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Microbiological Analysis of Poultry Feeds and Drinking Water Source of Poultry Pens

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Abstract

The production of poultry feeds requires microbiological safety regulations to minimize microbial contamination and eventual marketing of the bagged feeds. Samples were collected from four different poultry pens within Ilorin metropolis. A total of 18 bacterial species which included genera of *Staphylococcus , Micrococcus, Bacillus, Citrobacter, Pseudomonas, Enterobacter, Proteus , Corynebacterium, Klebsiella, Aeromonas* and *Klebsiella* were isolated and identified while 7 fungal species including *Aspergillus niger, Cladosporium cladosporioides, Aspergillus flavus, Fusarium oxysporium, Penicillium italicum,Mucor mucedo* and *Saccharomyces cerevisae* were isolated and identified. Total bacterial counts ranged between $1.0 \times 10^2 - 8.2 \times 10^3$ cfu/ml for water samples and $1.5 \times 10^3 - 9.4 \times 10^3$ cfu/ml for feed samples, fungal count ranged from $1.0 \times 10^2 - 1.1 \times 10^3$ cfu/ml for water samples and $3.0 \times 10^2 - 2.0 \times 10^3$ cfu/ml for feed samples.

Keywords: Poultry feeds, water quality, microbiological, hygiene, health

Introduction

Poultry feeds are food materials used in raising poultry birds. In Nigeria, the word poultry is gradually becoming synonymous with chickens but in actual fact, poultry includes all domesticated bird that can be used as a source of egg or meat production for human consumption. Such birds include chickens, turkeys, ducks and guinea fowl which are more acceptable to Nigerians than pea fowl, quail and pigeons which are popular in the western world. The raising of poultry birds on commercial level requires large scale use of commercially-prepared poultry feeds (Obi and Ozugbo, 2007).

The scale of feed production, as well as the possible occurrence of pathogenic bacteria, fungi and represents the most important potential risk to animal to animal and human health (Cegielska-Radziejewska *et al*., 2013). Poultry feeds have been identified as one of the sources of microorganisms to animals. Specifically, some of the additives have been incriminated amongst the principal sources of bacteria of public health concern. Various types of farmed animal diseases such as diarrheal diseases like bacillary dysentery, amoebic dysentery, fowl cholerae etc., salmonellosis, staphylococcosis, colibacillosis, erysipelas, listeriosis etc have been traced to the contamination of animal feeds (Uwaezuoke and Ogbulie, 2008).

Quality livestock feed is necessary for the maintenance of physiological functions and animal defense systems against diseases and parasites. Traditionally, feed quality has been specified on basis of the nutritional value of every individual feed component (Fink-Gremmels, 2004). Livestock feed quality may however be affected by various microorganisms such as bacteria and fungi growing in different parts of the world.

Water, on the other hand, makes up a large proportion of the body of the chicken, from 55% to 75%, therefore it is essential for life (Abbas *et al.*, 2008).

Although the importance of providing a sufficient amount of water or adequate access to it is well accepted, the importance of water quality on performance is often overlooked. Knowledge regarding water quality is important for poultry production as it provides producers with managerial information to prevent the potential adverse consequences of specific concentration of water constituents. These typically pertain to health and production parameters, the quality of the livestock product and the watering systems of intensive poultry production system. Water quality attributes can have a direct or indirect effect on performance. High levels of bacterial contaminants, minerals, or other pollutants in drinking water can have detrimental effects on normal physiological properties resulting in inferior performance (Blake, 2001).

Therefore, control measures must be considered as priority, in order to prevent the occurrence of diseases that are spread through water, and would certainly result in great economic losses.

In the rural areas, factors that increase the risk of occurrence of waterborne diseases include inadequate disposal of organic and inorganic residues from agriculture and livestock productions; the lack of concern regarding the quality control of the drinking water given to animals, resulting in the animals drinking water of very low quality. Diseases that can be transmitted to the bird flock through the drinking water may originate from water contamination by faeces and secretions of sick birds, or by the utilization of water already contaminated by pathogenic organisms that originate from other animal species and the man, such as in the case of *Salmonella* and

Escherichia coli, respectively. Diseases caused by bacteria, fungi, virus and protozoa are among the most common diseases in the poultry industry in which drinking water plays an important role (Blake, 2001).

The production of poultry feeds as well as drinking water for the birds requires microbiological safety regulations to minimize microbial contamination hence the need for microbiological investigation.

Materials and Methods

Sample Collection

Feed and water samples were collected three times each between 7a.m. and 9 a.m. using the methods described by Kator and Rhodes (2003) and Uwaezuoke and Ogbulie (2008), respectively. Feed samples were obtained from four different poultry pens within Ilorin metropolis viz. Faculty of Agriculture broiler pen, University of Ilorin (denoted *Site A*); Sani's poultry, Sanrab area, Ilorin (denoted *Site B*); Alao farms, Tanke, Ilorin (denoted *Site C*); and Ayo Poultry, Oke Odo, Ilorin (denoted *Site D*). Water samples were collected from both taps and wells.

Physicochemical parameters of the poultry feeds

pH

One gram of each sample was dissolved in 10ml of distilled water and the pH determined using a pH metre. The electrode was calibrated using buffer solutions of pH 4, 7, and 9 before every measurement. The electrode was immersed into a test tube containing some amount feed solution and left till the reading is constant (APHA, 1985).

Moisture content determination

This was carried out to determine amount of water present in the feed sample. The moisture content was determined by weighing 5g of the sample into a crucible which has been previously weighed. The feed and the crucible were dried in the oven at 105˚C to a constant weight and then cooled for 15 minutes in a dessicator each time before weighing.

Percentage moisture content is calculated as follows = Moisture content/Weight of sample x 100

Weight of crucible = W_1

Weight of crucible + sample = W_2

Weight of crucible + dried sample = W_3

Weight of dried sample = $(W_3 – W_1)$

Weight of wet sample = $(W_2 – W_1)$

Moisture content = $(W_2 – W_1)$ - $(W_3 – W_1)$

$$\text{Percentage moisture content} = \frac{(W_2 – W_1) - (W_3 – W_1)}{(W_2 – W_1)} \times 100$$

(Prabakaran and Dhanapal, 2009).

Ash Content Determination

The ash content of a biological material is an analysis term used for inorganic residues (minerals) in the materials after the organic portion has been burnt off. The weight of feed sample in a crucible was determined and then dried to a constant weight to remove available moisture**;** it is then ignited in a furnace at a temperature of 600˚C to 650˚C for 3hours. The residue was cooled in a desiccator and weighed. The weight of the ash was expressed as a percentage of the fresh sample. The ash content was calculated as follows:

Weight of crucible =W_1; Weight of crucible + sample = W_2; Weight of crucible + dried sample =W_3; Weight of the crucible + ash =W_4; Weight of sample = $(W_2 - W_1)$; Weight of dried sample = (W_3-W_1);Weight of ash = (W_4-W_1)
Ash content = $(W_4-W_1)/ (W_2 - W_1)$ X 100 (Prabakaran and Dhanapal, 2009).

Physicochemical Parameters of the Drinking Water

Temperature

Temperature of each sample was determined using mercury-bulb thermometer. The thermometer was inserted into a test tube containing some quantity of water sample and left for some minutes until the reading is constant (APHA, 1985).

Turbidity

Turbidity for each of the sample was determined using Genesys spectrophotometer with wavelength of 460nm. The blank was set to zero with distilled water. Each water sample is added into the cuvette of the spectrophotometer and absorbance reading which displayed on the screen was taken (APHA, 1985).

pH

The values of samples were determined using a pH metre. The electrode was calibrated using buffer solutions of pH 4, 7, and 9 before every measurement .The electrode was immersed into a test tube containing some amount water sample and left till the reading is constant(APHA, 1985).

Total Hardness

The total hardness of each sample collected was determined. A conical flask containing 100ml of the sample was supplemented with four drops of 25% of ammonia and swirled; this was followed by addition of an indicator, Erichrome Black-T indicator. The solution was titrated against 0.1M Ethylene Diamine Tetra Acetic Acid (EDTA) until the purple colour changed to blue. Hardness was obtained as follows:

Total hardness (mg/l) = Titre value x 100 (APHA, 1985).

Total Suspended Solids

For determination of the total suspended solids of each sample, a filter paper was oven-dried at 105˚C for 1 hour and the weight recorded as W_1, 100ml of sample was filtered through the paper and dried again in the oven for 1 hour at 105˚C. It was then weighed and recorded as W_2.Total suspended solids was determined as:

Total suspended solids (g/100ml) = $W_2 – W_1$ (APHA, 1985).

Total Chloride Content

One hundred millimeter of water was measured using a measuring cylinder. It was poured in a conical flask that has been properly rinsed with distilled. Some amount (2ml) of 5% potassium chromate was added as an indicator and the solution turned yellow. It was titrated with 0.1M silver nitrate until there was a faint pink colouration.

Total chlorine = Titre value of 0.1M $AgNO_3$ x 3.55 (APHA, 1985).

Microbiological Analysis

Estimation of Total Bacterial and Fungal Counts

The bacterial and fungal count of the feed samples and water samples collected were determined; serial dilution of the feed and water samples was made up to 10^{-2} . One gram of each of the feed sample was added in 9ml of sterile distilled water which

was shaken to make 10^{-1} and a sterile pipette was used to add 1ml of the 10^{-1} dilution into another test tube containing 9ml of sterile distilled water to make dilution up to 10^{-2}.

Sterile pipette was used to add 1ml of each of the raw water sample into 9ml of distilled water. It was shaken to make10^{-1} dilution. Another sterile pipette was used to take I ml of 10^{-1} dilution and added to another 9ml of sterile distilled water. It was shaken to make a10^{-2} dilution. One ml of appropriate dilutions was pipette into each sterile labelled Petri dish. For bacteria count, 10^{-2} dilutions were used for feed and water samples respectively and for fungal count, 10^{-2} dilutions was used for both feed and water sample. Sterile, cooled molten nutrient agar(NA) for bacteria and potato dextrose agar (PDA) for fungi was poured aseptically into the labelled Petri dishes, swirled gently to mix the content properly and allowed to set before incubation.

The bacterial plate was incubated in inverted position at 37˚C for 24hours while the fungal plate was incubated at room temperature for 72hours. After incubation, the plates were examined and the number of colonies counted with the use of colony counter (Salle, 1991).

Enumeration of Total Coliform Count

Presumptive and confirmed tests were employed for the enumeration of the most probable number (MPN) of coliform bacteria in the samples. The presumptive test was carried out using 3-3-3 regimen. The first three set of sterile single strength macConkey broth was inoculated with 0.1ml of water sample; the second three set of sterile single strength macConkey broth was inoculated with 1ml of water sample and the last three set of sterile double strength macConkey broth was inoculated with 10ml of sample. The broth bottles were incubated at 37˚C for 48hours to observe for acid and change in colour of the macConkey broth from red to yellow and accumulation of gas in the inverted Durham tubes. The most probable number of coliform/100ml of water sample was determined by making reference to MPN index table (Salle, 1991).

Enumeration of Faecal Coliform

One ml of aliquot of each water sample was inoculated unto the sterile Petri dish. Cooled and molten sterile Eosin methylene blue agar (EMB) was poured aseptically into the labelled Petri dishes and swirled to mix the content. The plate was incubated in an inverted position at 37˚C for 24hours. The number of colony formed was counted. This was further confirmed by the ability of the isolates to produce acid and gas at 44˚C in MacConkey broth (Gurgaon and Ashutosh, 2000).

Characterization and identification of isolates

Bacterial isolates were characterized and identified based on their colonial morphology, cellular morphology, and biochemical reactions (Fawole and Oso, 2004). Fungal isolates were identified on the basis of their colonial morphology and cellular morphology (Onions *et al*., 1981).

Physicochemical parameters

The average pH values of water samples ranged from 7.16 – 7.43 while those of the feeds ranged from 5.91 – 6.50.

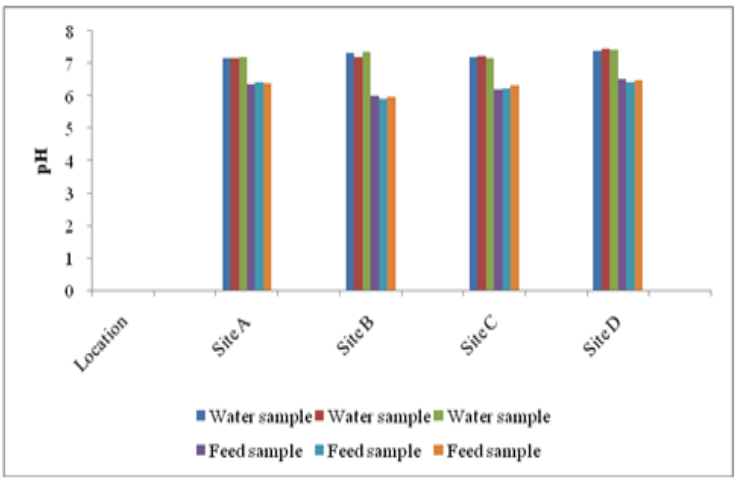


Figure 1: Average pH of water and feeds from the poultry pens

Temperature

Average temperature values were within the range of 26^{o}C to 30^{o}C. Figure 2 indicates temperature of the water samples.

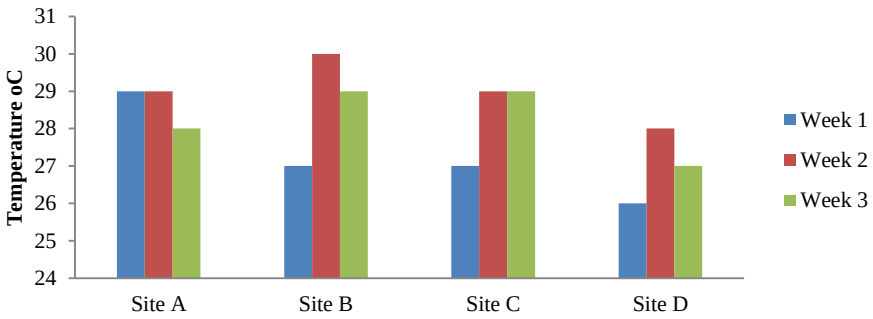


Figure 2: Average temperature of water sample from the poultry pens.

Turbidity

Turbidity trend observed over the sampling duration was uniform across all sampling location with a sharp rise and a steady decline after the first and second week respectively as shown in Figure 3. Turbidity ranged from 0.002 – 0.035 nm

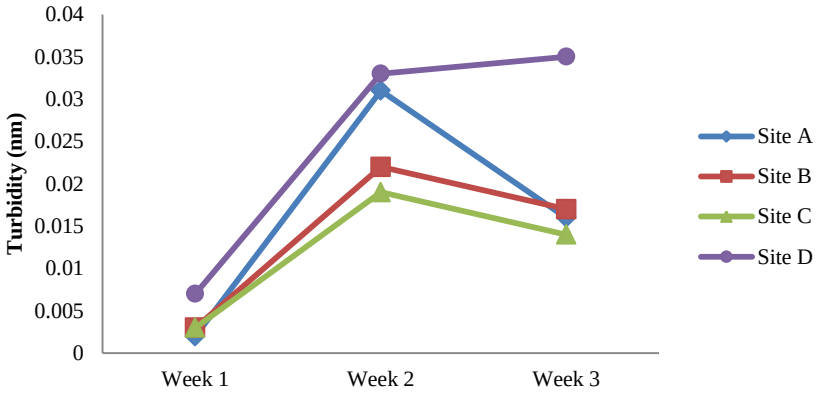


Figure 3: Average turbidity of water samples from the poultry pens

Total chloride content

Figure 4 indicates the average total chloride content levels which ranged from 3.4 – 4.97 mg/l for water samples from tap.

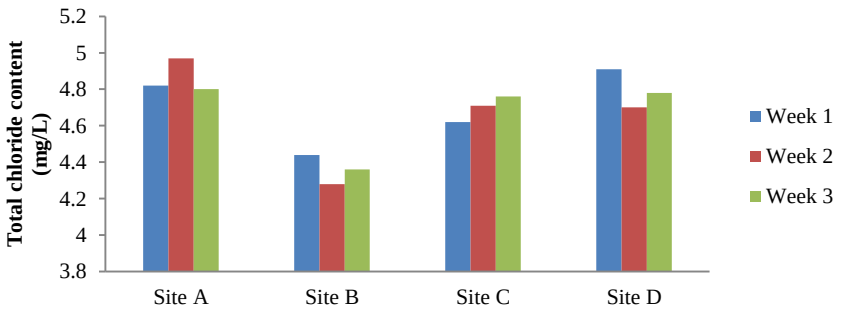


Figure 4: Average total chloride content of water sample from the poultry pens

Total suspended solids

Average total suspended solid for the water samples ranged between 0.015g/100ml to 0.470g/100ml.

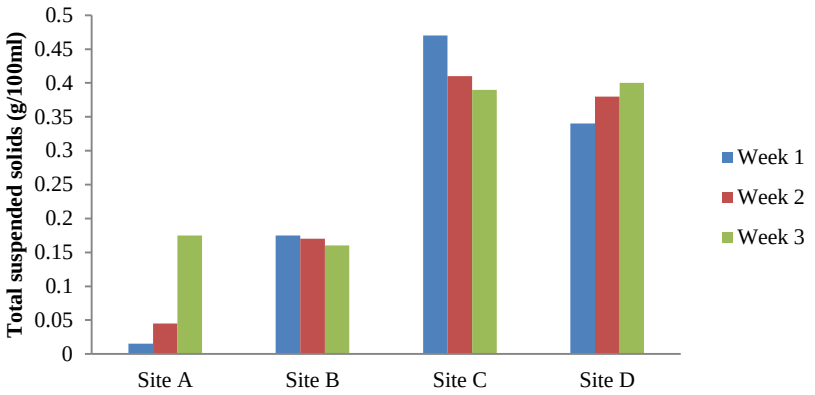


Figure 5: Average total suspended solids of water sample from the poultry pens.

Total hardness

Figure 6 shows that the average total hardness of water samples ranged from 60mg/l to 145mg/l.

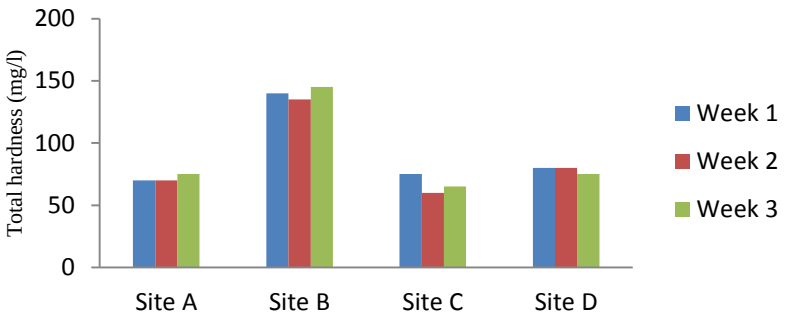


Figure 6: Average total hardness of water samples from the poultry pens (mg/l).

Moisture content of feeds

The average moisture content of the feeds ranged from 7 to 10% as shown in Table 2

Table 2: Moisture content (%) of feed sample from the poultry pens

Site	Week		
	1	2	3
A	8.27	7.90	8.23
B	9.31	10.00	9.06
C	7.00	7.35	7.39
D	7.77	7.94	8.24

Ash content of the feed

Figures obtained for average total ash content, in feed samples, did not vary widely with values ranging between 5.4% and 6.04% as shown in Table 3.

Table 3: Ash content (%) of feed sample from the poultry pens

Site	Week		
	1	2	3
A	5.90	5.91	5.73
B	6.04	5.90	5.93
C	5.70	5.40	5.05
D	5.96	6.00	6.02

Microbiological Analysis

Total bacterial count

Total bacterial counts for water ranged from $1 \times 10^2 - 8.2 \times 10^3$cfu/ml while that of feeds ranged from $1.5 \times 10^3 - 9.4 \times 10^3$cfu/ml. High bacterial count was obtained from Site D while Site A had high fungal population (Table 4).

Table 4: Total microbial count of water and feed samples

Location	Total bacteria count (n x 10^2cfu/ml)						Total fungal count (n x 10^2cfu/ml)					
	Water sample			Feed sample			Water sample			Feed sample		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
A	17	2	7	63	60	55	11	11	6	9	5	3
B	24	3	2	84	72	40	1	6	1	3	8	4
C	13	4	3	19	15	16	6	4	2	5	15	20
D	82	24	8	94	70	75	1	2	4	7	6	3

Total coliform count ranging from 3 to 1100MPN/100ml obtained is not surprising considering sanitary appraisal of the environments. It is however puzzling that figures obtained from Site A was constant throughout the period under review (Table 5).

Table 5: Total coliform (MPN/100ml) and faecal coliform count (cfu/ml) of water samples

Location	Week 1		Week 2		Week 3	
A	1100	33	1100	84	1100	50
B	1100	0	9	0	9	0
C	3	0	3	0	3	0
D	3	3	9	0	3	3

A total of 18 bacterial isolates were identified based on morphological, cellular and biochemical characteristics and included *Staphylococcus hominis, Staphylococcus saprophyticus, Micrococcus luteus, Staphylococcus aureus, Bacillus pasteurii, Citrobacter freundii, Pseudomonas aeroginosa, Enterobacter intermedius, Proteus vulgaris, Corynebacterium xerosis, Klebsiella edwasii, Micrococcus varians, Bacillus cereus, Corynebacterium kutscheri, Bacillus subtilis, Aeromonas salmonicida, Enterobacter aerogenes* and *Klebsiella aerogenes* while 7 fungal isolates identified on the basis of the colonial, morphological and microscopic characteristics of their vegetative bodies and spores were *Aspergillus niger, Cladosporium cladosporioides, Aspergillus flavus, Fusarium oxysporium, Penicillium italicum,Mucor mucedo* and *Saccharomyces cerevisae.*

Discussion

Result of pH investigation indicates that while water samples were generally neutral, poultry feeds were slightly acidic. The acidic nature of the feeds is not far-fetched as the maize grains, which forms components of the feed, are slightly acidic as supported by research work of Cegielska *et al*. (2013). Values obtained for water samples (7.16 – 7.43) were similar to that obtained by Abass *et al*. (2008) where pH values ranged from 7.0 and 7.5. This is within the 6.8 and 7.5 limit recommended by Carter and Sneed (2007) for poultry. Chloride content, ranged from 4.28 to 4.97 mg/l which is however different from that obtained for broiler chicks by Abass *et al*. (2008) who reported values ranging from 8 to 16 mg/l in a similar investigation.

This detail would be of significant economic importance to the poultry farmer who would invariably prefer to lower the cost of production.

Since standards for animal drinking-water indicate that there should be fewer than 100 bacteria of all types per millilitre (ml) of water and fewer than 50 coliform bacteria per ml (Carter and Sneed, 2007), the samples had total bacterial count ranging from1.0 x 10^1 - 8.2 x 10^3cfu/ml thus, fall within the specifications stated by the aforementioned standard. However, coliform count ranging from 3 - 1100MPN/100ml is a cause for concern. Bacterial count in feed sample, ranged from 1.5 x 10^3 - 9.4 x 10^3 cfu/ml signifies the need for stricter hygiene practices in these poultries. This result is significantly different from that reported by Obi and Ozugbo (2007) where values between 1.13 x 10^1 - 3.0 x 10^8cfu/ml were obtained but similar to those reported by Cegielska-Radziejewska *et al*. (2013) where 1.9 x 10^1 - 3.7 x 10^3cfu/ml were reported.

The implication of these values is in the associated health risk to the poultry and, of course, the consumers. Crump *et al*. (2002) and Cox *et al*. (2013) reported cases of obvious link between human food-borne illness and contaminated feeds thus this fear is not unsubstantiated. Its significance is even further reiterated by the bacteria and fungi isolated from the feed samples.

The presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Fusarium oxysporium*, *Aspergillus niger*, and *Mucor mucedo*, whose sources in the poultry feeds have been attributed to the either cereal grain, air or human handlers is not surprising as reported also by Cegielska-Radziejewska *et al*. (2013). This is also corroborated by Obi and Ozugbo, (2007), and Uwaezuoke and Ogbulie, (2008) in similar researches on the microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. The presence of the isolates signifies the conditions and standard of storage strategies employed. The farmer, on the other hand, should ensure proper disposal of poultry droppings to prevent transmission of pathogens from the droppings to the feed (Obi and Ozugbo, 2007).

The presence of *Aspergillus* is particularly alarming because of its involvement in the production of aflatoxins, which have been found to be carcinogenic, teratogenic and mutagenic in humans and birds (Obi and Ozugbo, 2007). In view of this, it is crucial to control the level of mycotoxins in feeds.

Aesthetics is a consumer's first hygiene standard, the poultry farmers are advised to improve significantly on the hygiene of the water source. Similarly, government regulatory agencies should present appropriate guidelines, enforce the guidelines, and ensure strict adherence to the guidelines.

Conclusion

Contaminaton of the various water samples sources and feeds in this study may be as a result of unhygienic handling, processing and purification procedures of water or lack of it,production of feeds and inadequate sanitary conditions. Workshops should be organised for poultry workers on adequate daily hygiene practices. Government and public health parastatals should raise public awareness on the human diseases that could be possibly contracted from poultry birds that consume contaminated feeds and much needed microbial safety in commercial poultry farms in the state.

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