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Qualities of poultry feeds produced by local small-scale feed mills in Ekiti State, Nigeria: A public health and feed safety study

[1*]David, O.M. and [2]Ogunlade, J.T.

[1]Departments of Microbiology, and [2]Animal Production, Ekiti State University, PMB 5363, Ado-Ekiti (360101) Nigeria

Abstract

Poultry feed has been reported to be of potential risk to the animals consuming it and also to man. In the recent time, there is an increase production of food animals and this consequently informed the rise in the production of feeds. The quality of feeds produced by local small scale feed mills in Ekiti State, Nigeria was investigated using standard microbiological and chemical methods. The total bacterial counts of the ten locally produced poultry feed samples ranged between 7.58 X 10^5 and 6.36 x 10^6 CFU/g. Bacterial count on Bile aesculin agar was lower than counts on both MacConkey and Salmonella-Shigella agars. The quantitative enumeration of fungal load showed that Growers mash (FDM) had the highest amount of fungal propagules followed by Grower mash (AMF). Five genera of fungi were isolated from the feeds comprising *Aspergillus* (3), *Abscidia* (1), *Fusarium* (1), *Penicillium* (3) and *Rhizopus* (1). At least one mycotoxigenic strain was isolated from all the feeds. In this study the values of the bacteria and mould in the feeds were below the maximum allowable loads. Out of the nine fungal species isolated *Aspergillus flavus* had the highest relative density of 70% and isolation frequency of 20.59%. The occurrence of aflatoxin B1 was highest in all the feed screened while aflatoxin G2 was not detected in any of the ten feeds screened. The level of all the heavy metals in the samples was within the tolerable levels for poultry. The microbiological and heavy metal qualities of poultry feeds from small scale poultry mills was within the international standard while aflatoxin levels in some of the feeds were above the maximum allowable levels.

Keywords: Poultry; Feeds; aflatoxin; contaminants; pathogens; heavy metals

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Introduction

Poultry feed is an important component in birds' production all over the world. Their composition includes mixtures of different raw materials from both plant and animal origins (Cox et al., 1983). Feeds and feed ingredients comprise a large variety of products like cereals, soybeans, sunflower supplemented by fat, vitamins, minerals, antioxidants and meat meal (Maciorowski et al., 2006; Cabarkapa et al., 2009).

Feed has been reported to be a major vector for transmission of pathogens to farms and processing plants (Rosa et al., 2006). The quality of poultry feed is of public health importance because it affects the quality of flock and the wholesomeness of a flock's meat and eggs consumed by man. Both pre-harvest and post-harvest biological contaminants can be transmitted via feed ingredients to the mixed feed and finally to live poultry.

Contaminated feed reduces the feed quality and can contribute to food-borne human illnesses through the feed-animal-food-human-chain. Contamination in animal feed is often originating from ingredients from both plant and animal sources, storage and unhygienic manufacturing processes ((Krytenburg et al., 1998; Beg et al., 2006). The quality and quantity of biological contaminants are largely affected by temperature and humidity.

Bacteria, moulds, secondary metabolites of microorganisms and heavy metals are major contaminants of public health importance, found in

Corresponding author: OM David, Department of Microbiology, Ekiti State University, PMB 5363, Ado-Ekiti (360101) Nigeria

animal feeds (Rosa et al., 2006). Poultry feeds are often contaminated with important human food-borne bacterial pathogens (Krytenburg et al., 1998) and the pathogens enter at various stages of feed production (Maciorowski et al., 2006). Most of these bacteria belong to the family *Enterobacteriacea* and are associated with environmental contamination of feed ingredients (Cox et al., 1983). *Salmonella* spp, *Enterococcus* spp. and *Esherichia coli* have been isolated from animal feeds (Sargeant et al., 2000) and multiple antibiotic resistant strains have been reported among these isolates recovered from the feeds (Davis and Wray, 1997; Chapin et al., 2005).

Fungal growth in feed leads to the loss of nutrients and production of mycotoxins that can be harmful to animal and man. Mycotoxins differ in their toxicological effects and are usually found in mixed form (Alkhalaf et al., 2010). On ingestion, mycotoxin may lead to decreased productivity, chronic damage to vital organs and tissues, and immunosuppression (Magan and Aldred, 2007). Mould infections and mycotoxin contamination of cereal grains used in producing animal feeds can occur and increase from the field to all stages of feed production (Okoli et al., 2006; Saleemi et al., 2010). The occurrence of mycotoxigenic fungi is widespread in tropical countries due to favourable environmental conditions (Okoli et al. 2006; Rosa et al., 2006; Krnjaja et al., 2009). The contamination of animal feed and animal meat by mycotoxins poses a serious threat to the health of both animal and human (Hussein and Brasel, 2001).

Heavy metal is one of the commonest forms of environmental pollutants (Lone et al., 2008; Jing et al., 2007). These biologically non-degradable contaminants ultimately end up in plants which are consumed by animals. When accumulated in cereals they get into the livestock as they are fed, and accumulate causing side effects (Jarup, 2003; Igwe et al., 2005).

In recent time, in Ekiti State, Nigeria, there is proliferation of local feed mills to meet up with the demand. Determination of the microbiological quality, heavy metal level and amount aflatoxins in the selected poultry feeds produced by local small scale feed mills form the aims of this study.

Materials and methods

Sample collection

Ten different poultry feeds were collected within Ekiti State, Nigeria. The feed samples collected include growers finisher (1), growers marsh (4), layer mash (4) and starter concentrate (1) from six different manufacturers. The samples were collected in sterile polythene bags and carried to the laboratory for analyses.

Determination of bacterial load

One gram of each of the feed samples was aseptically weighed and homogenized in 9 ml of sterile normal saline. Thereafter, ten-fold serial dilutions were done using the same diluents. One ml of appropriate dilution was aseptically plated, using pour plate technique, onto sterile plates of Plate Count Agar (Oxoid), MacConkey (Oxoid), Salmonella-shigella Agar (Oxoid) and Bile Aesculin Azide Agar (Oxoid) and incubated at 37 ^{o}C for 24 h. After incubation the colonies developed were counted and recourded.

Fungal cultivation and isolation

Modified method of Campos et al. (2008) was used for the quantitative enumeration of fungal propagules in the feeds. Feed samples were serially diluted and plated onto Potato Dextrose Agar and incubated at room temperature for 5 days at the end of which they were examined for fungal growth. Fungal isolates were sub-cultured onto fresh media for another 5 days to obtain pure cultures. Taxonomic identification of the fungal isolates was achieved through macroscopic and microscopic studies following the schemes proposed by Samson and Varga (2007), Samson and van Reenen-Hoekstra (1988) and Pitt and Hocking (1997). The isolation frequency (Fr) and relative density (RD) of the fungal species were calculated according to Gonzalez et al., (1995) as follows:

Fr (%) = (Number of samples with a species or genus / Total number of samples) × 100

RD (%) = (Number of isolates of a species or genus / Total number of fungi samples) × 100

Aflatoxin and heavy metals analyses of feed samples

Twenty five grams of each sample was added into 100 ml of 85 % methanol, the mixture was blended and filtered and the aflatoxins were detected and quantified according to the methods of Vogas and Gastro (2001) and Ghanem and Shuaib (2008) respectively. Heavy metal contents were analyzed in the feed samples using the method of AOAC (2005) and standardized by the method of Techtron (1975). All the mineral values were reported in mg/100g.

Results

A total of ten feed were screened in this study, they were produced by six local poultry manufacturers. The total bacterial count of the feed ranged between 7.58 x 10^5 and 6.36 x 10^6 CFU/g as shown in Table 1. The bio-burden of the screened poultry feeds was shown in Table 2. The count on MacConkey agar ranged from 2.85 x 10^2 and 4.40 x 10^4 CFU/g. Growers mash (FDM) had the highest load followed by Growers mash (TF).

Bacterial count on Bile aesculin agar was lower than counts on both MacConkey and Salmonella shigella agar. It values ranged between 1.12 x10^2 and 8. 30 x 10^2 CFU/g of the feeds. The quantitative enumeration of fungal load load showed that Growers mash (FDM) had the highest amount of fungal propagules followed by Growers mash (AMF).

Table 1: Total microbial load (CFU/g) of poultry feeds produced by small scale feed mills in Ado-Ekiti

Feed Samples	Microbial Load
Layers mash (FKA)	7.58 X 10^5
Layers mash (AMF)	1.80 X 10^6
Growers mash (AMF)	9.64 X 10^5
Layers mash (VTF)	1.22 X 10^6
Growers mash (FDM)	8.84 X 10^5
Starter concentrate (AC)	3.40 x 10^6
Growers mash (FKK)	9.60 x 10^5
Layers mash (TF)	5.12 x 10^6
Growers mash (TF)	6.36 x 10^6
Finisher concentrate (AC)	3.00 x 10^6

Table 3 shows the distribution of fungal genera isolated from poultry feeds screened. Five genera were isolated from the feeds. The genera were *Aspergillus* (3), *Abscidia* (1), *Fusarium* (1), *Penicillium* (3) and *Rhizopus* (1). All samples obtained at least one strain belonging to the main mycotoxigenic genera. The occurrence of mycotoxigenic species in the feeds ranged between 11.11 and 66.67 %. The highest value was recorded in Layers mash (FKA) while the least was observed in Growers mash (FDM). Out of the nine fungal species isolated, *Aspergillus flavus* had the highest relative density of 70 % and isolation frequency of 20.59 % followed by *Aspergillus niger*. *Aspergillus glaucus*, *Penicillium italiculum* and *Penicillium chalybeum* had the least occurrence with each of them having relative density and isolation frequency of 20 % and 5.88 % respectively.

From Table 4, compared to other classes of aflatoxin, the occurrence of aflatoxin B1 was highest in all the feeds screened. Aflatoxin G2 was not detected in any of the ten feeds screened while aflatoxin B2 was detected only in Growers Marsh (FKK) at an amount of 17.72 µg/g. Layers mash (FKA) and Growers mash (FDM) were the two feeds that has aflatoxin G1 at a detectable levels of 7.00 and 6.43 µg/g respectively. None of the four types of aflatoxins tested for was present in Layers mash (TF). Aflatoxin B1 has the highest occurrence in the feeds followed by G1. There was significant difference between aflatoxin B1 and B2 at $P<0.05$.

Copper was detected in five out of the ten poultry feeds screened with value ranging between 0.01 and 0.02mg/kg while Ni was detected in only two samples at 0.001 mg/kg as shown in Table 5. Lead was not detected in any of the feeds whereas the level of Zn contamination in the feeds was relatively high. The metal (Zn) was detected in all the feed samples with a range between 0.38 and 0.52 µg/g. Layers mash (VTF) recorded the highest level of Zn followed by Layers mash (FKA). Manganese (Mn) was not detected in three out of the ten feeds screened. The amount of Fe detected in the feeds ranged between 0.001 and 0.03 µg/g.

Discussion

Nutrients in animal feeds make microorganisms thrive when other environmental condition is favourable. The total bacterial count of the feed ranged between 7.58 x 10^5 and 6.36 x 10^6 CFU/g. The quality and quantity of microbes in animal feeds may be due to their natural occurrence, contamination of feed ingredients or unhygienic processing methods (Cabarkapa et al., 2009). The microbial load of feed screened in this study was lower than earlier report of Okonko et al. (2010), that reported the total bacteria count range between 1.03 x 10^8 and 1.232 x 10^9 CFU/g. The count on MacConkey agar ranged from 2.85 x 10^2 and 4.40 x 10^4 CFU/g. The level of contamination of bacteria in this study is an indication of poor hygiene and lack of good manufacturing environment.

The quantitative enumeration of fungal load showed that Growers mash (AMF) had the highest amount of fungal loads followed by Layers mash (FKA). Five genera were isolated from the feeds which

Table 2: Microbial quality of different poultry feeds produced by small scale feed mills in Ado-Ekiti (CFU/g)

Feed sample	Count on MacConkey agar	Count on Salmonella Shigella Agar	Count on bile esculin agar	Fungal Load	Yeast Count
Layers mash (FKA)	8.83 x 10^3	9.20 x10^2	1.12 x10^2	2.4 x10^4	1.60 x10^3
Layers mash (AMF)	6.40 x 10^2	1.74 x10^3	6.1 x10^2	1.44 x10^3	1.64 x10^3
Growers mash (AMF)	5.84 x 10^3	9.40 x10^2	4.7 x10^2	4.8 x10^4	6.80 x10^2
Layers mash (VTF)	9.92 x 10^2	9.83 x10^2	5.2 x10^2	4.8 x10^3	8.80 x10^2
Growers mash (FDM)	2.85 x 10^2	3.62 x10^2	6.5 x10^2	5.2 x10^5	5.60 x10^2
Growers mash (FKK)	6.90 x 10^3	1.24 x 10^2	5.00 x 10^2	4.80 x 10^3	1.28 x 10^3
Starter concentrate (AC)	4.40 x 10^4	1.40 x 10^1	8. 00 x 10^2	2.00 x 10^3	7.20 x 10^2
Finisher concentrate (AC)	3.50 x 10^4	5.80 x 10^2	1. 00 x 10^2	5.20 x 10^3	1.32 x 10^3
Layers mash (TF)	1.52 x 10^4	8.80 x 10^2	2. 00 x 10^2	1.60 x 10^3	7.20 x 10^2
Growers mash (TF)	3.56 x 10^4	1.68 x 10^3	2. 00 x 10^2	5.60 x 10^3	2.40 x 10^3

Table 3: Fungi isolated from different poultry feeds produced by small scale feed mills in Ado-Ekiti

Poultry feeds	Fungi isolates									Mycotoxigenic genera (%)
	AF	AN	AG	AS	FM	PI	PC	PE	RS	
Layers mash (FKA)	+	+	+	-	+	+	+	-	+	66.67
Layers mash (AMF)	+	-	+	-	-	-	-	+	-	33.33
Growers mash (AMF)	+	+	-	-	+	-	-	-	+	33.33
Layers mash (VTF)	-	+	-	+	-	-	-	+	-	22.22
Growers mash (FDM)	-	-	-	-	+	-	-	-	-	11.11
Starter concentrate (AC)	+	+	-	+	-	-	-	-	-	22.22
Growers mash (FKK)	+	+	-	+	-	-	+	-	-	33.33
Layers mash (TF)	-	-	-	-	-	+	-	+	-	22.22
Growers mash (TF)	+	+	-	-	+	-	-	-	-	33.33
Finisher concentrate (AC)	+	-	-	+	+	-	-	-	+	22.22
Relative density (%)	70	60	20	40	50	20	20	30	30	
Isolation frequency (%)	20.59	17.65	5.88	11.76	14.71	5.88	5.88	8.82	8.82	

Key: + Present; - Absent; AF: *Aspergillus flavus;* AN: *Aspergillus niger;* AG: *Aspergillus glaucus;* AS: *Abscidia* sp; FM: *Fusarium moniliforme;* PI: *Penicillium italiculum;* PC: *Penicillium chalybeum*; PE: *Penicillium expansum;* RS: *Rhizopus* sp

Table 4: Aflatoxin contents of poultry feeds produced by small scale feed mills in Ado-Ekiti (µg/Kg)

Samples	Aflatoxins					
	B1	B2	G1	G2	B (Sum)	G (Sum)
Layers mash (FKA)	27.18	0.00	7.00	0.00	27.18	7.00
Layers mash (AMF)	12.32	0.00	0.00	0.00	12.32	0.00
Growers mash (AMF)	4.67	0.00	0.00	0.00	4.67	0.00
Layers mash (VTF)	18.40	0.00	0.00	0.00	18.40	0.00
Growers mash (FDM)	29.66	0.00	6.43	0.00	29.66	6.43
Starter concentrate (AC)	7.87	0.00	0.00	0.00	7.87	0.00
Growers Marsh (FKK)	114.08	17.72	0.00	0.00	131.80	0.00
Layers mash (TF)	0.00	0.00	0.00	0.00	0.00	0.00
Growers mash (TF)	5.95	0.00	0.00	0.00	5.95	0.00
Finisher concentrate (AC)	13.07	0.00	0.00	0.00	13.07	0.00
Total	233.2	17.72	13.43	0.0	250.92	13.43
Mean	23.32	1.77	1.34	0.0	25.09	1.34

Table 5: Heavy metal content poultry feeds produced by small scale feed mills in Ado-Ekiti

Feed Samples	Heavy metals					
	Cu	Fe	Mn	Ni	Pb	Zn
Layers mash (FKA)	0.02	0.02	0.01	ND	ND	0.48
Layers mash (AMF)	0.02	0.02	0.01	0.001	ND	0.42
Growers mash (AMF)	ND	0.01	0.001	ND	ND	0.39
Layers mash (VTF)	0.02	0.03	0.01	ND	ND	0.52
Growers mash (FDM)	ND	0.001	0.001	ND	ND	0.38
Starter concentrate (AC)	ND	0.02	0.01	ND	ND	0.38
Growers mash (FKK)	0.01	0.02	ND	ND	ND	0.41
Layers mash (TF)	ND	0.01	0.001	ND	ND	0.44
Growers mash (TF)	0.01	0.02	ND	0.001	ND	0.41
Finisher concentrate (AC)	ND	0.02	ND	ND	ND	0.38
NRC Maximum tolerable level	0.30	1.00	2.00	0.30	0.03	1.00

ND = not detected

include *Aspergillus* (3), *Abscidia* (1), *Fusarium* (1), *Penicillium* (3) and *Rhizopus* (1). These fungi, apart from producing mycotoxin deplete the nutrients and spoil feeds (Pitt and Hocking, 1997). The values of the bacteria and mould in this study were below the maximum allowable load of 1.00×10^7 and 5.0×10^3 CFU/g respectively as reported by Cabarkapa et al. (2009). All samples screened had at least one species belonging to the mycotoxigenic genera. The occurrence of mycotoxigenic species in the feeds ranged between 11.11 and 66.67 %. Out of the nine fungal species isolated *Aspergillus flavus* had the highest relative density of 70 % and isolation frequency of 20.59% followed by *Aspergillus niger*. This trend was also observed by Fraga et al. (2007) and Pereyra et al. (2011) who reported high percentages of *A. flavus* in poultry feeds.

Despite the fact that most of the feed samples had fungal load that falls below the proposed limits (1×10^4 CFU/g) of Good Manufacturing Practices (GMP, 2008), mycotoxins were still detected in them. Mycotoxins pose a serious threat to the health of the poultry and

lead to serious economic losses. From Table 4, the occurrence of aflatoxin B1 was highest in all the feed screened. This findings are in agreement with Alkhalaf et al. (2010) who reported that aflatoxin B1 had a greatest occurrence and wide distributed in feed stuff than other classes. The level of aflatoxin detected in the samples was much higher than the international maximum allowable level of 5 ng/g (Van Egmond, 1995).

The level of heavy metals in the poultry feeds sampled differs. The difference could be due to the concentrations in the components especially those of plants origin as suggested by Ona et al. (2006) and/or environmental pollutions (Aluko et al., 2003). The level of all the heavy metals tested was lower than the maximum tolerable levels for poultry (NRC, 1980) and lower than the values reported by Alexieva et al. (2007).

The microbiological quality of poultry feeds from small scale poultry feed mills industry was within the international standard; however, the presence of aflatoxin in some of the feed exceeds the maximum allowable level. The microbial contaminations of the feeds should be reduced to the barest minimal. Since the climatic condition of the study area favours the growth of mycotoxicogenic fungi, production and storage must be appropriate bearing in mind the risks contaminations pose on the safety of the animals and public health.

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