

**BACTERIOLOGICAL QUALITY OF COMMERCIALY PREPARED AND SELF
COMPOUNDED POULTRY FEEDS IN SOKOTO METROPOLIS, SOKOTO, NIGERIA**

Aliyu¹, R. M^{*}, Egwu¹, E. O., Abubakar¹, M. B., Adamu², A. Y., Salihu³, M. D., Dabai⁴, A. I. and
¹Tambuwal, F. M.

¹Department of Veterinary Microbiology, Faculty of Veterinary Medicine

²Department of Veterinary Medicine, Faculty of Veterinary Medicine

³Department of Veterinary Public health and Preventive medicine, Faculty of Veterinary Medicine,

⁴Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University Sokoto.

* Corresponding author (rabiualbul2005@yahoo.com)

ABSTRACT: Evidences arising from epidemiological studies as well as from detailed experimental investigations have indicated that there is strong relationship between certain feed ingredients and incidence of bacterial infections. A total of two hundred and thirty nine (239) Poultry feed samples comprising of two hundred and four (204) commercially prepared feed and thirty five (35) self compounded feed were collected from seventy six (76) identified poultry farms in Sokoto metropolis over a period of 12 months for assessing their microbiological (bacterial) quality. Of the total 80 questionnaires administered, 76 (95.00%) were responded to. Of the respondents, 53 (69.74%) indicated using commercially prepared feed while 23 (30.26%) compounded the feed by themselves. Similarly, 30.57% of the farms visited store their feed either within the poultry pen or in an open space. Out of total ($n=239$), commercially prepared ($n=204$) and Self compounded ($n=35$) feed samples, 217 (90.79%) samples yielded positive bacterial growth. Based on culture and identification, 263 bacterial species/genus were identified which include the following: *Corynebacterium pyogenes* (9; 3.42%), *Bacillus subtilis* (60; 22.81%), *Enterobacter arrogenes* (6; 2.28%), *Escherichia coli* (57; 21.67%), *Listeria monocytogenes* (19; 7.22%), Coagulase-negative *staphylococcus* (8; 3.04%), *Pasturella multocida* (3; 1.14%), *Pseudomona aerogenosa* (7; 2.66%), *Proteus mirabilis* (1; 0.38%), *Proteus vulgaris* (17; 6.46%), *Salmonella spp.* (10; 3.80%), *Staphylococcus aureus* (44; 16.73%), *Streptococcus pyogenes* (17; 6.46%), *Yersinia enterocolitica* (3; 1.14%) and (2; 0.76%) unidentified bacterial species. The presence of the above bacteria in all the feed samples calls for attention in the storage methods employed by the poultry and other livestock farmers, the warehouse condition, distributors and the sellers. This result could be used as a baseline data in setting public health standard for poultry feeds to achieve food security concern issues.

Keywords: Poultry feeds, Microbiological Quality, listeria, *Yersinia enterocolitica*

INTRODUCTION

The importance of poultry to the national economy cannot be overemphasized, as it has become a popular industry for the small holders and this has contributed sparingly to the economy of this country (FAO, 2000). The industry has assumed greater importance in improving the employment opportunities and animal feed production in Nigeria (FAO, 2000). Poultry production has a significant effect on the national economy. An earlier report by Okonkwo and Akubuo (2001) showed that about ten (10) percent of the Nigerian population is engaged in poultry production, mostly on subsistence and small or medium-sized farms.

Poultry production in the study area is on the increase because of the mobilization and sensitization from the state and local government authorities. Bearing this in mind, there is therefore the need to conduct the present study.

More so, poultry farms are getting involved in commercial broiler and egg production utilizing a variety of growth poultry feeds (SSIPC, 2008). However, the level of poultry feed contamination with fungal and bacterial organisms remains unknown in the study area.

Consequently, poultry feed have being implicated in several poultry diseases with varied pathological manifestations. These diseases are of viral (e.g. Avian influenza, Newcastle disease), Bacterial (e.g. Salmonellosis and infectious coryza) and fungal origin. The involvement of poultry feeds in the transmission of Aflotoxicosis which is the most prevalent and economically significant mycotoxin is of great health concern to the poultry farmers and extended consumers.

There are numerous ways contaminating microorganisms can affect feed quality negatively including reducing dry matter and nutrients, causing musty or sour odours, and causing caking of the feed and producing toxins. Finally, feed can act as a carrier for animal and human pathogens. The type of feed, processing treatments and storage conditions can all be factors that influence the population levels and types of microorganisms present (Maciorowski *et al.*;2006).

Study of prevalence of toxigenic mycobiota of animals/poultry feeds is regularly and frequently reported from many countries including Japan (Furuta *et al.*, 1980), France (Bauduret, 1990), Portugal (Da costa *et al.*, 2007), Bangladash (Akong *et al.*, 2009).

In Nigeria However, Arotupin *et al.*, 2007; Obi and Ozugbo, 2007; Uwaezuoke and Ogbule 2008; Adebayo-tayo and Ettah, 2010 and Ezekiel *et al.*, 2011 independently reported the isolation of pathogenic bacterial genera and species in the poultry feed samples sold in parts of Western and Eastern Nigeria.

The production of poultry feeds for local and commercial farmers in the developing countries including Nigeria requires an above average microbiological safety regulations to escape microbial contamination of the product (Obi and Ozugbo; 2007). Thus the present study is to investigate the bacterial contamination of poultry feeds used in poultry farms in Sokoto metropolis.

MATERIALS AND METHODS

Study Area

The study area is Sokoto metropolis the capital of Sokoto state, located at latitude 13°N and between longitudes 4° 8' E and 6° 54' E in the North Western part of Nigeria, the extreme Northwest of Nigeria. It covers approximately an area of 56,000 square kilometers (Roger, 1999). The state shares border with Niger Republic to the north, Kebbi State to the south and Zamfara State to the east. Based on the 2006 population census, It has a projected population of about 4,244,399 as at 2009 (NPC, Nigeria; 2006). Sokoto State is endowed with livestock resources; indeed the state is placed second with regard to livestock population which has a mean livestock population for cattle (3 million), Goat (4 million), Sheep (3.85 million), Camels (0.8 million) and 1 million Poultry (SSIPC 2008).

Sokoto state is located in the Sudan savanna Zone, grass vegetation, sandy soil, and humidity, which is usually below 40% except in few wet months when it approaches 60 %. There are two major seasons namely wet and dry. The dry season starts from October, and lasts up to April, in some parts and may extend to May. The wet season on the other hand begins in May and lasts up to September, or October. The mean annual rainfall ranges between 500mm and 550mm. Hammatan, a dry, cold and fairly dusty wind is experienced in the state between November and February. These extreme weathers are inimical to effective poultry and feeds management.

Study Design and Sampling Procedure

The study is a cross-sectional Study that covered a period of 12 months (May 2010 to April 2011). It involved microbiological analysis of poultry feeds from 76 (95%) of the identified 80 poultry farms located in the area of study. From each visited farm, feed present in the farm were sampled by selecting 20-25% of the bags present in the farm by simple random sampling.

Sample Collection and Processing

During sampling visits of the 76 participating farms, a total of 239 poultry feed samples comprising of commercially prepared feed ($n=204$) and self-compounded feed ($n=35$) were collected. Samples were collected during the rainy season ($n=102$), the cold-dry season ($n= 64$) and during the hot-dry season ($n=73$). For each sample, 5-10 gram of feed was collected in a polythene bag and then collectively transported to the Veterinary Microbiology laboratory for immediate processing. In some cases the feed samples were stored at room temperature (22-25°C) for a maximum of 24 hours prior to inoculation onto culture media.

For each feed sample obtained, a ten-fold serial dilution of 1gram of feed was carried out using sterile distilled water and 0.1ml of the dilution was cultured by spread plate technique into nutrient agar, MacConkey agar and Blood agar. The inoculated plates were incubated at 37°C for 24 hours. Further identification of bacterial isolates were done following a series of biochemical tests which included, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, hydrolysis tests catalase, urea hydrolysis, lactose fermentation, nitrate reduction and sugar fermentation tests according to standard microbiological procedure (Carter *et. al.*; 1991 and Cheesbrough; 2000).

Data Presentation and Analysis

The Data generated in the study were presented as frequency distribution in the form of tables and figures using Frequency Distribution. Chi-square was used to test if there is any statistical association between seasons of the year, feed sample type and the rate of isolation of the bacteria respectively (Araoye, 2003).

RESULTS

Out of the total number of 239 samples tested, 217 (90.8%) yielded positive bacterial growth. Bacterial isolates were found among 184 (90.2%) of the 204 commercially prepared feeds and 33 (94.3%) of 35 self-compounded feeds (Table 1).

Table 1: Percentage of poultry feed samples positive for bacterial organisms in Sokoto and its environs

Feed type(s)	No. of Feed Samples collected	No. of Samples Positive for Bacteria	% Positive
Commercial	204	184	90.2
Self-Compounded	35	33	94.3
Total	239	217	90.8

Association between feed types and frequency of isolation of bacteria was not statistically significant, $\chi^2 = 0.025$; $p > 0.05$

The rate of isolation of bacteria in feed samples were found to be highest (100%) in the months of May, June, July, August and September, followed by November (94.4%) and the least rate (73.3%) was in the month of October (Table 2).

Table 2: Monthly distribution of bacteria in poultry feed samples collected from Sokoto and its environs

Months	No. of Feed Sample Tested	No. positive for growth (%)
June	23	23 (100.0)
July	15	15 (100.0)
August	16	16 (100.0)
September	16	16 (100.0)
October	15	11 (73.3)
November	18	17 (94.4)
December	22	19 (86.4)
January	24	21 (87.5)
February	20	17 (85.0)
March	26	20 (76.9)
April	27	25 (92.6)
May	17	17 (100.0)
TOTAL	239	217 (90.8)

Seasonally, the rate of isolation of bacteria in feed samples was found to be highest (95.3%) in rainy season, followed by cold-dry season (92.2%) and the least (83.3%) was hot-dry season, as illustrated graphically in Figure 1.

Of the bacterial species isolated from commercially prepared feeds, *Escherichia coli* (53; 23.6%) occurred most frequency, followed by *Bacillus subtilis* 49 (21.8%) and the least was *Proteus mirabilis* (1; 0.4%). The self-compounded feeds yielded *Bacillus subtilis* (10; 26.3%) as the highest rate of occurrence in feeds (Table 3).

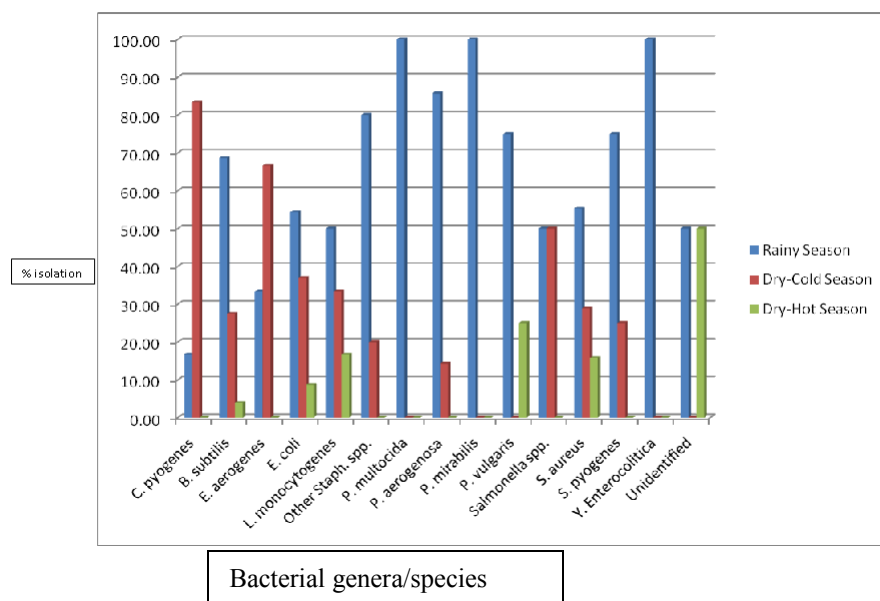


Figure 1: Distribution trends of bacteria isolated from poultry feed samples in Sokoto and its environs
Association between seasons and frequency of isolation of bacteria was not statistically significant, $\chi^2 = 0.7$; $p > 0.05$

Table 3: Frequency of bacterial genera/species isolated from poultry feeds by feed types in Sokoto and its environs

Bacterial Species	Frequency of isolation (%)		Total
	Commercially prepared (%) (n = 225)	Self compounded (%) (n = 38)	
<i>Bacillus subtilis</i>	49(21.8) ^a	10 (26.3) ^a	59
<i>Actinomyces pyogenes</i>	9 (4.0)	0 (0.0)	9
<i>Enterobacter aerogenes</i>	5 (2.2)	1 (2.6)	6
<i>Escherichia coli</i>	53 (23.6) ^a	4 (10.5) ^a	57
<i>Listeria monocytogenes</i>	18(8.0) ^a	4 (10.5) ^a	22
<i>Pasturella multocida</i>	1 (0.4)	2 (5.3)	3
<i>Proteus mirabilis</i>	1 (0.4)	0 (0.0)	1
<i>Proteus vulgaris</i>	13 (5.8) ^a	3 (7.9) ^a	16
<i>Pseudomona aeruginosa</i>	6 (2.7)	1 (2.6)	7
<i>Salmonella spp.</i>	9 (21.8)	1 (2.6)	10
<i>Staphylococcus aureus</i>	36 (16.0) ^a	7 (18.4) ^a	43
coag. neg. <i>Staphylococcus</i>	6 (2.7)	2 (5.3)	8
<i>Streptococcus pyogenes</i>	15 (6.7)	2 (5.3)	17
Unidentified	2 (0.8)	0 (0.0)	2
<i>Yersinia enterocolitica</i>	2 (0.8)	1 (2.6)	3
Total	225 (85.6)	38 (14.4)	263

The different between rates of isolation of bacteria with same superscript is not statistically significant ($p = 0.4676$; $p > 0.05$)

DISCUSSION

In total, 12 bacterial species and 2 bacterial genera were isolated from poultry feeds in the study area. The five most frequently isolated species are *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Proteus vulgaris* (Table 3).

The presence of *Listeria*, *Bacillus*, *Pseudomonas*, *Escherichia coli* and *Salmonella* species may suggest faecal as well as environmental contamination. Some of these organisms are well known pathogens of birds and farm animals. (Mallinson, 1984). For instance, *E. coli* is implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas *Salmonella*, *Listeria* and *Staphylococcus aureus* are capable of producing acute and chronic infections in all or most types of birds and other animals (Mallinson, 1984). The study showed that the percentage occurrence of *Listeria* spp was 7.2%. These organisms can survive and multiply at refrigerator temperatures and in a wide range of pH, hence even a small amount of contamination may be significant.

Statistical analysis of the isolates revealed no significant association between Feed types and the isolation rate of each of the bacterial organisms isolated from poultry feeds (χ^2 tabulated at df 1 = 0.3841 and at $p < 0.05$). This implies that the rate of isolation of each of the bacterial species does not depend on whether the feed is commercially prepared or self compounded poultry feeds. Similarly, there is no significant association between the seasons of the year and proportional rate of isolation of bacteria in feeds where p values of each statistically analyzed variables are greater than 5% ($p < 0.05$). This implies that seasonal variation has no influence on the rate of isolation of bacteria.

CONCLUSION

The high bacterial recovery in this study may indicate a potential hazard to both animals, and humans. The high occurrence of bacterial species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological contaminated feed or their toxins by farmed animal and subsequent public health problem. Due to this fact, regular microbiological analysis should be necessary methods for determination of quality and safety of poultry feed.

Acknowledgement

The authors are grateful to Lawali Ibrahim Kanoma of veterinary microbiology laboratory, UDUS for his technical assistance.

REFERENCES

- Abarca, M.L., Bragulat, M.R., Castella, G. and Cabanes, F.J., (1994) Mycoflora and aflatoxin-producing strains in animal mixed feeds. *J. Food Prot.* **57**, 256–258
- Accensi, E., Abarca, M.L. and Cabanes, F.J. (2004) Occurrence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce ochratoxin A. *Food Microb.*, **21**:623-627
- Adeboyega, M.A. (1999) *Serological Evidence of Newcastle Disease and Infectious Bursa Disease Agents in Ducks and Guinea Fowls*. Africa Press Ibadan. PP: 5-7
- Anon (2001): Sokoto state – The seat of the Caliphate. In Sokoto State Diary. Pp2-8
- Araoye, M. O. (2003) *Research Methodology with Statistics for Health and Social Sciences*. Nathadex Publishers, Ilorin
- Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni P. and Kozakiewicz, Z. (2003) Occurrence of ochratoxin A producing fungi in grapes grown in Italy. *J. Food Prot.*, **66**: 633-636
- Bejaoui, H., Mathieu, F., Taillandier P. and Lebrihi A. (2006) Black *Aspergilli* and ochratoxin A production in French vineyard. *Int. J. Food Microbiol.*, **111**: 546-552
- Carter, G. R. and Cole, J. R. (1990) *Diagnostic Procedure in Veterinary Bacteriology and Mycology*. 5th Ed. Academic Press Inc. 372-373
- Cheesbrough, M. (2002) *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press. 62-70.

- Chelkowsky, J. (1991) Mycological quality of mixed feeds and ingredients. In: *Cereal grain, mycotoxins, fungi and quality in drying and storage*. (Ed.): J. Chelkowsky. Elsevier, Amsterdam, pp. 217-227
- Dalcero, A., Magnoli, C., Chiacchiera, S., Palacios G. and Reynoso M. (1997) Mycoflora and incidence of aflatoxins B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. *Mycopathologia*, **137**: 179-184
- Hanif, N.Q., Naseem, M., Khatoon S. and N. Malik. (2006) Prevalence of mycotoxins in poultry rations. *Pak. J. Sci. Indust. Res.*, **49**: 120-124
- Leistner L. (1984) Toxinogenic penicillia occurring in feeds and foods: A review. *Food Technology in Australia*, **36**, 404-413
- Maciorowski, K.G., Herrera P., Jones F.T., Pillai S.D, and Ricke S.C (2006) *Effects on poultry and livestock of feed contamination with bacteria and fungi*. Elsevier B.V. 109-136
- Mallinson, E. T. (1984) Infectious diseases. In: *Animal Health*. Jack Hayes (ed). Bureau of Animal Industry (Publisher), Maryland, U.S.A
- Martins, M., Martins H.M. and Bernardo, F. (2003) Fungal flora and mycotoxins detection in commercial pet food. *Revista Portuguesa de Ciências Veterinárias*, **98**: 179-183
- Obi C. N. and I. J. Ozugbo, (2007) Microbiological analysis of poultry Feeds sold in Umuahia main market, Abia State, Nigeria. *Res. J. of Appl. Sci.* **2**(1): 22-25
- Ogbulie, J.N., Uwaezuoke, J.C. and Ogiehor, S.T. (1998) *Introductory Microbiology Practical*. Springfield Publishers, Owerri, pp 162
- Oliveira, G.R., Ribeiro, J.M., Fraga, M.E., Cavaglieri, L.R., Direito G.M., Keller, K.M., Dalcero A.M. and Rosa, C.A.R. (2006) Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia*, **162**: 355-362
- Osho, I.B., Awoniyi, T.A.M. and Adebayo, A.I. (2007) Mycological investigation of compound poultry feeds used in poultry farms in south west Nigeria. *African J. Biotech.*, **6**: 1833-1836
- Rosa, C.A.R., Riberio, J.M.M., Fraga, M.J., Gatti, M., Cavaglieri, L.R., Magnoli, C.E., Dalcero, A.M. and Lopes, C.W.G. (2006) Mycoflora of poultry feed and ochratoxin- producing ability of isolated *Aspergillus* and *Penicillium* species. *Vet. Microb.*, **113**: 89-96
- Russell, R. and Peterson, M. (2007) Aflatoxin contamination in chilli from Pakistan. *Food Control*, **18**: 817-820
- Saleemi, M. K., Khan, M. Z., Ahrar, k. and Javed, I. (2010) Mycoflora of Poultry Feeds and Mycotoxins Producing Potential of *Aspergillus* Species. *Pak. J. Bot.*, **42**(1): 427-434, 2010
- Saleemullah, A. Iqbal, I.A. Khalil and Shah, H. (2006) Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chem.*, **98**: 699-703
- Shareef, A. M. (2010) Molds and Mycotoxins in Poultry Feeds from Farms of Potential Mycotoxicosis. *Iraqi J of Vet. Sci.* **24** (1) 17-25
- Simas, M.M., Botura, S., Correa, B., Sabino, M., Mallmann, C.A., Bitencourt, T.C.B.S.C. and Batatinha. M.J.M. (2007) Determination of fungal microbiota and mycotoxins research in brewers grain used in cattle feeding in the state of Bahia, Brazil. *Food Control*, **18**: 404-408
- Somashekar, D., Rati, E.R., Anad S. and Chandrashekar, A. (2004) Isolation, enumeration and PCR characterization of Aflatoxigenic fungi from food and feed samples in India. *Food Microb.*, **21**: 809-813
- SSIPC (2008) Beef up Your Profits by Investing in Sokoto State's Livestock Sector, 1-8.