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Microorganisms Associated with Poultry Feeds in South West, Nigeria

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Abstract

The rate of mortality of poultry as a result of contaminated feeds is of the increase in the recent times. This study was carried out to determine the level of contamination, microbial loads and spoilage of selected poultry feeds in south west Nigeria. Rabiu Feeds, Caps Feeds, Ayo Best Feeds and Top feeds were selected and sampled. Standard pour plate methods were used for the analyses. Results obtained revealed average range of total viable count, coliform counts, *Staphylococus* counts and fungal counts of 2.35 - 7.10x 10^4 Cfu/g, 0.55-2.65x10^4, 0.50-2.90 Cfu/g and 1.30-3.30 x 10^4 Cfu/g respectively. Microorganisms obtained include fifteen bacteria, eight yeast and five mould isolates. The genera are *Bacillus, Pseudomonas, Klebsiella, Enterobacter, Escherichia, Staphylococus, Micrococcus, Alcaligenes, Acinetobacter, Salmonella, Serratia, Corynebacterium, Clostridium, Erwinia, Flavobacterium*, *Saccharomyces, Candida, Geotrichum, Rhodotorula, Kluyveromyces, Torulopsis, Hansenula, Pichia*, *Aspergillus*, *Rhizopus, Fusarium, Mucour* and *Talaromyces.* Fifty percent of the isolates were known pathogenic microorganisms. This study therefore, concludes and recommends that stringent hygienic measures during production and storage of poultry feeds should be followed and enforced to the later. Constants inspection by the Standard Regulatory Bodies to the production sites should be encouraged.

Introduction

Poultry are birds of economic value contributing significantly to human food as a primary supplier of meat, egg, raw materials to industries (feathers, waste products), source of income and employment to people compared to other domestic animals (Demeke, 2004; Onajobi et al., 2020). Food for farm fowl, such as chickens, ducks, geese, and other domestic birds, is known as poultry feed (Bonnie, 2013).

Prior to the 20th century, grain, kitchen scraps, calcium supplements like oyster shell, and garden waste were frequently added to chicken diet as supplements. To maintain healthy birds, the feeds are kept fresh as much as possible at all times. However, in Southwest of Nigeria, it is quite difficult at times to maintain the freshness of the feed, where high temperature and oxidation destroy certain vitamins.

Feed spoilage is caused by the growth of undesirable molds and bacteria. Poultry feed spoilage reduces the feed value and palatability. Poultry feed is known to contain Salmonella, and other microorganisms which are detrimental to the health of poultry animals (Eugene, 2012). As a result, this study aim is to evaluate the microbial quality of poultry feeds in south west region of Nigeria.

Materials and Methods

Study area

The study area is three reputable commercial poultry feed Companies within the southwest Nigeria. These includes; Rabiu Feeds – Ijebu-Ode, Ogun State, F.A. Feeds–Ijebu–Ode, Ogun States, Hybrid Feeds–Osogbo, Osun State and Top Feeds–Ibadan, Oyo State.

Sample collection

A total of 12 samples were used. Samples consisted of Chicks Mash, Grower Mash, and Layers Mash from each commercial poultry feed companies respectively. The samples were collected in a clean polythene bag and transported to Federal Institute of Industrial Research, Oshodi, Lagos State (FIIRO) for further analysis. Each selected commercial feed depot was visited two times for sample collections during the study period.

Preparation of media diluents

23 g of Nutrient agar, 67g of MarConkey agar and 38g of Potato dextrose agar is weighed using a digital chemical balance and suspended into 1 liter amount of distilled water homogenized on hot plate magnet stirrer to form a uniform solution. Diluents (dilution blanks 0.1%) made up of 90ml and 9ml amount of distilled water were made sterilized at 121℃. 15 pounds per pressure (PSI) for 15 minutes in the autoclave. At the end of the sterilization period, media were cooled to 45^{o}C in water bath preset at 45℃ order to inhibit bacterial growth, streptomyan (0.14w/v) was aseptically weighed and added to potato dextrose agar only.

Isolation of microorganisms from sample

Ten grams (10g) of sample were weighed with sterile spatula using chemical balance. The samples were put into a sterile pestle and mortar, crushed with 90 milliliters of sterile distilled water. The sample was aseptically poured into the bottle of 90ml of sterile distilled water above burner. This was properly mixed together-1ml portion from the above dilution was aseptically taken with a sterile pipette and introduced into 9ml-amount of sterile water 10^{-1} dilution and from this dilution the samples were serially diluted up to the required dilution 10^{-5} dilutions according to Onajobi et al. (2015).

Disposable petri dishes were set out and labeled accordingly while inoculation was carried out using pour plate method. From the 10^{-4} and 10^{-5} dilutions, avqurt (1.0ml) of inoculums was aseptically pipette and inoculated into sterile petri dishes, cool molten of Nutrient agar, MacConkey agar and potato dextrose agar poured onto the inoculums respectively and mixed clock wisely and anticlockwise for evenly distribution of the inoculums. The plates were allowed to set properly and incubated in an incubator at 35+2℃ for 24 hours for bacteria and 28 +2℃ for 3 – 5 days for fungi.

At the end of incubation period, the colony observed on the culture plates is counted using coulter colony counter. The colony or viable count per ml was calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution. Report as Colony forming units/ml (Cfu) or (Cful/g) was according to Onajobi et al. (2017).

Casein hydrogen

Nutrient agar (250ml) was prepared only 1%w/v (2.5g % casein) casein powder was added to Nutrient agar homogenized on hot plate magnetic stirrer. The medium was sterilized in an autoclave at 115^{o}C for 10 mins allowed to cool to about 45–50^{o}C and poured aseptically in petri dishes. The plates were allowed to set and dry at 45^{o}C. Fresh culture or isolation of 18 -24 hours were inoculated into plates of casein agar. Incubated at 35+/-2^{o}C for 5 days. Plates were examined for clearing of the medium around the bacteria growth using 20% and mercuric chloride (HCl and $HgCl_2$) solution (Onajobi et al., 2020).

Identification of moulds

The observed moulds growth was subcultured on fresh potato dextrose agar (PDA) plates and incubated at 28+2^{o}C 5 days and therefore an accurate description of the fungus as grown on the medium was observed and examined at frequent intervals for colonial or cultural characteristics.

The colonial morphology of the mold isolates was performed based on the size, colour and aerial mycelia growth. Microscopy morphology is determined using blue stain. The fungal growth was stained using wet mount techniques. With a sterile inoculating needle, mycelia growth is picked from the culture plates and placed onto cleaned grease free glass slide on which a drop of saline water had been dropped. The fungal mycelia were teased out properly. One drops of lactophenol cotton blue stain was added and the preparation was covered with clean cover slips. The preparation was subsequently viewed under the X40 microscope objective (Cheesbrough, 2010).

Screening of poultry samples for toxins

Chromatographic method was used to screen for poultry samples for the presence of toxins. 50 ml of 80% methanol was added to 10g of inoculated poultry feed each and were grinded into fine particles using a high-speed blender for 3 minutes. They were transferred back into conical flask and was shaken for 30 minutes on a shaker. The mixture was then filtered.

through Whitman paper and the extract collected in a 250ml flask, 20ml distilled water was added to ease separation. 15ml dichloromethane was added and shaken for proper mixture.

After separation, dichloromethane layer was filtered out through 20g of anhydrous sodium Sulphate to remove residual H_20. The extraction was collected in polypropylene cup and evaporated to dryness in a fume cupboard. The residue was redissolved in 1ml of dichloromethane. Aflatoxin standards and extracts were separated on thin layer chromatography plate. Aflatoxin plate was observed under long wavelength U.V light pitted in a black cabinet (Cheesbrough, 2010).

Characterization and identification of bacterial isolates

Pure cultures of bacterial isolates from feeds are identified based on their colonial morphology, cellular morphology and biochemical characteristics whereby the following analysis were carried out gram and spore staining, catalase production (Ramachandran et al., 2014), gelatin hydrolysis, starch hydrolysis, carbohydrate utilization oxidase test, indole production, nitrate reduction, coagulase test, urease test and methyl-red voges proskauer test (Cheesbrough, 2010).

Results and Discussion

Average range of count of 2.35-7.1×10^4cfu/g for total viable bacteria 0.55-2.65 x 10^4 for coliforms counts, 0.5-2.9 Cfu/g for *Staphylococcus* and 1.3-3.3×10^4Cfu/g for fungi (yeast counts were recorded respectively in all the samples analyzed from all poultry feeds investigated in table 1, 2 and 3 below. Slight variations were observed amongst the group of microorganisms within each poultry feeds. The average rate of occurrence and distribution of ten (10) members of the fungi group (Yeasts) were significantly different from the bacteria group.

Table 4, 5, 6 and 7 revealed vast array of microorganisms were detected and isolated among the various groups of microorganisms isolated were *Bacillus* species, *Corynebacterium* species, *Clostridium* species, *Flavobacterium* species, *Pseudomonas* species, *Micrococcus* species, *Alcaligenes* species, *Acinetobacter* species, *Proteus* species, *Staphylococcus* species, *Erwinia* species, *Enterobacter* species, *Klebsiella* species, *Serratia* species, *Citrobacter* species, *Salmonella* species, *Escherichia* species, *Sporosarcina* species and *Xanthomonas* species were among the bacteria group while the fungi group included: *Saccharomyces cererisiae*, *Saccharomyces exigins*, *Saccharomyces rouxii*, *Candida* species, *Pichia* species, *Geotrichum* species, *Rhodotorula glutinis*, *Hansenula anomala*, *Torulopsis stellate* and *Kluyveromyces maxians*.

Table 1. Physicochemical characteristics of poultry feed samples

Sample Location	Types of Feed	Sample No	Dtae of Collection	Moisture Content	PH	Colour	Texture	Odour
Rabiu Feeds	i.Layers Mash	1	1 – 8 – 16	1. 16.5%	6.6	Light	Coarse	Faint
(Ijebu Ode)	ii.Grower Mash	2	"	2. 17.0%	6.5	Brown	Coarse	Faint
Ogun State	iii.Chick Mash	3	"	3. 15.5%	6.7	Light Brown Light Brown	Coarse	Very Faint
F. A Feeds	i.Layers Mash	4	"	1. 16.3%	6.6	Brown	Coarse	Faint
(Ijebu Ode)	ii.Grower Mash	5	"	2. 16.7%	6.5	Brown	Coarse	Faint
Ogun State	iii.Chick Mash	6	"	3. 16.0%	6.6	Brown	Coarse	Faint
Hybrid Feeds	i.Layers Mash	7	"	1. 16.6%	6.7	Light	Coarse	Faint
Osogbo Osun	ii.Grower Mash	8	"	2. 17.0%	6.4	Brown	Coarse	Faint
State	iii.Chick Mash	9	"	3. 15.8%	6.7	Light Brown Light Brown	Coarse	Faint
Top Feeds	i.Layers Mash	10	"	1. 16.8%	6.4	Brown	Coarse	Faint
(Ibadan)	ii.Grower Mash	11	"	2. 16.4%	6.5	Brown	Coarse	Faint
Oyo State	iii.Chick Mash	12	"	3. 16.0%	6.6	Brown	Coarse	Faint

Table 2. Total mesophile aerobic microbial population of poultry fee

Sample Location	Types of Feed	Total Viable Count	Coliform Count	*Stapylococcus* Count	Fungi Count
Rabiu Feeds (Ijebu Ode) Ogun State	i. Chick Mash	71×10^3	11×10^3	29×10^3	33×10^3
	ii. Layer Mash	31×10^3	09×10^3	07×10^3	17×10^3
	iii. Grower Mash	24×10^3	06×10^3	05×10^3	14×10^3
F. A Feeds (Ijebu Ode) Ogun State	i. Chick Mash	33×10^3	06×10^3	10×10^3	16×10^3
	ii. Layer Mash	47×10^3	17×10^3	13×10^3	22×10^3
	iii. Grower Mash	64×10^3	23×10^3	21×10^3	25×10^3
Hybrid Feeds Osogbo Osun State	i. Chick Mash	30×10^3	09×10^3	07×10^3	13×10^3
	ii. Layer Mash	31×10^3	13×10^3	19×10^3	21×10^3
	iii. Grower Mash	46×10^3	10×10^3	14×10^3	25×10^3
Top Feeds (Ibadan) Oyo State	i. Chick Mash	41×10^3	11×10^3	10×10^3	20×10^3
	ii. Layers Mash	47×10^3	20×10^3	12×10^3	20×10^3
	iii. Grower Mash	57×10^3	27×10^3	14×10^3	27×10^3

Table 3. Mean of total mesophile aerobic microbial population of poultry feeds

Sample Location	Types of Feed	Total Viable Count	Coliform Count	*Stapylococcus* Count	Fungi Count
Rabiu Feeds (Ijebu Ode) Ogun State	1. Chick Mash	39×10^3	10×10^3	09×10^3	19×10^3
		42×10^3	12×10^3	10×10^3	21×10^3
	2. Layer Mash	48×10^3	19×10^3	11×10^3	18×10^3
		45×10^3	20×10^3	12×10^3	22×10^3
	3. Grower Mash	55×10^3	25×10^3	13×10^3	25×10^3
		58×10^3	28×10^3	15×10^3	28×10^3
F. A Feeds (Ijebu Ode) Ogun State	1. Chick Mash	28×10^3	09×10^3	06×10^3	12×10^3
		32×10^3	08×10^3	07×10^3	14×10^3
	2. Layer Mash	53×10^3	14×10^3	20×10^3	20×10^3
		48×10^3	11×10^3	18×10^3	22×10^3
	3. Grower Mash	49×10^3	12×10^3	12×10^3	24×10^3
		42×10^3	08×10^3	14×10^3	26×10^3
Hybrid Feeds Osogbo Osun State	1. Chick Mash	68×10^3	13×10^3	26×10^3	30×10^3
		74×10^3	09×10^3	32×10^3	36×10^3
	2. Layer Mash	33×10^3	10×10^3	05×10^3	18×10^3
		28×10^3	08×10^3	08×10^3	16×10^3
	3. Grower Mash	25×10^3	07×10^3	04×10^3	13×10^3
		22×10^3	04×10^3	06×10^3	15×10^3
Top Feeds (Ibadan) Oyo State	1. Chick Mash	31×10^3	05×10^3	10×10^3	15×10^3
		34×10^3	07×10^3	09×10^3	17×10^3
	2. Layers Mash	46×10^3	15×10^3	12×10^3	20×10^3
		48×10^3	18×10^3	13×10^3	24×10^3
	3. Grower Mash	66×10^3	24×10^3	18×10^3	26×10^3
		62×10^3	22×10^3	24×10^3	23×10^3

Amongst the bacterial group *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus polymyxa*, *Staphylococcus aureus*, *Staphylococcus albus, Staphylococcus hominis, Micrococcus luteus*, *Micrococcus roseus*, *Enterobacter cloacea*, *Escherichia coli*, *Klebsiella oxytoca*, *Euterobacter intermedius*, *Alcaligenes faecalis*, *Acinetobacter mallei*, *Klebsiella aurogenes*, *Klebsiella liquefascieus*, *Pseudomonas aeruginosa* and *Flavobacterium rigense* were most prevalent while *Saccharomyces cerevisiae*, *Saccharomyces rouxii*, *Candida utilis*, *Hansenula anomala* and *Candida parapsilosis* were most prevalent among the fungi group.

Thirteen species of *Bacillus* were encountered and they were identified. They were all Gram-positive rods, catalase positive, motile, oxidase positive, most are citrate, starch, gelatin, casein and Proskaeur positive. Most species fermented glucose, sucrose, lactose, mannitol, fructose, Arabinose and Xylose (Table 4). They were mostly present in all poultry feeds samples.

Two species of *Clostridium* were isolated and identified as *Clostridium tertium* and *Clostridium septicum*. They were all gram-positive rods, catalase, oxidase, indole, methyl red, voges proskaeur, citrate, urease negative. They were both motile, casein, positive spore formers. *Clostridium tertuim* reduced nitrate to nitrite, fermented glucose, sucrose, lactose mannitol, maltose and fructose, while *Clostriduim septicum* liquefied gelatin, NO_3 reduction negative, fermented glucose, sucrose, lactose, xylose and fructose. The organism identified as *Flavobacterium* species, was yellow/orange, short rods, gram negative rods, catalase, oxidase positive *Flavobacterium rigense* is motile, urease positive and liquefied nutrient gelatin, fermented glucose, sucrose/mannitol.

Five species of *Pseudomonas* were encountered and they were identified as *Pseudomonas aeruginosa*, *P. mendocina*, *P. cepaciae*, *P. mallie* and *P. fluorescens*. They were all Gram-negative rods, catalase and oxidase positive, motile, urease negative. Most species liquefied nutrient gelatin. The species did not hydrolyse starch. Most species reduced nitrate to nitrite. *P. mendocina* fermented only glucose, other species fermented glucose, sucrose, mannitol, Arabinose / raffinose. *P. aeruginosa* did not ferment xylose, lactose and salicin whereas *P. fluorescens* fermented xylose, mannitol and salicin.

The next genus was identified as *Alcaligenes*, it was isolated from the five samples. The colonies were white, entire and raised. Cellular observation showed that they were coccibacilli in shape and Gram negative. The cells were motile, catalase, oxidase, citrate utilization and voges proskaeur positive, urease, indole, methyl red, starch and gelatin tests were all negative. The organisms did not ferment lactose, xylose, salicin, sorbitol, mannitol, maltose, arabinose, raffinose and fructose. It was subsequently identified as a strain of *Alcaligenes faecalis*.

The organism identified as *Acinetobacter iumiwoffi* was short rod, gram negative and non-motile. It was catalase, urease and citrate test positive and did not produce acid from most carbohydrate sugars tested, except glucose and mannitol sugars.

The next species were *Eriwinia*, they were identified as *Eriwinia carotovora* and *E. uredovor.* They were gram negative rods, motile, catalase positive, and voges proskaeur positive, liquefied nutrient gelatin, nitrate reduced. They both fermented carbohydrate sugars such as glucose, xylose, salicin, mannitol and arabinose, *E. uredovora*did not ferment sucrose, lactose sorbitol, maltose, raffinose and fructose. The organic identified as *Xanthomonas campestris* was yellow in colour, gram-negative rods in shape, motile, catalase positive, starch hydrolyzed and liquefied nutrient gelatin, they fermented glucose, sucrose, maltose, arabinose and fructose. They did not ferment lactose, xylose, sorbitol, Salicin, mannitol, and raffinose.

Two species of *Staphylococcus* were isolated and identified as *Staphylococcus albus*, and *S. ariettae*. Both were Gram-positive cocci, catalase positive, non-motile, oxidase negative except *S. albus*, indole, methyl red, citrate utilization tests negative, they did not hydrolysed and liquefied nutrient gelatin as shown in table 6. Both were voges proskaeur test positive and Urease positive. *Staphylococcus albus* did not ferment xylose, ribose, galactose, raffinose, arabinose, but *Staphylococcus ariettae* fermented almost all the sugars except galactose in which acid was not produced. *Micrococcus* species (*Intense, Candidus* and *Roseus*). They were all gram-positive cocci, catalase positive, oxidase positive except *Micrococcus roseurs* which was oxidase negative. *Micrococcus candidius* was urease, Voges proskaeur positive and liquefied nutrient gelatin. They all fermented glucose, sucrose, xylose and maltose sugars. Out of 12 poultry feed samples investigated, a total of 24 isolates were obtained. Gram negative isolates were about 12 isolates. Table 7 shows the identified fungi isolates (yeast isolates) *Saccharomyces* species (*cerevisiae, rouxii*, and *exigus*) *Candida* species (*parapsilosis, utilis, castelli, sphaenical,* and *glabaruta*) *Geotrichum* species (*Klebahnii* and *capitatum*) *Torulopsis stellate*, *Kluyveromyces maxicans*, *Hansenula anomola*, *Pichiaohmeri*, and *Rhodotorula glutinis*. They were all catalase positive, motile. Nitrate was not reduced except *Hansenula anomalla* in which nitrate was reduced to nitrite. *Saccharomyces* species, *Kluyveromyces* species, *Pichiaohmeri*, *Hansenula* species produced/formed ascospores from their asci. Carbohydrate sugars were fermented by the most yeast species. The fungi (molds) groups were significantly differently from the bacteria group and were in the order *Aspergillus niger*, *Rhizopus stolonifer*, *Mucorplumbeus*, *Fusarium oxysporium*, *Aspergillus chevalieri*, *Rhizopus arrhizus*, *Nigrospora oryzae*, *Absidiaspinosa*, *Aspergillus flavus*, *Aspergillus amstelodami*. Among the fungi (moulds) the most prevalent species were *A. niger*, *A. flavus*, *Fusarium oxysporum*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, and *Absidias spinosa* as shown in Figure I.

Table 4. Biochemical test on isolated bacteria

Isolate code	Colour/ pigment	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl/ red	Vp-voges prosphase	Urease	Citrate utilization	Gelutin hydrohyes	Stenrch hydrolyis	No_3 reduction	Probable identification
Top Feed															
1	Cream	+ve	Rods	-	-	-	+	-	-	-	-	-	-	+	*Clostridium tertium*
1	Cream	+ve	Rods	-	-	-	+	-	-	-	-	+	-	-	*Clostridium septicum*
2	Yellow Orange	-ve	Rods	+	+	-	+	-	-	+	+	+	-	-	*Flavobacterium rigense*
2	Yellow Orange	-ve	Rods	+	+	-	-	-	-	-	-	-	-	+	*Pseudomonas mendocina*
3	Cream White	+ve	Cocci	+	+	-	-	-	+	-	-	-	-	-	*Staphylococcus albus*
3	Cream White	-ve	Rods	+	+	-	-	-	-	-	-	+	-	+	*Pseudomonas cepaciae*
Hybrid Feed															
1	Yellow	+ve	Cocci	+	+	-	-	-	-	+	-	+	-	-	*Micrococcus intense*
1	Cream	-ve	Rods	+	-	-	+	-	+	-	-	+	-	+	*Erivinia carotovora*
2	Pinkish	-ve	Rods	+	-	-	-	-	-	+	+	-	-	+	*Acinetobacter iwoffi*
2	Orange Yellow	+ve	Cocci	+	-	-	-	-	+	-	-	-	-	-	*Staphylococcus ariettae*
3	Red	+ve	Cocci	+	-	-	+	-	+	-	-	-	-	+	*Micrococcus roseus*
3	Yellow	-ve	Rods	+	-	-	+	-	-	-	-	+	+	-	*Xanthomonas campestris*
Rabiu Feed															
1	Green	-ve	Rods	+	+	-	+	-	-	+	-	+	-	-	*Pseudomonas mallei*
1	Green	-ve	Rods	+	+	-	+	-	-	-	+	+	-	+	*Pseudomonas aeruginosa*
2	Bluish Green	-ve	Rods	+	+	-	+	-	-	-	-	+	-	+	*Pseudomonas fluorescens*
2	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
3	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
3	Pink	+ve	Cocci	+	-	-	+	-	+	-	-	-	-	+	*Micrococcus roseus*
F.A. Feed															
1	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
1	Yellow	-ve	Rods	+	-	+	+	-	+	-	-	+	-	+	*Erivinia uredovora*
2	Cream	-ve	Rods	+	-	-	+	-	+	-	-	+	-	+	*Erivinia carotovora*
2	Pinkish	-ve	Rods	+	+	-	+	-	+	-	-	-	-	-	*Alcaligenes faecalis*
3	Cream Butter	+ve	Rods	+	+	-	+	-	+	-	+	+	+	+	*Bacillus cereus*
3	Cream Butter	+ve	Rods	+	+	-	+	-	+	-	+	+	+	+	*Bacillus cereus*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 5. Biochemical test on isolated coliforms

Sample location	Isolate code	Colour/ pigment	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl /red	Vp-voges prosphase	Urease	Citrate utilization	Starch	No_3 reduction	Probable identification
Top Feed															
	1	Pink	-ve	Rods	+	-	+	-	-	+	+	+	+	+	*Klebseiella oxytoga*
	1	Pink	-ve	Rods	+	-	-	+	-	+	+	-	+	-	*Enterobacter aerogeres*
	2	Pink	-ve	Rods	+	-	-	-	+	-	+	+	-	-	*Klebseiella liquefezium*
	2	Black	-ve	Rods	+	-	-	+	-	-	-	+	-	-	*Salmonella arizonic*
	3	Black	-ve	Rods	+	-	+	+	+	-	-	-	-	+	*Escherichia coli*
	3	Red	-ve	Rods	+	-	-	+	+	+	-	+	-	-	*Serratia liquefasciens*
Hybrid Feed															
	1	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter intermedines*
	1	Cream	-ve	Cocci	+	-	+	+	+	-	-	+	-	+	*Citrobacter diversus*
	2	Cream	-ve	Rods	+	-	+	-	-	-	-	+	-	-	*Citrobacter koseri*
	2	Pinkish	-ve	Rods	+	-	-	+	-	+	+	+	+	-	*Enterobacter cloacae*
	3	Pinkish	-ve	Rods	+	+	-	+	-	-	-	+	-	-	*Alkaligenes faecalis*
	3	Pink	-ve	Rods	+	-	-	-	+	+	+	+	+	+	*Klebseiella planticola*
Rabiu Feed															
	1	Cream	-ve	Rods	+	-	-	+	+	-	+	+	-	-	*Citrobacter freundii*
	1	Pinkish Cream	-ve	Rods	+	-	-	-	-	-	-	+	-	-	*Acinetobacter mallei*
	2	Pink	-ve	Rods	+	-	-	+	-	+	-	-	-	+	*Enterobacter amigenus*
	2	Black	-ve	Rods	+	-	-	+	+	-	-	+	-	+	*Salmonella bougori*
	3	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter agglomerans*
	3	Black	-ve	Rods	+	-	+	+	+	-	-	-	-	+	*Escherichia coli*
F.A. Feed															
	1	Cream	-ve	Rods	+	-	-	+	+	+	-	+	+	-	*Proteus mirabilis*
	1	Black	-ve	Rods	+	-	+	-	+	-	-	-	-	+	*Escherichia coli*
	2	Pink	-ve	Rods	+	-	+	+	-	+	+	+	+	+	*Klebseiella oxytoca*
	2	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter intermedium*
	3	Cream	-ve	Rods	+	+	-	+	-	-	-	+	-	-	*Alcaligenes faecalis*
	3	Pink	-ve	Rods	+	-	-	+	-	+	+	+	+	-	*Enterobacter cloacae*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 6. Biochemical characterization of isolated *Staphlococcus* species

Staphlococcus	Colour	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl/ red	Vp-voges prosphase	Urease	Citrate utilization	No3 reduction	Probable identity
Isolate Code													
Top Feed													
1	White	ve	Cocci	+	+	-	-	-	+	-	-	-	*Staphlococcus albus*
1	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	White	ve	Cocci	+	+	-	-	-	+	-	-	+	*Staphlococcus albus*
2	Orange	ve	Cocci	+	-	-	-	-	+	-	-	+	*Staphlococcus ariettae*
3	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus carnosus*
3	White	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus simulans*
Hybrid Feed													
1	Yellow	ve	Cocci	+	+	-	-	-	-	-	+	+	*Micrococcus varians*
1	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	Yellowish	ve	Cocci	+	+	-	-	-	+	+	-	+	*Micrococcus kristinae*
2	White	ve	Cocci	+	+	-	-	-	+	-	-	+	*Staphlococcus albus*
3	Yellow	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus epidermid*
3	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
Rabiu Feed													
1	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
1	Yellow	ve	Cocci	+	+	-	-	-	+	+	-	+	*Micrococcus candidus*
2	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
2	Red	ve	Cocci	+	-	-	+	-	+	-	-	+	*Micrococcus roseus*
3	Yellow	ve	Cocci	+	+	-	-	-	-	+	-	+	*Micrococcus luteus*
3	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
F.A. Feed													
1	Red	ve	Cocci	+	-	-	+	-	+	-	-	+	*Micrococcus roseus*
1	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
2	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	Yellow	ve	Cocci	+	+	-	-	-	-	+	-	+	*Micrococcus luteus*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 7. Biochemical characterization of isolated yeasts

Yeast	Colour	Cellular morphology	Catalase test	No3 reduction	Ascospaze formation	Motility test	Urease test	Psedomycillui m production	Glucose	Maltose	Meubrose	Probable Oranism
ISOLATE CODE												
Top Feed												
1	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomyes cerevisiae*
1	Cream dull	Oval	+	-	-	+	-	-	+	+	-	*Candida parapsilopsis*
2	Cream rough	Cylindrical	+	-	-	+	-	-	+	-	-	*Geotrichum klebahnii*
2	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Ellipsoidal	+	-	-	+	-	-	+	-	-	*Saccharomye s rouxi*
3	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
Hybrid Feed												
1	Cream dull	Oval budding (small)	+	-	+	+	-	-	+	+	-	*Candida utilis*
1	Cream	Round budding	+	-	+	+	-	-	+	-	-	*Saccharomye s exigus*
2	Cream	Round	+	-	-	+	-	-	+	-	-	*Torulopsis stellata*
2	Cream	Round	+	-	+	+	-	-	+	-	-	*Kluyveromyc es maxians*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
Rabiu Feed												
1	Cream	Round budding	+	+	+	+	-	-	+	-	-	*Hansenula anomola*
1	Cream	Round budding	+	-	+	+	-	-	+	-	-	*Pichiaohmeri*
2	Cream	Oval small	+	-	+	+	-	+	+	-	-	*Candida castelli*
2	Cream	Ellipsoidal	+	-	+	+	-	-	+	-	-	*Saccharomye s cerevis*
3	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
F.A. Feed												
1	Red/Pink	Elongated	+	-	-	+	-	-	+	-	-	*Rhodoforula glutinis*
1	Cream	Cylindrical	+	-	-	+	-	-	+	+	-	*Geotrichum capitatum*
2	Cream	Oval small	+	-	-	+	-	-	+	+	-	*Candida sphaxrica*
2	Cream	Cylindrical	+	-	-	+	-	-	+	-	-	*Candida glabrata*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

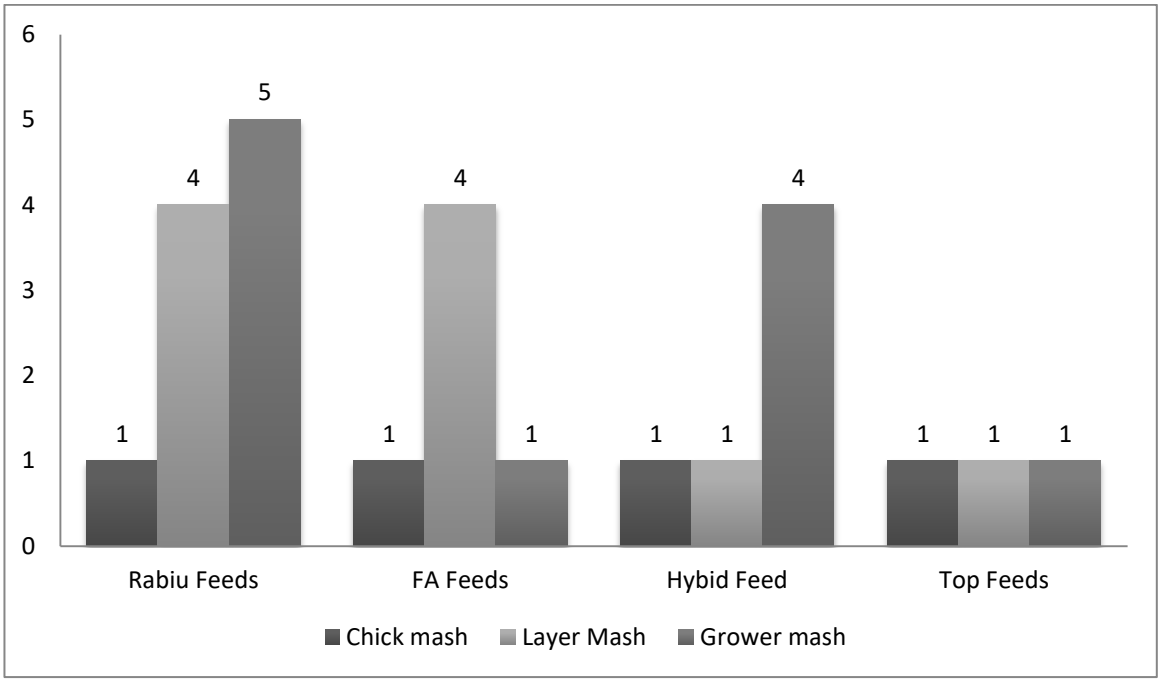

Figure 1. Histogram showing level of afflatoxins in (3) different poultry feed

Keys: 1 – No Aflatoxin detected, 4 – Aflatoxin (B1) detected, 5 – Aflatoxin (G1) detected
NOTE: X- Axis – Sample Names (Rabiu feeds, FA feeds, Hybrid feeds and lastly Top feeds)
Y- Axis- Toxins Status (1- Not detected, 4- AF(B1), 5- AF (G1)

This study revealed the microorganisms associated with poultry feeds that are commonly used within the south western region of Nigeria. The presence and isolation of these microorganisms depict that they are the causal agents responsible for the spoilage of poultry feeds. The slightly high viable staphylococcus counts bacteria, fungi (yeast/moulds), recorded may be associated with inadequate post-processing handling practices as spreading on the floor, mat and sometimes on high density polythene spread on the floor during and after pre-mixed bagging and packaging and during haulage and storage. These may also be responsible for the vast array of microorganisms detected and isolated. These finding corroborate with the report of Lund et al. (2000).

Low counts of coliforms and *Salmonella* were detected. However, there presence appeared transient since no growth was detected on agar plate following analysis after 24 hours. This may be due to their inability to with stand the micro environmental condition. The high rate of occurrence and distribution of moulds such as a *Aspergillus, Fusarium, Rhizopus, Taloromyces, Absidia* and others may be traced to the inadequate post-processing handling practices, storage in high environmental conditions, the ubiquitous nature of their moulds and their ability to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of the poultry feeds (Beatriz and Eliana, 2000).

It is a mandate of the World Health Organization (WHO) that chicken products be safe for human consumption. Important potential route for infections to enter the supply of food for humans is through microbial contamination of chicken feed (Kashiwazwki, 1999). To ascertain the load and the microorganisms connected with chicken diets in southwest Nigeria, this study was planned and executed. These are the primary industries that deal with poultry feeding. Despite the apparent similarity in contamination, market and factory-sourced feeds were analyzed independently due to the effects of storage, time, and environmental factors. A total of 132 isolates were discovered and acquired from the twelve (12) feeding samples that were analyzed. There were somewhat fewer Gram-negative isolates than Gram-positive ones. Gram-negative bacteria, particularly Salmonella, are more dangerous than Gram-positive bacteria, hence only a very small fraction of them were discovered here (Olajuyigbe et al., 2006 and Onajobi et al., 2017).

Salmonella arizonae and *Salmonella bongori* were both isolated from samples from Top feeds layer mash and Rabin feeds layer mash. This finding is consistent with previous work (Kidd et al., 2002). Feeds have been noticed to be the source of human infection due to eating chicken fed salmonella-contaminated feeds. Other feeds sources or samples were found free of salmonella but can be contaminated if stored in environment with about 20–25% moisture content.

The result showed that grower mash and chick mash were most contaminated due to period of feed storage and storage conditions are suspected to be behind the higher level of contamination. It was found that chick mash feed samples are the most contaminated followed by grower mash feed samples, followed by layer mash which was the least contaminated feed samples. This is mainly attributed to the high nutritive value of the feed samples (Sakazaki, 2000 and Onajobi et al., 2017).

Amongst Gram-negative bacteria *Escherichia coli*, followed by *Klebsiella* species *Enterobacter* species, *Citrobacter* species, *Pseudomonas* species, *Alcaligenes* species, *Acinetobacter* species, *Serratia* species, and *Proteus mirabilis* isolated from poultry feed was reported by Wadu (2002), additionally discovered in a poultry shop (Quinn et al., 1999).

The majority of feed sample isolates contained *Bacillus* species. According to, *Bacillus* spp. may be pollutants in poultry feed (Bryan and Doyl, 1995). In this experiment, the results were consistent with those established by Wadu (2002), who discovered that *Bacillus* species are the most prevalent isolate in chicken feed. Nada (2005) successfully isolated *Bacillus* species from chicken feed. The samples also included isolated *Staphylococcus* species, including *Staphylococcus aureus, Staphylococcus albus, Staphylococcus simulans*, and *Micrococcus* species. The public's health may be impacted by these organisms. The microflora in poultry feeds may be different and come from a variety of environmental factors, such as soil, temperature, dust, and insects. Pathogens may infect poultry feed ingredients at any moment when they are being grown, harvested, processed, or stored (Watkins et al., 2003).

Seventeen fungal isolates were recovered from the twelve feed samples. The yeasts found to spoil the feed samples were identified as *Saccharomyces roxii*, *Saccharomyces exigus*, *Geotrichum klebahaii*, *Candida utilis*, *Torulopsis stellata*, *Kluyveromyces maxians, Hansenula anomala*, *Candida castelli*, *Candida glabrata*, *Candida sphaerica*. *Geotrichum capitatum* and *Rhodotorula glutinis, Saccharomyces*, *Candida*, and *Geotrichum* species are dominant organisms in cereal-based foundation species of the genus *Saccharomyces* and *Candida* are widespread in nature and can be found on plants or material of plant origin in fermenting or spoiling food (Belgin and Kathryn, 2006).

Fungal colonies (moulds) selected from each plate were based on colony appearance. Colonies having characteristic features such powdery appearance, fluffy, velvety texture, low mycelia with colour ranging from white, gray to pinkish, pink, greenish yellow, black yellow, green, gray green and others were selected; fifteen fungal (moulds) isolated were selected, examined microscopically and identified by their cellular morphology and culture characteristics.

The moulds isolates encountered and identified are *Aspergillus niger*, *Tolaromyces thermophilus*, *Fusaruim oxysporium*, *Absidia spinosa, Mucor plumbeus, Aspergillus amstelodami, Nigrosporaoryzae*, *Aspergillus chevalieri, Rhizopus arrhizus* and others. These groups of moulds have been variously linked with the production of various types of mycotoxins under various condition (Tournas, 1994). Exposure of mycotoxins through ingestion of contaminated foods of poultry feeds by birds or chicken and inhalation to toxins produced have been linked to acute and chronic toxicity in animals. Since poultry feeds require little or no further processing or treatment prior to consumption by the chicken, there is the possibility of ingesting large dosage over a period of time with possible health hazards. Hence the need to develop adequate processing, handling and storage techniques for this relish poultry feeds (Kayode and Oworunubi, 1988 and Onajobi et al., 2020).

Conclusion

The present investigation or work revealed slightly high bioload and vast array of microorganism associated with poultry feeds and high rate of occurrence and prevalence of fungal producing mycotoxins. This is alarming and suggests early warning signals indicating the level of safety of available poultry feeds. It also warrants renewed vigilance on the efficacies of food processing conditions, feed handling and handlers' technical knowhow, hygiene practices and safety storage conditions.

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