Microbiological Assessment of Poultry Feeds within Ilorin, Nigeria

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Abstract

The poultry feeds were obtained from 20 different poultry pens and their microbial contents were assessed. The antibiotics resistance patterns of the bacterial isolates were also determined. The bacterial count ranged from $5.0 \times 10^5$ to $1.76 \times 10^6$ cfu/g while the fungal count ranged from $3.5 \times 10^4$ to $1.9 \times 10^5$ cfu/g. The bacterial species isolated were Staphylococcus aureus, Staphylococcus pyogenes, Micrococcus luteus, Micrococcus varians, Micrococcus roseus, Staphylococcus hominis, while the fungal species isolated were Saccharomyces cerevisiae, Fusarium oxysporum, Penicillium sp., Humicola grisea, Aspergillus fumigatus, Hansenula sp., and Humicola fuscoatra. All the bacterial isolates were resistant to ceftazidime and cefuroxime and all the isolates were resistant to at least three antibiotics. Ofloxacin produced the highest zone of inhibition, followed by gentamicin, and then erythromycin. The presence of some pathogenic microorganisms in the poultry feeds revealed high level of contaminations. It is recommended that poultry feeds should be made from good quality grains and it should be prevented from environmental or other contamination.

Keywords: antibiotics, assessment, contaminants, microbial content, poultry feeds

Introduction

Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Raloff, 2003). For the development of healthy poultry, the poultry farmers should formulate a feed that will give the best possible result at the least possible cost (Lossli et al., 1999). Poultry feeds are food materials used in raising poultry birds and are designed to contain all the nutritional materials needed for proper growth, meat and egg production in birds. Antibiotics such as bacitracin, tetracycline, oxytetracycline, chlorotetracycline have been incorporated into poultry feed formulations usually at low prophylactic level to prevent minor diseases and enhance efficient growth (Smith, 2005). The feeds for poultry production are composed largely of grains such as corn, wheat or barley, oil seeds, cake meal, sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal’s and feather meal (Bale et al., 2002).

The poultry industries rely on the supply of ready-to-use feed from feed mills (Agnaga et al., 2000). These packaged feeds from feed mills constitute the main source of feeds for poultry farmers. Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes et al., 2003). Consequently, poultry feed has been implicated in several poultry diseases with varied pathological manifestations. These diseases might be of viral (Avian influenza, Newcastle disease), bacterial (Salmonellosis and infectious coryza) or fungal origin. The involvement of poultry feeds in the transmission of aflatoxicosis, which is the most prevalent and economically significant mycotoxin, represents also the main concern to the poultry farmers and extended consumers (Aliyu et al., 2016).

From an ecological point of view, harvested grains are not only ingredients for livestock diets, but can act as substrate for the transmission of vectors of simple unicellular prokaryotic and eukaryotic organisms. Feeds may contain diverse microflora that is acquired from environmental sources, including dust, soil, water, and insects. Feed materials may be inoculated at any time during growing, harvesting, processing, storage and transportation of the feed.

The 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 of animal and human pathogens.

The type of feed, processing treatments and storage conditions are some of the factors that influence the population levels and types of microorganisms present in poultry feeds (Zhao and Xiuping, 2014).

In Nigeria, Obi and Òzogbo (2007), Uwaezuoke and Ogbule (2008), Adedoyi-Tayo and Ettah (2010) independently reported the isolation of pathogenic bacterial genera and species in the poultry feed samples sold in Western and Eastern parts of the country.

The current research was conducted to determine the quality of poultry feeds collected from various poultry pens...
within Ilorin (Nigeria). Hence, it is necessary to assess the hygiene and safety of these feeds. Therefore, the objectives of the research were to determine the bacterial and fungal loads of poultry feeds; to isolate, to characterize, and to identify the bacterial and fungal species in these products; to determine the absence or presence, as well as the count of specific bacterial pathogens present in poultry feeds and to determine the antibiotic susceptibility patterns of the bacterial isolates.

Materials and Methods

Collection of poultry feeds and the counting of microorganisms

The poultry feeds were collected from 20 different poultry farms located in Ilorin metropolis. They were aseptically collected using sterile spatula into sterile black polythene bags. The collected feeds were coded A to T.

One gramme of the feed sample was serially diluted and 1 ml aliquot was inoculated into sterile Petri dishes using pour plate technique. Nutrient agar was used for the bacteriological analysis.

For fungal counts, 0.1 ml of the serially diluted aliquot was plated using spread plate method. The sterile potato dextrose agar supplemented with streptomycin was used for fungal isolation. After incubation, the colonies were counted and expressed in cfu/g (Fawole and Oso, 2004).

Total and faecal coliform counts

Isolation of total and faecal coliforms were carried out by inoculating 0.1 ml of aliquot from 10^1 to 10^3 dilutions on MacConkey agar (MA) and eosin methylene blue agar (EMB) plates respectively using spread plate method. After the incubation period, typical pinkish colonies was counted and recorded as total coliform on MA, while colonies with greenish metallic sheen were counted and recorded as faecal coliform (E. coli). The colonies were further confirmed by biochemical tests (Fawole and Oso, 2004).

Isolation of specific pathogenic bacteria

In order to observe the pathogenic bacteria Staphylococcus aureus, Salmonella, Shigella and Pseudomonas aeruginosa mannitol salt agar, Salmonella-Shigella agar and cetrimide agar respectively were used. Plating of 0.1 ml aliquot from 10^1 to 10^3 dilutions was done using spread plate method. After incubation, typical colonies were observed on the media and they were further confirmed by suitable biochemical tests (Collins and Lyne, 1970; Willey et al., 2008).

Then, isolation of pure culture of microorganisms was performed by subculturing until pure culture was obtained. The pure cultures were then stocked in agar slant and kept in a refrigerator at 4-8 °C (Fawole and Oso, 2004).

Characterization and identification of isolates

The bacterial isolates were characterized and identified mainly on the basis of their colonial morphology, cellular morphology and biochemical reactions. Identification was based on standard texts such as Cowan and Steel (2005).

The fungal isolates were identified based on their macroscopic and microscopic features and making reference to standard texts (Onions et al., 1981).

Antibiotics susceptibility test

Normal saline broth culture of each bacterium was prepared and standardized using 0.5 MacFarland’s standard. The standardized inocula were then used to seed the surface of a plate of Mueller Hinton agar and antibiotic disc was placed on the surface of the inoculated medium. Incubation was done at 37 °C for 24 hours after which the diameter of the zone of inhibition was measured in mm (CLSI, 2005).

Statistical analyses

Statistical analysis package SPSS 15.0 was used to determine the mean, the range and the standard deviation. The differences within the means were expressed using one way analysis of variance (SPSS, 2010) and the means were compared by Duncan’s test, α ≤ 0.05.

Results

Microbial counts of poultry feeds

The bacterial and fungal counts of the poultry feeds ranged from 5.0 × 10^3 to 1.76 × 10^6 cfu/g and 3.5 × 10^3 to 1.59 × 10^6 cfu/g respectively (Table 1). The total coliform and Staphylococcus aureus counts ranged from zero to 3.0 × 10^5 cfu/g and 1.9 × 10^3 to 5.5 × 10^5 cfu/g respectively. There was a complete absence of faecal coliform, Salmonella, Shigella and Pseudomonas aeruginosa from the poultry feeds (Table 2).

Characterization and identification of isolates

After characterization, the bacterial isolates Streptococcus salivarius, Streptococcus pyogenes, Miroccocus luteus, Micrococcus varians, Micrococcus roseus, Staphylococcus aureus, Staphylococcus saprophyticas and Staphylococcus hominis were identified (Table 3). Likewise, fungi such as

Table 1. Total bacterial and fungal counts of poultry feeds

| Sampling sites | Bacteria | Fungus |
|----------------|----------|--------|
| A              | 22.0±1.0  | 13.2±0.0 |
| B              | 10.4±1.0  | 15.2±2.0 |
| C              | 4.9±1.0   | 15.3±3.0 |
| D              | 3.9±0.5   | 12.4±2.0 |
| E              | 0.5±0.0   | 9.3±2.0  |
| F              | 3.3±0.3   | 19.0±1.0 |
| G              | 0.8±0.1   | 18.5±1.0 |
| H              | 10.0±2.0  | 6.0±1.0  |
| I              | 21.0±2.0  | 7.7±2.0  |
| J              | 4.0±0.0   | 8.6±2.0  |
| K              | 17.3±1.0  | 4.9±2.0  |
| L              | 5.0±1.0   | 3.9±1.0  |
| M              | 92.0±8.0  | 9.9±3.0  |
| N              | 39.0±4.0  | 10.1±3.0 |
| O              | 49.0±4.0  | 6.1±1.0  |
| P              | 56.0±5.0  | 8.1±2.0  |
| Q              | 41.0±4.0  | 8.6±2.0  |
| R              | 63.0±5.0  | 8.1±2.0  |
| S              | 44.0±4.0  | 3.5±0.0  |
| T              | 51.0±5.0  | 6.1±1.0  |

Values followed by the same superscript within the same column are not significantly different at α ≤ 0.05 based on Duncan’s multiple range test.
Values followed by the same superscript within the same column are not significantly different at α≤0.05 based on Duncan’s multiple range test.
Saccharomyces cerevisiae, Fusarium oxysporum, Penicillium sp., Humicola grisea, Aspergillus fumigatus, Hansenula sp. and Humicola fuscoatra were identified, based on their macroscopic and microscopic features. All the bacterial isolates were susceptible to gentamicin and ofloxacin, but resistant to ceftazidime and cefuroxime. Each bacterial isolate was resistance to at least 3 of the antibiotics used (Table 6).
Discussion

Animal feed has been listed as one of the main sources of microbes in farm animals and poultry. The high occurrence of fungal and bacterial species is of public health concern and this may indicate obvious health hazard in terms of direct consumption of contaminated feed or their toxins by farm animals (Aliyu et al., 2016). The high counts of fungi in the current study indicate that more attention is needed in the storage strategies employed by the poultry feed manufacturers, or with the warehouse condition, as well as with handling of products and duration of storage. Arotupin et al. (2007) obtained bacterial and fungal count in the range of 6.6 x 10^3 - 2.5 x 10^4 and 1.5 x 10^3 – 7.4 x 102 cfu/g respectively. All the poultry feed samples examined showed the presence of microorganisms which included: Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pyogenes, Streptococcus salivarius, Micrococcus luteus, Micrococcus varians, Micrococcus roseus and Staphylococcus hominis. The presence of these microorganisms in the poultry feeds suggest that the feeds contain sufficient nutrients for the growth of the isolated organisms. The activities of these microorganisms on the feeds under the study may cause degradation, thereby reducing the nutrients for the livestock. The present findings are in agreement with the report of Aganaga et al. (2000) on poultry feeds and the sensitivity pattern of the associated microorganisms. These microorganisms may probably have originated from the raw materials from which the feeds were produced. In addition, most of the isolated microorganisms owned their origin from air and soils (Arotupin and Akinyosoye, 2001). Hancock et al. (1998) reported microbial contamination of poultry feeds of plant and animal origin to be due to climatic conditions encountered, harvesting, processing, storage and transport technologies employed. The test for specific bacterial pathogens revealed the high presence of total coliforms and Staphylococcus aureus. The presence of Staphylococcus aureus, a normal floral of the skin and nose suggests improper handling practices (Hancock et al., 1998), while members of total coliforms had probably environmental origin. Dhand et al. (1998) and Hancock et al. (1998) separately implicated Micrococcus luteus and Staphylococcus aureus in the microbial infection outbreak of poultry farming. The isolation of toxigenic mould, Aspergillus fumigatus, should be viewed with serious concern. This organism has been documented to be the most dominant of all the fungi in respect of aflatoxin production in poultry feeds (Henzler and Opitz, 1992).

Most of the bacterial isolates were resistant to at least one or more antibiotics especially ceftazidime, cefuroxime, ceftriaxone, cloxacillin and amoxicillin. However, they were susceptible to gentamicin and ofloxacin to different extents. Khan et al. (2002) reported the isolation of erythromycin resistant Staphylococci, Enterococci, and Streptococci from litters samples collected from poultry houses that added antibiotics to their feeds.

Aseptic handling and good storage condition which may prevent frequent exposure to the atmosphere is the basic step needed to minimize the contamination of feed product. High quality ingredients such as grains should be used for the manufacture of the poultry feeds.

Conclusions

The poultry feeds analysed in the hereby study contained high counts of bacteria and fungi. Specific pathogenic bacteria test revealed the presence of total coliforms and Staphylococcus aureus in addition to the isolation of aflatoxigenic fungus, Aspergillus fumigatus, in the poultry feeds. The poultry feeds investigated were free of faecal coliform, Salmonella, Shigella, and Pseudomonas sp.

References

Adebayo-Tayo, BC, Etraah AE (2010). Microbiological quality and aflatoxin B1 level in poultry and livestock feeds. Nigerian Journal of Microbiology 24(1):2145-2152.

Aganaga AA, Omphile UG, Malope P, Motsamai CH, Mostsumi LG (2000). Traditional poultry production and commercial broiler alternative for small-holder farmers in Botswana. Livestock Research for Rural Development 12:1-8.

Aliyu RM, Abubakar MB, Yakubu Y, Kasarawa AB, Lawal N, Bello MB, Fardami AY (2016). Prevalence of potential toxigenic Aspergillus species isolated from poultry feeds in Sokoto metropolis. Journal of Veterinary Sciences 14(1):39-44.

Arotupin DJ, Kayode RMO, Awojobi KO (2007). Microbiological and physicochemical qualities of selected commercial poultry feeds in Akure, Nigeria. Journal of Biological Sciences 7:981-984.

Arotupin DL, Akinyosoye FA (2001). Evaluation of microbial isolates from sawdust for cellulose hydrolysis. Nigerian Journal of Microbiology 15:97-102.

Bale OO, Sekoni AA, Kwanashie CN (2002). A case study of possible health hazard associated with Poultry house. Nigerian Journal of Animal Production 29:1022-1031.

Barnes M, Vallancourt JP, Gross WB (2003). Diseases of poultry. 11th Ed Iowa State University Press, Amesia, USA pp 110-150.

CLSI (2005). Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement, M100-S15, Vol 25, No 1. Clinical and Laboratory Standard Institute, Wayne, Pa.

Collins CH, Lyne PM (1970). Microbiological methods. Butterworths London, University Park Press, Baltimore pp 171-311.

Cowan ST, Steel KJ (1985). Manual for the identification of medical bacteria. 4th Ed. Cambridge University Press, London pp 217.

Dhand NK, Joshi DV, Dand SK (1998). Contamination of dairy feeds and their toxigenicity. Indian Journal of Animal Science 68:1090-1096.

Fawole MO, Oso BA (2004). Laboratory manual of microbiology. 4th Ed. Spectrum Books Limited Ilbadan pp 123.

Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE, Carpenter LV (1998). Multiple sources of Escherichia coli 0157 in feed lots and dairy farms in the Northern USA. Preventive Veterinary Medicine 35:11-19.

Henzler DL, Opitz HM (1992). The role of mice in the epizootiology of Salmonella enteriditis infections on chicken. Avian Diseases 36:625-631.
Khan AA, Nawaz MS, Khan SA, Steele R (2002). Detection and characterization of erythromycin resistant methylase genus in Gram positive bacteria isolated from poultry litter. Applied Microbiology and Biotechnology 59:377-381.

Loosli JK, Henry E (1999). The tropical environment and animal productivity. Animal production in the Tropics. Heinemann Education Books pp 34-50.

Maciorowski KG, Herrera P, Jones FT, Pillai SD, Rickie SC, Elsevier BV (2006). Effects on poultry and livestock of feed contamination with bacteria and fungi. Animal Feed Science and Technology 133(1-2):109-136.

Obi CN, Ozugbo IJ (2007). Microbiological analysis of poultry feeds sold in Umuahia main market, Abia State, Nigeria. Research Journal of Applied Sciences 2(1):22-25.

Onions AH, Allopp D, Eggins HO (1981). Smith’s introduction to industrial mycology, 7th Ed. Edward Arnold Limited, Bedford square, Britain.

Raloff EM (2003). Salmonella reservoirs in animals and feeds. Journal of Poultry Science 46(22):1-9.

SPSS (2010). Statistical Package for Social Scientists 15.0 for Window evaluation.

Smith WT (2005). Feed chickens properly co-operative extension. Extension service of Mississippi State University Service pp 58-60.

Uwaezuoke JC, Ogbu JN (2008). Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. Journal of Applied Science Environmental Management 12(1):113-117.

Willey JM, Sherwood LM, Woolverton CJ (2008). Prescott, Harley and Klein’s microbiology, 7th Ed. Mc Graw-Hill, Washington DC pp 550-556.

Zhao C, Xiuping J (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A review. Agriculture 4:1-29.