabrum*           | 0       | 6        | 0    | 0       | 6     | 3.6         |
| *F. oxysporium*        | 14      | 4        | 6    | 4       | 8     | 10.8        |
| *A. oryzae*            | 4       | 0        | 2    | 4       | 0     | 3.0         |
| *A. flavus*            | 16      | 8        | 8    | 6       | 4     | 12.7        |
| *A. parasiticus*       | 6       | 2        | 0    | 0       | 2     | 2.5         |
| *Alternaria* sp        | 10      | 10       | 0    | 4       | 0     | 7.2         |
| *P. citrinum*          | 16      | 8        | 12   | 2       | 6     | 13.3        |
| *Stachybotrys* sp      | 0       | 4        | 0    | 4       | 0     | 2.5         |
| *Cladosporium* sp      | 2       | 0        | 0    | 6       | 4     | 3.6         |
| *Aureobasidium* sp     | 6       | 0        | 4    | 0       | 0     | 3.0         |
| *Eurotium* sp          | 8       | 0        | 0    | 0       | 2     | 3.0         |
| *A. versicolor*         | 10      | 8        | 0    | 4       | 8     | 9.0         |
| *A. fumigatus*         | 6       | 0        | 2    | 8       | 4     | 6.0         |
| *A. niger*             | 14      | 0        | 4    | 8       | 4     | 9.0         |
| **Total**              | 124     | 60       | 44   | 56      | 50    | 100         |

### Table 4. Isolation frequency of aflatoxigenic fungi from commercial fish feed outlets

| Commercial fish feed outlet | Mould     | % isolation frequency |
|-----------------------------|-----------|-----------------------|
|                            | *A. flavus* | *A. parasiticus* | *A. niger* |
| *Egetuki Aquashop*          | 13.1      | 4.9                  | 11.5       | 29.5 |
| *Dombetty Enterprises*      | 13.3      | 3.3                  | -          | 16.6 |
| *Zamo Enterprises*          | 18.2      | -                    | 9.1        | 27.3 |
| *Enochem Enterprises*       | 10.7      | 14.3                 | -          | 25.0 |
| *Jumbo Enterprises*         | 8.0       | 4.0                  | 8.0        | 20.0 |

### Table 5. Percentage frequency of fungal isolates from home-made fish feeds

| Fungal species     | Occurrence | % frequency |
|--------------------|------------|-------------|
| *Trichoderma* spp  | 10         | 7.8         |
| *Rhizopus* spp     | 30         | 23.4        |
| *Penicillium* spp  | 16         | 12.5        |
| *Fusarium* spp     | 8          | 6.3         |
| *Aspergillus* niger | 8         | 6.3         |
| *Aspergillus* flavus | 20      | 15.6        |
| *Cladosporium* spp | 20         | 15.6        |
| *Aureobasidium* spp | 16        | 12.5        |
| **Total isolates** | **128**    | **100**     |
All fish feeds (100 %) had moisture content of above 10 %, had high CFUs and were not properly stored. This concurs with the findings by [30] who found out that moisture levels in feeds above 14 % and improper storage favour the growth of aflatoxin producing moulds. When such feeds are stored improperly and for long, the growth of fungi in them is accelerated. The high moisture content could be due to absorption of moisture from the atmosphere during the two rainy seasons of the year when atmospheric humidity increases to 76 %. As observed by [30], such humidity levels that are greater than 62 % favour growth of moulds and aflatoxin production. When exposed to the atmosphere for long, the feeds absorb moisture from the surrounding affirm[31]. This is probably why the sampled fish feed had high moisture content and presence of fungi.

Proper feed storage is important in ensuring both feed quality and safety. This is because improperly stored feed can lead to spoilage causing huge economic losses. It is important that feed safety be ensured by maintaining proper storage conditions for fish feeds. [32] have observed that certain environmental factors such as high humidity, temperature and poor aeration result in increased contamination of stored feeds by fungi. The extent of contamination by fungi will also differ with geographic location and the susceptibility of commodities to the penetration of fungi during storage and processing periods in different weather conditions. This is because fungi are found everywhere and may be transmitted by humans, rodents, insects and animals, resulting in contamination, observed [25]. Moreover, many fungi grow more rapidly at temperatures between 15 °C and 40 °C. This temperature range is termed as temperature danger zone. Foods and feeds should be handled so that the amount of time the food is in the temperature danger zone is kept to a minimum to minimize multiplication of the fungi, the [33] advised.

Both commercial and home-made fish feeds were packed in gunny bags. This could have contributed to both fungal contamination and high moisture content of the feeds. [34] have observed that the type of storage material used affect the aflatoxin levels in the stored feed especially if they were stored for a long period of time with moisture content above 10 %. Storage materials such as jute bags absorb moisture from the surrounding and this increases the moisture content of the stored feed or food. Other storage
4. CONCLUSION

The fish feeds sold in Kisii County are contaminated with moulds. These moulds include mycotoxin producing species such as Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Fusarium and Alternaria. Other moulds associated with fish feeds sold in kisii County are Mucor spp, P. glabrum, F. oxysporium, A. oryzae, P. citrinum, Stachybotrys spp, Cladosporium spp, Aureobasidium spp, Eurotium spp, A. versicolor and A. fumigatus. This contamination could be due to use of mould contaminated ingredients. The presence of mycotoxigenic moulds in the fish feeds poses a health risk to both fish and consumers of fish fed on the contaminated feeds. There is therefore need for frequent detection of mycotoxigenic moulds in fish feeds and sensitization on ways of minimizing mould contamination and proper storage of fish feeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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During storage of feeds effort should be made to prevent moisture migration into the stored feed through leaking roofs and condensation resulting from inadequate ventilation as reported by [40]. This will ensure maintenance of the quality of feeds and reduced risk of fungal and consequently aflatoxin contamination as reported by [41]. The government of Kenya should also ensure that the established maximum allowable aflatoxin levels in feeds is enforced both at the national and especially the county level. Cheap and simple aflatoxin analysis methods such as ELISA can be used for frequent surveillance of aflatoxin levels in foods and feeds. Strengthening of policy and adherence to proper packaging and storage should be enforced at all times.
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