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Assessment of microbial profile of selected proprietary broiler chicken feeds sold in Abeokuta, South-West, Nigeria

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***Target audience:** Nutritionists, Feed producers, Farmers, Distributors / Sales outlets, Extension agents*

Abstract

The growth in poultry industry has resulted in heavy dependence on finished feeds supplied by feed millers, the quality of which determines the profit margin of the farmers. Presence of pathogenic and toxigenic microbes, however, tends to deplete the nutritive value of poultry diets. This study, therefore, focused on assessing the incidence and concentration of micro-organisms in five selected brands of proprietary broiler feeds sold in Abeokuta, South-West, Nigeria. None of the feed samples investigated was devoid of pathogenic and toxigenic pathogens of public health concern. Bacterial and fungal isolates recovered include Escherichia coli, Staphylococcus albus, Bacillus subtilis, Micrococcus spp, Pseudomonas spp, Aspergillus fumigatus, A. flavus, A. niger, Fusarium spp, Mucor spp, and Penicillium notatum. Total microbial counts were significantly different ($P<0.05$) among the selected feed brands for both Broiler Starter and Finisher diets. Occurrence and high level of pathogenic microbes observed in this study indicated that quality of finished feeds offered to broilers chickens could predispose them to health hazard with resultant economic loss. Strategies to improve the shelf life of commercial finished feeds, such as inclusion of toxin binders, are hereby advocated.

Keywords: *Broiler, Feeds, Quality control, Bacteria, Fungi, Mycotoxins*

Description of Problem

Majority of poultry farmers in Nigeria depend largely on finished feeds produced by feed millers. Hence, the quality of feed supplied to the farmers determines the profitability of their farming enterprise because feed quality has been identified as a major factor that has positive correlation with adequate nutrition and high productive performance of animals (1). The rapid growth in poultry industry with consequent increase in demand for feed as well as seasonal availability of conventional feed ingredients used in ration formulation warrants their storage to ensure availability during off-season. However, improper storage can result in feed value deterioration (2).

Several factors that affect loss of feed value have been identified and categorised as physical, biological and chemical (2, 3). Deterioration of compounded feeds due to

microbial contamination could be as a result of various ingredients that are blended together, inadequate biosecurity measures during production, packaging at the mill, and poor storage condition before utilization. The extent of damage done to the feed and birds depends largely on the types and population of microbes present including their degree of virulence, and duration of exposure to the animal (3).

Presence of toxigenic pathogens usually results in toxins production and ingestion of such mycotoxin-contaminated feeds have been reported to cause feed refusal with resultant reduced growth efficiency, reproductive disorders, increased susceptibility to diseases, reduced vaccination efficacy, as well as induced pathologic damage to the liver (3, 4). Occurrence of micro-organisms in some selected poultry feedstuffs and finished feeds has been previously investigated (1, 5, 6, 7). Some of the micro-organisms (Bacteria and Fungi) isolated were found to be of serious concern to poultry health. Due to public health implication, this study was, therefore carried out to determine the presence and assess the level of pathogenic micro-organisms in selected proprietary broiler feeds sold in Abeokuta, South-West, Nigeria

Materials and Methods

Experimental site and Sample collection

The study was carried out at Animal Nutrition Laboratory of College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Five brands of broiler starter and finisher diets commonly patronized were used in this study. They were coded as A, B, C, D, and E. Details of the feeds were not specified in this report for obvious reasons. The bulk representatives were drawn from 2 standard commercial feed bags (25 kg each) as described by (5). The samples were designated as AS, BS, CS, DS, and ES as well as AF, BF, CF, DF, and EF indicating Broiler Starter and Finisher diets A, B, C, D, and E, respectively. All samples were homogenized and divided to obtain a 1kg of working sample for analysis. They were then taken to the laboratory for microbiological analysis.

Table 1: Biochemical test for Bacteria identification

Gram	Motility	Glucose	Lactose	Mannitol	Maltose	Indole	Methyl Red	Vogesproskauer	Citrate	H_2S	Sucrose	Urea	Oxidase	Coagulase	Catalase	ISOLATE
-	+	+	+	+	+	+	+	-	-	-	NA	-	-	NA	+	*Escherichia coli*
-	+	+	-	+	-	-	+	+	+	+	+	+	+	NA	+	*Pseudomonas spp*
+	+	+	+	+	+	NA	-	+	NA	NA	+	-	-	NA	+	*Bacillus subtilis*
+	-	+	+	+	+	NA	+	-	+	+	+	+	-	-	+	*Staphylococcus aureus*
+	-	+	+	+	+	NA	+	-	+	+	+	+	+	-	+	*Micrococcus spp*

+ = Positive, - = Negative, NA = Not applicable

Isolation, Enumeration and Identifi-cation of Bacteria

The dilute plate technique was used for enumeration and isolation of bacteria as described by (5). One gram of each sample was measured and homogenized with 9mls

of sterile water to make 1/10 dilution under aseptic condition prior to serial dilution. One ml of aliquot was diluted with 9mls of sterile water in different test tubes to give 1:9 dilution. From this, ten-fold serial dilutions were made up to 10^{-4}. One ml of the sample was plated on nutrient agar for bacteria. All the plates after inoculation were incubated at 37^0C for 24 hrs and the number of colonies formed after the incubation period were counted, recorded and expressed as standard numbers of colony forming unit per millilitre (cfu/ml). The discrete colonies that grew were sub-cultured on fresh media to obtain pure cultures which were maintained at 4^oC and used for further characterization and identification. Each bacterial colony was identified by their gram staining property and was biochemically characterized using Sugar fermentation, coagulase, indole, citrate, oxidase, hydrogen sulphide production and urea tests.

Table 2: Biochemical test for *Saccharomyces cerevisiae* identification

Tests	Results
Utilization of carbon:	
Glucose	+
Fructose	+
Sucrose	+
Galactose	+/-
Raffinose	+
Lactose	-
Starch	+
Utilization of nitrogen:	
Peptone	+
Asparagine	+
Ammonium sulphate	+
Nitrate	-
Acid production	+
Urea hydrolysis	-
Ester production	+

+ = Positive, - = Negative

Isolation, Enumeration and Identifi-cation of Fungi

The dilute plate technique was used for enumeration and isolation of fungi (8). Ten grams of each feed sample was mixed with 90 ml of 0.1% peptone and shaken on a horizontal shaker for 20 minutes. Then, 0.1 ml of this dilution was inoculated on each of three different media: Dichloran Rose Bengal Chloramphenicol Agar (DRBC) to enumerate total culturable fungi, Dichloran 18% Glycerol Agar (DG18) to enumerate xerophilic fungi, and Dichloran Chloramphenicol Peptone Agar (DCPA) for selective isolation of *Fusarium* species. Plates were incubated at 25°C for 7 days. The DCPA plates were incubated under 12 hours of light: 12 hours of darkness. For counting, plates containing 10–100 colonies were used and the results were expressed as colony-forming units per gram of sample (CFU/g). Individual CFU/g counts for each colony type, considered to be different, were recorded. Representative colonies of each type were transferred for sub-culturing onto plates with Malt Extract Agar (MEA) or Water Agar (WA), for molds suspected to belong to *Fusarium* genera. The isolates were identified based on their morpholo-gical and microscopic features.

Aspergillus and Penicillium isolates were preserved on agar slants of Malt Extract Agar (MEA) and Fusarium isolates on Potato Dextrose Agar (PDA) at 4°C and cryopreserved in 18% glycerol at “20°C. Meanwhile, following serial dilutions and incubation at 37^oC for 3 days, colonies of yeasts (*Saccharomyces cerevisiae*) were subjected to biochemical tests such as Carbon source fermentation, Nitrogen source utilization, acid production from fermented sugars, Ester production, and Urea hydrolysis as previously described (9). The fungal isolates were counted, recorded and expressed as standard numbers of colony forming unit per milliliter (cfu/ml)

Table 3: Micro-organisms isolated from the Broiler Starter and Finisher Feeds

Microbial isolates	Starter Feeds					Finisher Feeds				
	AS	BS	CS	DS	ES	AF	BF	CF	DF	EF
Bcateria										
Escherichia coli	-	-	+	-	+	+	+	+	-	+
Staphylococcus aureus	-	+	+	-	-	+	-	+	+	-
Pseudomonas spp	-	-	-	+	+	-	-	-	-	-
Micrococcus spp	-	+	-	+	-	-	-	-	-	+
Bacillus subtilis	+	+	+	-	+	+	+	+	+	+
Fungi										
Aspergillus fumigatus	+	-	+	+	+	-	-	-	+	-
A. flavus	-	-	+	-	-	-	-	+	-	-
A. niger	-	+	-	-	+	-	-	-	+	-
Fusarium spp	+	+	+	-	-	-	-	+	-	-
Mucorspp	-	-	+	+	-	-	-	-	-	-
Penicillium notatum	-	-	-	-	-	-	-	-	-	-
Sacchromyces cerevisiae	+	+	+	+	-	-	+	+	+	+

+ = Present, - = Absent

Table 4: Total microbial counts of Broiler Starter feeds

Microbial counts	Starter Feeds					Overall Mean ± SD	SEM	P-value
	AS	BS	CS	DS	ES			
TBC (x10^{4}cfu/ml)	2.52^c	1.73^d	6.10^b	7.51^a	2.34^c	4.02 ± 2.41	0.070	0.0142
TFC (x10^{6}cfu/ml)	0.21^b	0.20^b	0.32^a	0.31^a	0.30^a	0.26 ± 0.06	0.017	0.0026
TScC (x10^{6}cfu/ml)	0.64^d	0.72^c	3.21^a	2.40^b	0.43^e	1.46 ± 1.17	0.030	0.0038

TBC = Total Bacteria Counts, TFC = Total Fungi Counts, TScC = Total *Saccharomyces cerevisiae* Counts, cfu/ml = coliform forming unit per millilitre.

^{a,b,c,d,e} Means on the same rows having different superscripts are significantly (P<0.05) different.

Statistical analysis

The total microbial counts of feed types from different feed manufac-turers were compared by One-way ANOVA and statistically separated by Duncan's Multiple Range Test at 5% level of significance using General Linear Model procedure of Statistical Analysis System (10).

Table 5: Total microbial counts of Broiler Finisher feeds

Microbial counts	Finisher Feeds					Overall Mean ± SD	SEM	P-value
	AF	BF	CF	DF	EF			
TBC (x10^{4}cfu/ml)	8.04^a	6.02^b	8.31^a	1.45^c	1.24^c	4.98 ± 3.25	0.292	0.0024
TFC (x10^{6}cfu/ml)	0.31^{ab}	0.32^{ab}	0.45^a	0.43^a	0.26^b	0.32 ± 0.03	0.035	0.0047
TScC (x10^{6}cfu/ml)	4.03^a	3.64^b	4.82^a	0.31^d	3.12^c	3.16 ± 1.60	0.139	0.0205

TBC = Total Bacteria Counts, TFC = Total Fungi Counts, TScC = Total *Saccharomyces cerevisiae* Counts, cfu/ml = coliform forming unit per millilitre.

^{a,b,c,d} Means on the same rows having different superscripts are significantly (P<0.05) different.

Results and Discussion

The results of biochemical test for identification of bacteria and *Saccharomyces cerevisiae* present in the feed samples are presented in Tables 1 and 2, respectively. The isolated bacteria were *Escherichia coli, Staphylococcus albus, Bacillus subtilis, Micrococcus spp,* and *Pseudomonas spp.* Table 3 shows the microbial isolates identified from the feed samples. Incidence of micro-organisms was observed in all the broiler diets examined. A total of 13 micro-organisms (5 bacteria spp and 6 fungi spp) were isolated. At least, one bacteria spp was found in each sample. However, no fungi spp was isolated in feed AF.

Most of the bacterial isolates in these feeds are of poultry health concern. Generally, *E. coli* infections are called colibacillosis (7) which has also been implicated for such diseases as omphalitis, aerosacculitis, salpingitis, polyserositis, panophthalmitis, septicaemia, and other mainly extra intestinal diseases in chickens (11). *Staphylococcus aureus* is known to cause food poisoning and has been implicated in osteomyelitis, arthritis, synovitis, septicaemia, and cellulitis (12).

Bacillus subtilis, however, is a potent probiotic whose effectiveness as broad microbial activity against several strains of Campylobacter and Clostridium *spp* has been reported. (13) reported reduction in intestinal microbial counts with resultant improved gut health in broiler chickens. In a related study, (14) also found out that a single dose of *B. subtilis* reduce bacteria load (*Salmonella enterica serovar Enteriditis*) in broiler droppings. This will invariably improve food safety by preventing cross-contamination during processing. In the same vein, recombinant *B. subtilis* endospores have also been used to confer immunity against tetanus, anthrax, *Clostridium perfringes* alpha-toxin, and necrotic enteritis in poultry (15). Therefore, dietary inclusion of *Bacillus subtilis* as feed additive should be encouraged because it would help to reduce pathogenic microbial load in the chicken's gut through competitive exclusion mechanism (13, 14).

Fungi from the genera *Aspergillus, Fusarium, Mucor* and *Penicillium* were identified with *Aspergillus* having the highest level of occurrence. This agrees with the report of (1), (5), (6) and (7) who reported *Aspergillus* spp as the most prevalent fungi present both in the feed ingredients and compounded feeds. Higher number of fungal isolates unlike bacterial isolates in the feed samples, which is also in consonance with the report of (5), could be attributed to the fact that moulds has the capacity to survive and grow under relatively low moisture content hence its ability to remain inactive for a long time until conditions become favourable (16).

Some of the isolated fungi are highly pathogenic, for example, *Aspergillus* has been implicated for Aspergillosis through its metabolic activity. Some fungi are known to produce toxins, which have deleterious effects on animals. For example, Fusarium toxins possess a pronounced caustic effect, resulting in necroses and crusts of the buccal mucosa in poultry birds. This was, detected in some brands of feeds used. However, *Saccharomyces cerevisiae* is a potential probiotic, which survives transit through the GIT and produces inhibitory substances that prevent the growth of pathogenic bacteria. The mechanism involved is that its outer membrane is rich in mannose which allowing pathogens such as *Salmonella typhimurium*, *E. coli*

and others that contain mannose-binding fibres in their structure to bind to this mannose-rich membrane, thereby preventing such harmful bacteria from adhering to intestinal cells (9). Furthermore, *S. cerevisiae* releases polyamines, which help in repairing mucous membranes. These polyamines increase the activity of short chain fatty acids (SCFA) and disaccharide enzymes (lactase, maltase, sucrase). Polyamines also stimulate the repair of intestinal cells and the growth of colonic mucosa (9).

Table 4 shows the total bacteria counts (TBC), total fungi counts (TFC), and total *Saccharomyces cerevisiae* counts (TScC) for starter diets. TBC was within the range of $1.73x10^4$ – $7.51x10^4$cfu/ml with feeds DS and BS having the highest and lowest values, respectively while TScC ranged from $0.43x10^6$ to $3.21x10^6$cfu/ml with highest and lowest values recorded for feeds CS and ES, respectively. However, the values obtained for TFC were in close range ($0.20 x10^6$ – $0.32 x10^6$cfu/ml).

The data in Table 5 represent the total microbial counts for Broiler finisher diets. TBC ranged from $1.24x10^4$ to $8.31x10^4$cfu/ml with feeds CF and EF having the highest and lowest values, respectively while TScC values obtained ranged between $0.31x10^6$ and $4.82x10^6$cfu/ml with highest values also recorded in CF while DF had the lowest values. Though significantly different among feed types, TFC had similar values ($0.26 x10^6$ – $0.45 x10^6$cfu/ml) when compared with Broiler Starter diets.

The variation observed in the total microbial counts among the feeds from different manufacturers could be attributed to the variation in the gross composition of the feed. This could also be a pointer to the level of biosecurity measures employed during production process, packaging, transportation and storage. The common feeds available within Abeokuta metropolis are those from multi-national companies that take the issue of biosecurity in production process serious. Hence, cross-contamination might have occurred during transportation and/or storage condition when it has been delivered to the feed distributors.

Results obtained in this study is higher than that of (5) who reported TBC values of $2.50x10^4$ and $1.46x10^4$cfu/ml for Broiler Starter and Finisher diets, respectively, and TFC values of $3.60x10^2$ and $2.3x10^2$cfu/ml for Broiler Starter and Finisher diets, respectively. Meanwhile, mean bacterial and total fungal counts recorded in this study were lower than those reported by (6). This could be due to variation in the feed brands investigated. The authors were silent on the brands of poultry feeds examined, whether they were broiler starter, broiler finisher or chicks mash. Therefore, appropriate quality control measures should be taken during feed production process to minimize the microbial loads in broiler feeds to the lowest concentration possible. In addition, inclusion of toxin binders (such as activated charcoal, Zerotox®, Tox-O®) into compounded feeds is hereby suggested in order to reduce the production of mycotoxins and increase the storage/shelf life, particularly, for mash feeds.

From this study, it was observed that the total microbial counts were significantly ($P<0.05$) higher in finisher diets than in starter diets. This could be as a result of differences in the composition of the diets. It is believed that higher amounts of such ingredients as maize, groundnut cake (GNC), soybean meal (SBM), Fish meal (either local or

imported), and probably, palm kernel cake (PKC) would be used in formulating finisher diets compared to starter diet. (1) had earlier reported that these feedstuffs had high number of isolates due to their nature and nutritive value. The nutritive value of commercial feeds may, therefore, be a pointer, directly or indirectly, to the quality and quantity of microbial load. This implies that broiler finisher diets are more prone to feed value deterioration than starter diets due to the supposedly higher rate of microbial activity during storage. This could, invariably, pose a lot of threat on the animal's performance when fed with such a diet.

Conclusions and Applications

1. Bacterial and fungal isolates recovered from broiler finished feeds include *Escherichia coli, Staphylococcus albus, Bacillus subtilis, Micrococcus spp, Pseudomonas spp, Aspergillus fumigatus, A. flavus, A. niger, Fusarium spp, Mucor spp,* and *Penicillium notatum.*
2. Occurrence and high level of pathogenic microbes observed in this study indicated that quality of finished feeds offered to broilers could predispose them to health hazards with resultant economic losses.
3. Regular investigation of microbial profile of most vulnerable feedstuffs and that of finished feeds should be encouraged in order to ensure provision of safe feeds to poultry birds. Proper training should be organized for personnel at the feed mills on the issue of biosecurity and feed quality control.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Okoli, I.C., Ogbuewu, P.I., Uchegbu, M.C., Opara, M.N., Okorie, J.O., Omede, A.A., Okoli, G.C. and Ibekwe, V.I. (2007). Assessment of the Mycoflora of Poultry Feed Raw Materials in a Humid Tropical Environment. *Journal of American Science,* 3(1): 5-9
2. Uppal, D.S., Ilyas, S.M. and Sikka, S.S. (2004). Quality and safety of Animal Feeds in India. Available online at: http://www.unapcaem.org/Activities%20Files/A16/Animal%20Feed%20&%20Quality.pdf**.** Date assessed: October 16, 2014. 30p
3. Reddy, D.V. (2001). Principles of Animal Nutrition and Feed Technology. Oxford & IBH Publishing Company Pvt, Ltd. New Delhi, India. 2nd edition. 431p
4. Huwig, A., Freimund, S., Kappeli, O. and Dutler, H. (2001). Mycotoxin detoxification of animal feeds by different adsorbents. *Toxicology Letters,* 122: 179-188
5. Arotupin, D.J., Kayode, R.M.O. and Awojobi, K.O. (2007). Microbiological and Physicochemical Qualities of Selected Commercial Poultry Feeds in Akure, Nigeria. *Journal of Biological Sciences,* 7(6): 981-984
6. Uwaezuoke, J.C. and Ogbulie, J.N. (2008). Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. *Journal of Applied Sciences and Environmental Management,* 12(1):

113-117

7. Aliyu, R.M., Egwu, E.O., Abubakar, M.B., Adamu, A.Y., Salihu, M.D., Dabai, A.I. and Tambuwal, F.M. (2012). Bacteriological quality of commercially prepared and self-compounded poultry feeds in Sokoto metropolis, Sokoto, Nigeria. *International Journal of Applied Biology and Pharmaceutical Technology,* 3(1): 345-350
8. Grevo, M.V., Franshi, M.L., Golba, S.L.R., Pardo, A.G. and Pose, G.N. (2014). Mycotoxins and Mycotoxigenic Fungi in Poultry Feed for Food-Producing Animals. *The Scientific World Journal,* 2014: 1-9. Article ID: 968215. Doi: 10.1155/2014/968215
9. Zubaidy, Z.M. and Khidhr, K.O. (2014). Isolation and identification of *Saccharomes cerevisiae var boulardii* and its uses as a probiotic (in vitro). *Rafidain Journal of Science*, 25(1): 1-11
10. SAS, (1999). Statistical Analysis System User Guide: Statistics. Cary, NC, USA
11. Dho-Moulin, M. and Fairbrother, J.M. (1999). Avian Pathogenic Escherichia coli (APEC). *Veterinary Research,* 30(2-3): 299-316
12. Hazariwala, H., Sanders, Q., Hudson, C.R., Hofacre, C., Thayer, S.G. and Maurer, J.J. (2002). Distribution of staphylococcal enterotoxin genes among staphylococcus aureus isolates from poultry and humans with invasive staphylococcal disease. *Avian Diseases,* 46(1): 132-136 [PubMed]
13. Jayaraman, S., Thangavel, G., Kurian, H., Mani, R., Mukkari, R. and Chirakkal, H. (2013). Bacillus subtilis PB6 improves intestinal health of Broiler Chickens challenged with Clostridium perfringes-induced necrotic enteritis. *Poultry Science,* 92(2): 370-374
14 Knap, I., Kehlet, A.B., Bennedseri, M., Mathis, G.F., Hofacre, C.L., Lumpkins, B.S., Jensen, M.M., Raun, M. and Lay, A. (2011). *Bacillus subtilis* (DSM17299) significantly reduces Salmonella in Broilers. *Poultry Science,* 90(8): 1690-1694
15. Hoang, T.H., Hong, H.A., Clark, G.C., Titball, R.W. and Cutting, S.M. (2008). Recombinant *Bacillus subtilis* expressing the *Clostridium perfringes* alpha toxoid is a candidate orally delivered vaccine against Necrotic Enteritis. *Infection and Immunity,* 76(11): 5251-5262
16. Maciorowski, K.G., Herrera, P., Jones, F.T., Pillae, S.D. and Ricke, S.C. (2007). Effects on Poultry and Livestock of feed contamination with bacteria and fungi. *Animal Feed Science and Technology,* 133(1):109-136.