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Research article

Fungal Contamination and Characterization of *Aspergillus flavus* on Poultry Feeds and their Ingredients in North Sumatera

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Abstract

Poultry feeds mainly consist of cereal grains and crops that are vulnerable to fungal infection. Improper handling of the feeds increases spoilage and contamination by aflatoxigenic fungi. The aim of this study was to determine fungal contamination including characterization of toxigenic and non-toxigenic *Aspergillus flavus* strains in poultry feed and its ingredients. We collected 200 composite samples of compound poultry feeds and their ingredients sold by retailers at traditional markets. The fungal population was enumerated using a dilution followed by the pour plate in dichloran 18% glycerol agar medium. All isolated *A. flavus* were identified by culture technique and molecular approach. Aflatoxin production was determined in 10% coconut agar medium with thin layer chromatography. Regulatory (*aflR*) and structural (*nor-1*, *ver-1*, *omt-1*) genes of *A. flavus* strains were isolated using four sets of primers (aflR, nor-1, ver-1, omt-1). We found that all feeds were infected by fungi. *Aspergillus chevalieri* was the most found in all feeds, particularl on chick starter. Of the 200 samples of compound and poultry feed ingredients, 57 isolates of *A. flavus* were examined and 16 of them (28%) were toxigenic, each strain producing aflatoxin B_1 from <3.01 to 35.50 ppb. Among feeds, layer finisher, pellet starter and grain corn were the most infected by toxigenic *A. flavus*, respectively. The contamination occurred on feeds that contained corn as the main compound. Routine analysis of compound poultry feeds and their ingredients for aflatoxigenic fungi particularly *A. flavus* is compulsory.

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1. Introduction

Mycotoxins are secondary metabolites produced by certain species of fungi particularly *Aspergillus*, *Fusarium*, *Penicillium,* etc. They can contaminate our food chain by indirect contamination from the growth of toxigenic fungi [1, 2]. Among mycotoxins, aflatoxins are highly toxic, mutagenic, and carcinogenic produced by *Aspergillus flavus*, *A. nomius* and *A. parasiticus* which are especially abundant in warm and humid areas. Recently, the problem of food and animal feed contamination with mycotoxins, and in particular aflatoxins, has received attention worldwide [3-5]. Even at low concentrations, aflatoxins are hazardous to humans and livestock [5]. The occurrence of fungal infection on poultry feeds in tropical countries is a primary cause of deterioration. Feeds from cereal grains such as corn and rice bran, as well as crops (soybean, peanut) and the by-products are susceptible to infection by molds [6-8]. Some fungal genera such as *Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium*, and *Mucor* are commonly found contaminating poultry feeds [5-7], with *Aspergillus flavus* the most predominant species [5, 7]. Infection by molds also spoils and reduces the nutritional content. Among animals, poultry is the most sensitive to the toxic effects of aflatoxins [8]. Exposure to aflatoxins increase maturity in poultry [9]. Several studies have noted the presence of fungal mycotoxins, particularly aflatoxin residues, in the eggs and muscles of chickens that had consumed contaminated feeds, and these were associated with losses in productivity [10-12]. The presence of *A. flavus* in feeds occurs when toxigenic and non-toxigenic strains colonize the feed ingredients during harvesting and then grow during storage [13]. The potential for *A. flavus* to produce aflatoxins is determined by genetic analysis and testing for the presence of the enzymes involved in aflatoxin biosynthesis [14]. Culture and molecular techniques have been widely applied to identify aflatoxigenic and non aflatoxigenic *A. flavus* strains [15, 16].

Here, we aimed to enumerate fungal contamination and to characterize toxigenic *A. flavus* strains isolated from compound poultry feeds and the ingredients sold by retailers at traditional markets in North Sumatera, Indonesia.

2. Materials and Methods

2.1 Sample collection

A total of 200 samples of compound poultry feeds (chick starter, broiler finisher, layer finisher, pellet starter) and ingredients (paddy bran, grain paddy, milled corn and corn grain) were purchased (250 g per sample) from five retailers at five traditional markets in North Sumatera, Indonesia during the dry seasons (months of March and September 2018). The size of laboratory was 1250 g for each sample. Each sample then was packed under vacuum in a polyethylene bag and stored (-4°C) in a refrigerator for further use.

2.2 Moisture content determination

The moisture content (% wet basis) was determined using standard oven dry method. As much as 40 g of each poultry feed and the ingredients was dried in an oven at 130ºC for 2 h with three replicates per sample.

2.3 Fungal population and identification

The fungal population was determined by dilution followed by the pour plate method in dichloran 18% glycerol agar (DG18 medium) (Neogen, Lansing, MI 48912, USA). To the twenty-five grams

of each sample in a 1000 mL erlenmeyer, sterilized distilled water was added until the volume was up to 250 mL. The suspension was homogenized using a shaker (Gallenkamp, orbital shaker SG92, England) at 100 rpm for 2 min. Four dilutions were made (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) and 1 mL of each dilution was transfered onto a Petri dish (9 cm diameter) and pour plated in DG18 medium. Each sample was cultured in triplicate. The plates were incubated at ambient temperature (29±2ºC) for 6 days. All colonies were counted as colony forming unit per gram (CFU g^{-1}) of the sample. Every separate colony was isolated and cultured on Czapex yeast extract agar (CYA) and CYA+20% sucrose (CYA20S) and identified. Macroscopic and microscopic identification of each fungal species were conducted according to Pitt and Hocking [17].

2.4 *Aspergillus flavus* isolation and toxigenicity determination by culture technique

All separate colonies of *A. flavus* were further isolated on potato dextrose agar (PDA, Oxoid Ltd, Basingstoke, Hants, UK) in a Petri dish (9 cm diameter) and incubated for 7 days at ambient temperature (29±2ºC). The toxigenicity of each strain was determined using 10% coconut agar medium (CAM) (36 g L^{-1} bacto agar and 900 mL distilled water, 100 mL L^{-1} coconut cream extracted from freshly shredded coconut endosperm) and adjusted to pH 7 using 2 N NaOH [18]. The medium was sterilized for 20 min at 120ºC. Each isolate of *A. flavus* was then inoculated and incubated for 5 days at 29±2ºC. The presence of yellow pigment on the reverse side of the medium indicates an aflatoxin-producing strain [19]. Each colony was also observed under long wave (365 nm) UV light. The blue fluorescence colonies indicate a positive result. Medium with no colonies and non-toxigenic strains were used as a control. Plates containing aflatoxin-producing strain were further analyzed by thin layer chromatography (TLC).

2.5 Determination of aflatoxin B_1 production by TLC

A colony of *A. flavus* obtained from a CAM plate that showed a yellow pigment on the reverse side of the medium was mixed with 50 mL of ethanol in a waring blender, and the suspension was extracted for 30 min and filtered using Whatman # 1 filter paper. The filtrate was then placed into a 250 mL separating funnel and extracted two times with 50 mL of n-hexane and rinsed with 50 mL of chloroform. The extract was then dehydrated in a vial bottle and filtered using anhydrous sodium sulfate (Na_2SO_4). Ten mL of the residue (using microsyringe) was spotted onto a TLC plate (MERCK # 1.05554, Silica gel 60, F254) and run for 20 min. The developing solvent used was chloroform: acetone (9:1), the commercial aflatoxin standards used was from Sigma-Aldrich). The plate then was observed under UV light (365 nm).

2.6 Sclerotial production

For the sclerotial production, plates (9 cm diameter) containing PDA agar medium were inoculated with each strain of *A. flavus*. The plates were incubated in dark at 29ºC for 15 days. Sclerotia were harvested according to Novas and Cabral [20]. Sclerotia were obtained by scraping the surface of the medium and running water containing Tween 20 (100 µL L^{-1}) over Whatman filter paper No. 2. Sclerotia obtained were then rinsed with tap water and air dried. *Aspergillus flavus* strains producing numerous sclerotia (average diameter < 400 µm) were assigned to the S (small) type. Those with an average diameter > 400 µm were assigned to the L (large) type.

2.7 Isolation of the *A. flavus* genome

Mycelia for genome extraction were obtained by culturing each strain of *A. flavus* on a PDA plate (Difco Laboratories, Spark, MD, USA) and incubated for 2 days at 29ºC. Approximately 40 mg of fungal mycelia in a clean tube containing 600 µL nuclei lysis solution was ground using a micropestle. DNA was extracted using the Mini Kit (Promega, Madison, WI, USA) according to the manufacturer's procedures. The homogenized mycelia were incubated at 65ºC for 15 min and cooled at room temperature (29-30ºC) for 5 min. About 200 µL of protein precipitation solution was added, vortexed for 5 s, and centrifuged at 13000×*g* for 3 min. The supernatant was placed in a clean tube containing 600 µL isopropanol. The mixture was then centrifuged at 13000×*g* for 2 min. The supernatant was decanted and mixed with 600 µL of 70% ethanol and centrifuged at 13000×*g* for 2 min. The ethanol was aspirated. The air-dried pellet containing DNA was added with 50 µL DNA rehydration solution and vortexed for 5 s. As much as 0.5 µL RNase solution was added and incubated at 37ºC for 15 min. The DNA was rehydrated at 65ºC for 1 h or overnight at 4ºC. The DNA concentration was determined using nanophotometer (IMPLEN, Munich, Germany, serial no. 6042). Subsequently, DNA was examined by agarose gel (1%) electrophoresis (SCIE-PLAS, Ltd, Cambridge, England). The gel was stained with 0.1 mg^{-1} ethidium bromide. Electrophoresis was visualized using Gel Doc (Uvitec, Cambridge, Serial no. 13 200263) under UV light (303 nm).

2.8 Amplification of *A. flavus* genes to determine aflatoxin biosynthesis

Quadruplex-PCR was performed according to Criseo *et al*. [21]. The GeneAmp PCR Labcycler Gradient System (Sensoquest, Germany, Serial no. 1123280105) was used to amplify structural (*nor-1*, *ver-1*, *omt-1*) and regulatory (*aflR*) genes with fragments sizes of 400, 895, 1232, 1032 bp, respectively. The specific primer sets (Integrated DNA Technologies, Singapore) used were: nor-1-F (5′-ACCGCTACGCCGGCACTCTCGGCAC-3′), nor-1-R (5′-GTTGGCCGCCAGCTTCGACACTC CG-3′, ver-1-F (5′-TGTCGGATAATCACCGTTTAGATGGC-3′, ver-1-R (5′-CGAAAAGCGCCACC ATCCACCCCAATG-3′, omt-1-F (5′-GGCCCG GTTCCTTGGCTCCTAAGC-3′, omt-1-R (5′-CGCC CCAGTGAGACCCTTCCT CG-3′, aflR-F (5′-TATCTCCCCCCGGGCATCTCCCGG-3′, and aflR-R (5′-CCGTCAGACAGCCACTGGACACGG-3′). The amplification mixture consisted of 12.5 µL PCR Mix, 2.5 µL each of 10 µm F and R each primer, 2.5 µL nuclease free water, and 5 µL DNA template, in a final reaction volume of 25 µL. The PCR cycling parameters were: initial denaturation at 94ºC for 10 min followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 65ºC for 2 min, extension at 72ºC for 2 min, and a final extension at 72ºC for 7 min. A 10 µL aliquot of PCR products were subjected to electrophoresis (SCIE-PLAS, Ltd, Cambridge, England) on 1.2% agarose gel stained with ethidium bromide (0.1 mg^{-1}) and photographed under UV light (303 nm) (Uvitec, Cambridge, Serial no. 13 200263) adjacent to a 100 bp ladder.

2.9 Statistical analysis

The experiment was conducted using a factorial completely randomized design. The observed data were analyzed using analysis of variance (ANOVA) for statistically significant differences, followed by Duncan's multiple range test at the 5% probability level. Statistical analysis SPSS software version 22 (IBM Inc. New York, USA) was used.

3. Results and Discussion

3.1 Feeds moisture content and fungal population

The compound feeds and the ingredients sold by retailers in traditional markets are typically packaged in 50 kg polypropylene bags and stored in the open air. The moisture content of the feeds was presented in Table 1. It indicated that the moisture content of mashed feeds such as chick starter had the highest moisture content (12.81%) followed by broiler finisher (11.37%) and milled corn (11.02%). While pelleted feed, i.e. pellet starter had the lowest moisture content (7.5%). According to BSN [22], the moisture contents of poultry feeds for layer finisher and broiler were 13 and 14%, respectively. The moisture content of the feeds is related to fungal population (Table 2). Based on feed composition, it indicates that compound feeds (chick starter, broiler finisher, layer finisher, pellet starter) (Figure 1a) had more fungal populations than that of their ingredients (Figure 1b). Our results were in agreement with the findings of Ghaemmaghami *et al*. [23], who reported that mashed feeds had more fungal infection than pelleted feeds. Previous studies by Boroojeni *et al*. [24] and Ghaemmaghami *et al*. [25] reported that the compacted structure of pellet feeds that resulted from pressing and heating during processing reduced microbial contamination. Among the fungi, *Aspergillus* was the most common and found on all feeds. Recent studies by Monson [9] and Ochieng *et al*. [12] showed that *Aspergillus* and other mycotoxigenic fungi were the most commonly found on poultry feeds. Mokubedi *et al*. [26] studied molds and mycotoxins in poultry in South Africa. They reported that *Aspergillus* and *Fusarium* were the most frequent mycotoxin producers and were found in all samples. The results of our study further agreed with the reports of Ibrahim *et al*. [27], who studied the occurrence of mycotoxigenic fungi in poultry feeds at live-bird markets in Nigeria. They found that 78% of 300 feeds samples were infected by *Aspergillus*. A previous study by Nurtjahja *et al*. [28] showed that storage fungi, particularly *Aspergillus* and *Penicillium*, are able to grow at low moisture content. The presence of fungal populations at low moisture content points to the concerning potential for spoilage and mycotoxins contamination of feeds and their ingredients.

Table 1. Moisture content (percentage wet basis) of poultry feeds and their ingredients sold by retailers at traditional markets

Poultry feeds and their ingredients	Traditional markets/moisture content (% w.b)					Average MC*
	TM1	TM2	TM3	TM4	TM5	
Chick starter	12.95	12.73	12.73	12.78	12.85	12.80[a]
Broiler finisher	11.81	11.25	11.0	11.48	11.32	11.37[ab]
Layer finisher	10.05	9.32	9.00	9.30	9.25	9.38[bc]
Pellet starter	8.35	8,15	6.21	7.50	7.35	7.51[d]
Paddy bran	9.27	8.50	7.80	8.75	8.80	8.62[c]
Grain paddy	9.95	9.25	8.85	9.35	9.35	9.35[bc]
Grain corn	11.50	11.25	10.22	11.50	10.55	11.00[ab]
Milled corn	11.35	11.25	10.65	11.00	11.00	11.05[ab]

*Numbers followed by same letters are not significantly different (P<0.05) according to Duncan's multiple range test (DMRT)

3.2 Fungal population

A total of 13 different species of field and storage fungi were isolated and identified (Table 2). The highest number of the fungal isolates in compound feeds were found in chick starter (8 species), followed by layer (7 species) and broiler (5 species). Among feed ingredients, grain corn was the most infected by mold (5 species) (Table 2). A recent study by Shehu *et al*. [29] showed that stored corn was susceptible to contamination by fungi. *Aspergillus chevalieri* was the most frequent population found on chick starter (log 5.49 CFU g^{-1}) and milled corn (log 5.27 CFU g^{-1}), and *A. flavus*, *A. chevalieri*, *A. fumigatus*, and *R. stolonifer* were found in all feeds.

Among strorage fungi, *A. chevalieri* (formerly *Eurotium chevalieri*) had the highest population, particularly in chick starter (log 5.49 CFU g^{-1}) and milled corn (log 5.27^4 CFU g^{-1}). Our results were in agreement with the findings of Greco *et al*. [30], who studied mycotoxigenic fungi on commercial poultry feeds for food-producing animals, and found that 52.2% of the samples were infected by *Eurotium*, a storage fungi. *Aspergillus chevalieri* is capable of rapid growth above about 0.77 water activity (a_w) and slow growth at 0.75 a_w [31]. High moisture content both chick starter and milled corn may promote the growth of the fungus.

3.3 Toxigenicity *A. flavus* strains

A total of fifty seven strains of *A. flavus* were isolated from all feeds. All strains are biseriate at conidial heads and produce large (L) sclerotia (>400 µm in diameter). Sixteen of the *A. flavus* strains were toxigenic (aflatoxin producer) (Table 3). Based on the culture technique in 10% coconut agar medium (CAM), toxigenic *A. flavus* were indicated by the presence of yellow pigment on the reverse side of the medium and showed blue fluorescence under UV light (365 nm) around the colonies. As shown in Table 3, about 28% (16 of 57) of compound feeds and the ingredients were infected by toxigenic *A. flavus*. The most infection occurred on paddy grain (18 strains) followed by grain corn (17 strains). The most infection by toxigenic *A. flavus* occurred on layer finisher (5 strains) followed by grain corn (4 strains).

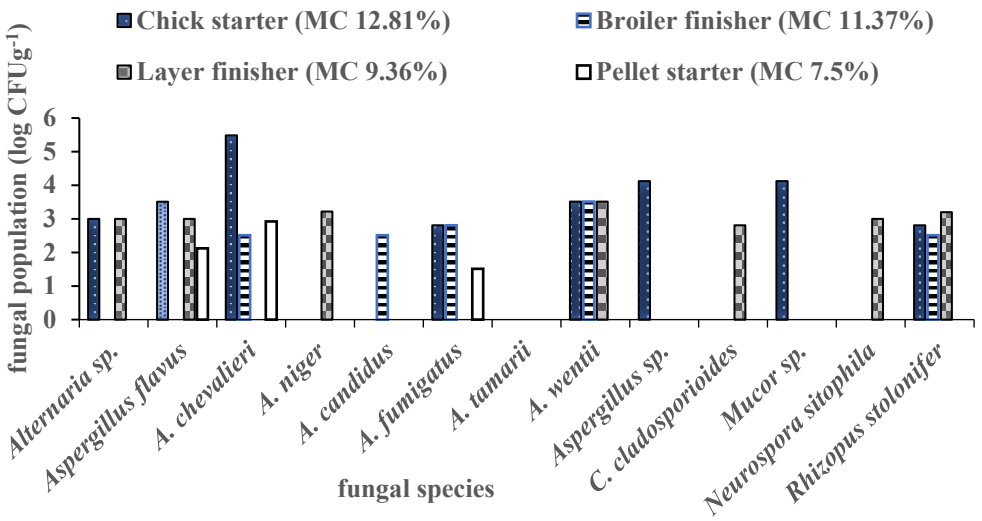
Based on aflatoxin B_1 (AFB_1) analysis, it showed that each toxigenic *A. flavus* produced aflatoxin B_1 (AFB_1) at different levels (Table 4). Strain $Afll_4$ isolated from layer finisher (35.50 ppb) and Afl_{c3} from grain corn (35.50 ppb) were the highest aflatoxin producers. Quadruplex PCR analysis showed similar results to qualitative toxigenicity determination using 10% CAM medium (Figure 2 and Table 4). Compound feeds, particularly layer finisher, were the most infected by toxigenic *A. flavus* which was followed by grain corn and pellet starter.

All *A. flavus* isolated were L (large) tipe sclerotia with > 400 µm in diameter. The present study also agrees with Okoth *et al*. [32] who observed sclerotial formation and aflatoxins production of *A. flavus* isolated from corn kernels. They conclued that L type *A. flavus* produces low aflatoxin level or non-toxigen, whereas S (small) types are toxigenic and produce high aflatoxin levels. The results related to *A. flavus* infection and high aflatoxin production in grain corn and layer finisher obtained in this study indicate that corn as main component of the feed promotes AFB_1 production. The results of this study were in line with Liu *et al*. [33], Nakavuma *et al*. [34] and Ghaemmaghami *et al*. [23] who reported that grains and in particular corn contain nutrients that promote mycelial growth of *A. flavus* and AFB_1 production.

Table 2. The average fungal population (CFU g^{-1}) isolated from compound commercial poultry feeds (chick starter, broiler finisher, layer finisher, pellet starter) and their ingredients (paddy bran, grain paddy, grain and milled corn) collected from retailers at traditional markets

Fungal genera	Fungal population (log CFU g^{-1})							
	Type of commercial commercial compound feeds				Feed ingedients			
	Chick starter (n=25)	Broiler finisher (n=25)	Layer finisher (n=25)	Pellet starter (n=25)	Paddy bran (n=25)	Grain paddy (n=25)	Grain corn (n=25)	Milled corn (n=25)
Alternaria sp.	3.0^{c}	0^{d}	3.0^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Aspergillus flavus	3.51^{bc}	0^{d}	3.0^{c}	4.12^{b}	4.42^{b}	4.77^{ab}	4.80^{a}	0^{d}
A. chevalieri	5.49^{a}	2.31^{cd}	0^{d}	2.92^{c}	3.81^{bc}	0^{d}	3.5^{bc}	5.27^{a}
A. niger	0^{d}	0^{d}	3.22^{c}	0^{d}	3.81^{bc}	3.51^{bc}	0^{d}	0^{d}
A. candidus	0^{d}	2.51^{cd}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
A. fumigatus.	2.81^{c}	2.81^{c}	0^{d}	1.51^{cd}	0^{d}	4.12^{b}	4.63^{ab}	3.51^{bc}
A tamarii	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	3.51^{bc}	0^{d}
A. wentii	3.51^{bc}	3.51^{bc}	3.51^{bc}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Aspergillus sp.	4.12^{b}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Cladosporium cladosporioides	0^{d}	0^{d}	2.81^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Mucor sp.	4.12^{b}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Neurospora sitophila	0^{d}	0^{d}	3.0^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Rhizopus stolonifer	2.81^{c}	2.51^{cd}	3.20^{c}	0^{d}	4.63^{ab}	3.81^{bc}	4.0^{b}	0^{d}

n = number of samples examined; CFU = colony forming unit
Numbers followed by same letters are not significantly different ($P<0.05$) according to Duncan's multiple range test (DMRT)



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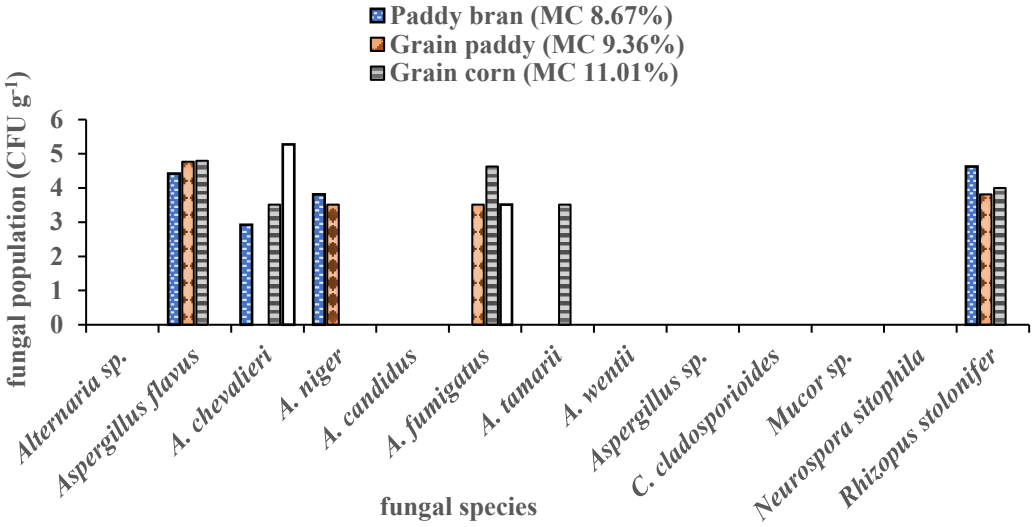

b

Figure 1. Fungal population in relation to moisture content
a. compound poultry feeds, b. feed ingredients, MC = moisture content

Table 3. *Aspergillus flavus* strains isolated from compound commercial poultry feed and the ingredients collected from retailers at five traditional markets (TM)

Poultry feed and feed ingredients	**Toxigenicity of *Aspergillus flavus* strains**	**Traditional markets**					**Total**
		TM1	**TM2**	**TM3**	**TM4**	**TM5**	
Chick starter	toxigenic	1	0	0	0	0	1
	non-toxigenic	0	0	0	0	0	0
Broiler finisher	toxigenic	0	0	0	0	0	0
	non-toxigenic	0	0	0	0	0	0
Layer finisher	toxigenic	2	0	2	0	1	5
	non-toxigenic	1	1	2	0	0	4
Pellet starter	toxigenic	0	1	1	0	1	3
	non-toxigenic	0	0	0	0	1	1
Paddy bran	toxigenic	0	0	1	0	0	1
	non-toxigenic	1	3	3	0	0	7
Paddy grain	toxigenic	0	0	2	0	0	2
	non-toxigenic	1	5	3	4	3	16
Grain corn	toxigenic	1	1	1	0	1	4
	non-toxigenic	4	2	3	4	0	13
Milled corn	toxigenic	0	0	0	0	0	0
	non-toxigenic	0	0	0	0	0	0
Total		11	13	18	8	7	57

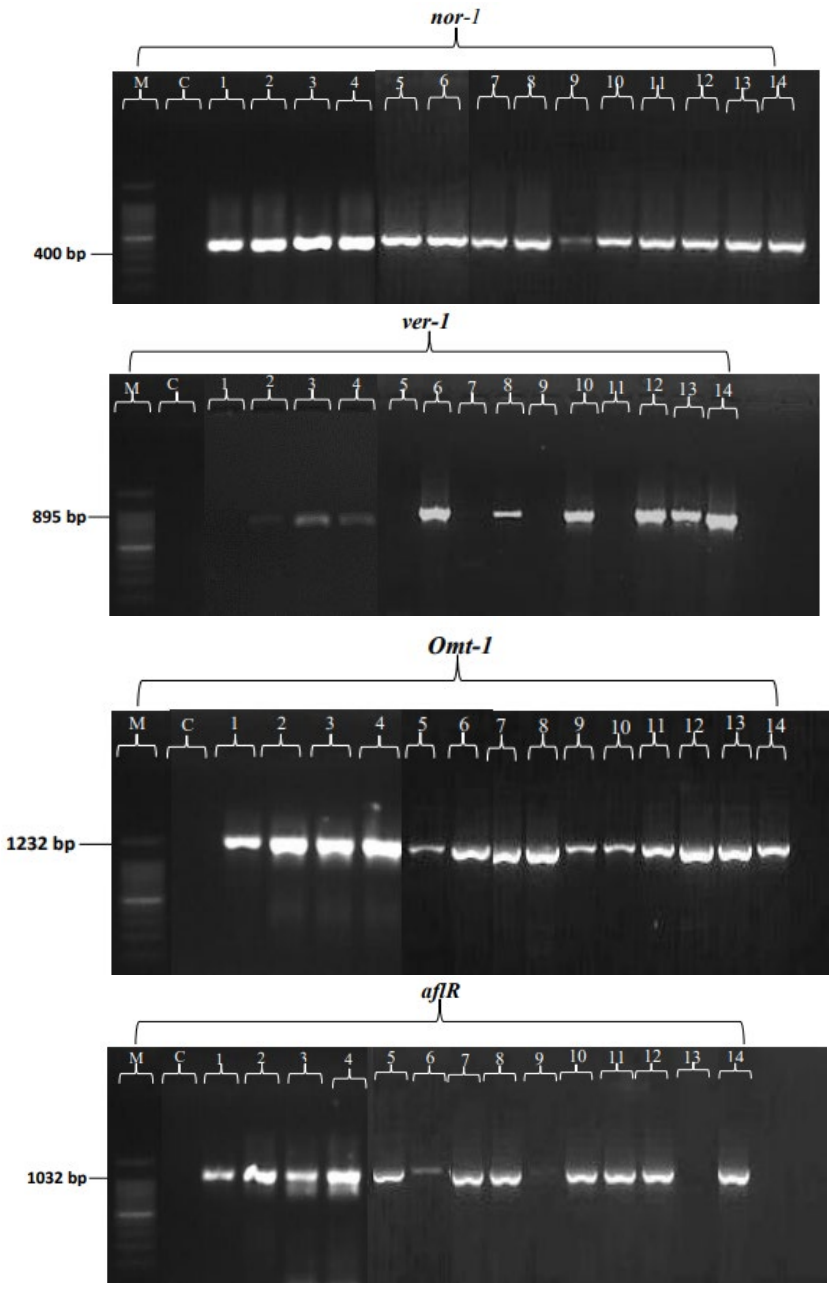

Figure 2. Quadruplex PCR of structural (*nor-1, ver-1, omt-1*) and regulatory (*aflR*) genes of 14 *Aspergillus flavus* strains isolated from compound feeds and their ingredients. M = 100 bp DNA marker, C = control; Afldoc (line 1); $Afll_1$ (line 2), $Afll_2$ (line 3); $Afll_3$ (line 4); $Aflb_1$ (line 5); $Aflb_2$ (line 6); $Aflc_1$ (line 7); $Aflc_2$ (line 8); $Aflp_1$ (line 9); $Aflp_2$ (line 10); $Afll_4$ (line 11); $Afll_5$ (line 12); $Aflpel_1$ (line 13); $Aflpel_2$ (line 14). *nor-1* = 400 bp; *ver-1* = 895 bp; *omt-1* = 1232; *aflR* = 1032 bp

Table 4. Representative toxigenicity and non-toxigenicity of *A. flavus* strains isolated from poultry feeds and the ingredients based on the presence of structural and regulatory genes

***Aspergillus flavus* code**	**Poultry feed**	**Structural and regulatory genes**				**Culture technique in CAM**	**AFB_1 production (ppb) by TLC analysis**
		nor-1	*ver-1*	*Omt-1*	*aflR*		
Afldoc	chick starter	+	-	+	+	negative	0.00
$Afll_1$	layer finisher	+	+	+	+	positive	< 3.01
$Afll_2$	layer finisher	+	+	+	+	positive	12.90
$Afll_3$	layer finisher	+	+	+	+	positive	< 3.01
$Aflb_1$	paddy bran	+	-	+	+	negative	0.00
$Aflb_2$	paddy bran	+	+	+	+	positive	27.50
$Aflc_1$	grain corn	+	-	+	+	negative	0.00
$Aflc_2$	grain corn	+	+	+	+	positive	35.50
$Aflp_1$	paddy grain	+	-	+	-	negative	0.00
$Aflp_2$	paddy grain	+	+	+	+	positive	< 3.01
$Afll_4$	layer finisher	+	+	+	+	positive	35.50
$Afll_5$	layer finisher	+	+	+	+	positive	< 3.01
$Aflpel_1$	pellet starter	+	+	+	-	negative	0.00
$Aflpel_2$	pellet starter	+	+	+	+	positive	30.50

+ = positive for an amplicon; - = negative for an amplicon. Positive and negative in CAM (coconut agar medium) is the toxigenicity of *A. flavus* as indicated by the presence of yellow pigment in the reverse side of the medium

4. Conclusions

Compound commercial poultry feeds and their ingredients that are sold by retailers at traditional markets can potentially be infected with molds and lead to deterioration and contamination by aflatoxin B_1. Therefore, steps to prevent fungal growth on feeds and their ingredients mut be taken and routine aflatoxin analysis of the poultry feeds and ingredients must be performed before consumption by poultry.

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