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Incidence of *Salmonella spp., Klebsiella spp., Proteus spp.* and Two Micronutrients (Zinc and Iron) Present in Poultry Feeds Collected from Sokoto, Nigeria

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Abstract

Mostly, poultry quality is determined by its feed. Thus, in this time of ravaging pollution and infectious diseases monitoring of quality of products related to poultry is eminent. This study aimed at determining the incidence of some microbes and micronutrients (zinc and Fe) in poultry feed in Sokoto, Nigeria. Standard methods and materials of analytical grade were used. Zinc and iron micronutrients were determined in feed and egg using the method of atomic absorption spectroscopy. Likewise, standard methods were used to determine Salmonella spp., Klebsiella spp. and Proteus spp. in feed. The most frequent bacteria were Salmonella spp., then Klebsiella, and lastly the Proeus spp. The concentrations of zinc, and iron micronutrients present in different poultry feeds and whole egg are as follows: the zinc values in different feeds ranges from 10.12 ± 3.5 to 20.60 ± 6.6 ppm; and likewise, the iron ranges from 14.6 ± 0.12 to 40.10 ± 2.5 ppm (P<0.05). The CDI for zinc and iron are lower than 1, the HQ for zinc is very elevated, and that of Fe is lower than 1. However, the HI for the micronutrients is of concern, because it is above 1. The found organisms may contaminate poultry products and adversely affect the public health. However, the micronutrients determined in different feeds were lower than standard, but the presence of these metals in the examined poultry egg could pose hazard to the public like the microbes.

Keywords: Poultry, gg, micronutrient, zinc, iron, hazard, microbes

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INTRODUCTION

Living organisms are distinguished from non-living things due to their high level pf organization displayed bin their ability to perform basic functions of life such as reproduction, growth, movement, irritability, excretion, respiration, and nutrition. All these processes of life are possible when there is energy and raw materials for biosynthesis within the body, which are only obtained from food, because humans cannot make their own food [1]. Food is anything that provide nutritional support for the body and usually hails from plants or other animals. Poultry is an example of food that is beneficial to humans [2]. Throughout the world, the poultry meat is about to be the most consumed after cattle. It is a source of economic, and nutritional benefits [3].

In Nigeria, there is high prevalence of malnutrition among the populace. There is shortage

of proteins, there is micronutrients deficiency, and shortage of vitamins intake in many circumstances [4]. Thus, there is need for the contribution of poultry in meeting up the demands for balanced nutrition among the population of the country. Egg remain a major product from poultry and is a common diet rich in nutrients that can serve in many purposes. There are different types of eggs sold in markets around the nooks and crannies of the state comparatively at lower price and accessible to consumers [5,6, 7,]. Eggs from poultry are widely used carbohydrate, lipids, minerals, and vitamins source, playing a major role among the populace [5]. Parable, an egg is composed of three parts, namely, yolk, white, and shell. About 11.0% protein is provided by a typical egg, and about 75 Kcal of energy is provided when taken in egg. likewise, enzymes that are useful to the biological system can be obtained from egg consumption as well [8].

However, there is concern about the feed consumed by poultry. The current rise in pollution has in turn put poultry feed at risk of being contaminated by microbes or metallic substances. Poor quality feed affects the health of poultry and humans as well [9,10]. Due to the nutritious components present in poultry feed and poor handling or processing, the microbes find the feed suitable as breeding ground to obtain nutritional food requirements [11].

Consequently, the microbes contribute to poor health in poultry and elicit public health concern in humans as well. Additionally, the colonization of poultry feed by the microbes reduces the nutrients availability in feed, thereby causing low nutrition in the affected poultry. Moreover, in the course of the fed lifecycle, micronutrients such bas zinc and iron are incorporated deliberately for nutrition reason or accidently through pollution or contamination effects [12,13,]. Zinc is an important player in biological processes such as growth, immunity, development and reproduction. Iron is needed by many enzymes and proteins [14,15]. (Sousa et al., 2019). Nevertheless, due tom pollution or poor preparation methods, micronutrients in poultry feed can also be surplus and that condition affect the health of poultry and ultimately affect humans as the consumers [14,15]. In this part of the country, there is poor monitoring of feeds quality and scarce information pertaining that. It is indeed imperative to unveil the quality of feeds regards to microbial and metallic contents to safeguard public health [12]. Thus, this study aimed at determining the incidence of some microbes and specific micronutrients (zinc and Fe) in poultry feed in Sokoto, Nigeria.

MATERIALS AND METHODS

Study Area

The present study was carried out at Microbiology laboratory in Umaru Ali Shinkafi Polytechnic, Sokoto State, Nigeria.

Collection of samples

The 8 types of poultry feed were properly collected under aseptic measures from some farms and markets located in Sokoto, Nigeria. These samples of different brands were labelled Sample A, Sample B, Sample C, Sample D, Sample E, Sample F, Sample G and Sample H were immediately taken to the Microbiology laboratory of Umaru Ali Shinkafi for further analysis.

Preparation of Media

Nutrient agar

28g of nutrient agar powder was weighed using weighing balance and poured into a conical flask. It was dissolved in 1000ml of distilled water and was placed on hot plate to dissolve properly. Then, autoclave aided in sterilization (at a temperature of 121 degree Celsius) for fifteen minutes. The media was allowed to cool and it was dispensed into sterile petri-dishes [16].

Sample processing

The peptone water was prepared by adding peptone (6.75gm) in 225ml distilled H_2O contained in 250ml flask. The flasks were gently swirled and covered with aluminium foil. After wrapping the mouth

and properly labelling, the flasks were autoclaved at 121°C for 15mins. The flasks were removed from the autoclave and were kept at room temperature. Twenty-five gram was weighed from each feed sample and inoculated into the flasks containing 225ml peptone water for the enrichment of the bacteria. These flasks were then incubated at 37°C for 24 hours in the incubator [17].

Inoculation

For culturing, 1 ml of the Enriched media was aseptically introduced into 9 ml of sterile water and mixed properly to give good homogenate used as stock. A ten-fold serial dilution was made for samples in appropriate dilution tubes which were done until the 5th serial dilution. Rod and plate technique and nutrient agar media were utilized to culture dilutions. Then incubation at 37°C was done. Each plate was observed after 24 hrs for visible growth. The colonies were counted as the Total Viable Count (TVC) Clinical and Laboratory Standards Institute (CLSI), (2008) [17].

For sub-culturing, the colonies on the NA media were inoculated in the selective media, for the identification of microbes from the same dilutions of the different feed samples and were incubated at 37°C overnight. Selective media were used for inoculation of nutrient broth media for the feed samples, then incubated at 37°C overnight. The bacterial load count of was carried out (Clinical and Laboratory Standards Institute (CLSI), (2008) [17].

Characterization Tests for Bacteria

Colonies to be identified were picked from each plate and kept on slants of nutrients agar medium for further biochemical analysis. Standard methods were used for the microscopic examination; Motility test, Indole test, Methyl red test, Voges Proskauer, Citrate test, Sugar fermentation test, Motility test and Oxidase test as described in Cheesbrough (2006) [16].

Bacteriological Analysis

The inoculation of study specimens into the blood agar was done. Then, plates were placed in inverted format and incubated at the presence of oxygen at 37°C for to stay for twenty-four hours for the purpose of growth examination. Bacteria cultures were purely and aseptically obtained through streaking representative colonies that are diverse in morphology on blood agar. The colonies were then examined with naked eye for their morphological properties and any change in the media. The isolates were then cultured to differentiate colonies of bacteria and a loop of the isolates were inoculated into nutrient broth for further investigation [16].

Serial Dilution

One germ of feed sample was added to test tube containing 9 ml sterile normal saline were prepared, by sterile tip on micropipette transferred 1 ml of dilution to labelled as the first dilution. Ten-fold serial dilutions of poultry feed samples were prepared. Four test tubes containing 9 ml sterile normal saline were prepared. Before holding a micropipette vertically, it was sterilized; then inserted in 3cm of the feed sample surface to obtain 1 ml and placed in first tube of the dilution series (touching of dilution fluid was avoided). The top part was discarded and labelled the initial tube as first dilution tube (10^{-1}). A new and sterilized strip aided in the mixing of the contents of the first dilution. Then, 1 ml from the first dilution series was placed in the second tube of the dilution series, the tip was discarded and labelled the tube the second dilution tube (10^{-2}). Further dilution of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared similarly. Ten samples from each feed source were randomly selected for viable count [16].

Grams staining

This method used to differentiate bacteria into Gram-negative (pink) and Gram-positive bacteria (purple). It was done based on the ability of the bacteria to retain the colour of stains used during gram reaction. Alcohol was utilized to remove colour from the gram-negative bacteria. However, the gram-negative bacteria were not decolourized. After decolourization step, a counter stain (carbol fuchsine) was used to impart a pink colour to the decolorized Gram-negative bacteria [16].

Biochemical tests

Catalase Test

Catalyst test was done by using a stick of broom to pick the bacteria, then a drop of hydrogen peroxide was allowed on a clean tile, and the broom containing the sample (bacteria) was mixed together with the hydrogen peroxide and if there is bubbles, it means its positive (+ve) and if bubbles does not form, it means it is negative (-ve) (Bashan et al., 2014) [18].

Coagulase Test

Coagulase is done using a clean tile also and stick of broom. The sample is picked using the stick of broom and a drop of the serum is allowed on the tile and the stick of broom containing the sample is used to mix the serum together with the sample and if there are bubbles, it means its positive (+ve) and there are no bubbles means its negative (-ve) (Bashan et al., 2014) [18].

Estimation of Human Health Risk

Human Health risk was calculated using different equations shown in this section.

$$CDI=CP\times IR\times EF\times ED/Bw\times AT$$

Where, CDI= Chronic Daily Intake, CP= concentration of metal in herbal snuff, IR=Ingestion Rate=1, EF= Frequency of Exposure=90 days, ED=Exposure Duration=30 days, Bw=weight=70 kg for adult and 30 kg for children AT= 2700 days.

Hazard Quotient= CDI/RfD

Where, RfD= Chronic Oral Reference Dose, Zn=0.003mg/kg, Fe=0.7 mg/kg

Transfer factor (TF)= Concentration of metal in egg/ concentration of metal in feed [19,20].

Statistical Analysis of Data

Data generated were statistically analysed using IBM - SPSS Statistics version 20 computer program. The one-way analysis of variance (ANOVA) and chi-square test were used to ascertain the prevalence of *microbial* species contaminations among the different collected from different farms; and the heavy metals measured in feed and egg at $P < 0.05$.

RESULTS AND DISCUSSION

The results of this study are presented in Tables 1-4.

Table 1. Morphological and Biochemical Characterisation of the bacteria isolated from the study area.

S.N.	Gram Reaction	Morphology	Cat	Coag.	Slant	Butt	Gas	H_2S	Isolated organisms
1.	-ve	Rod	+ve	-ve	A	A	+	-	*Klebsiella sp.*
2.	-ve	Rod	+ve	-ve	K	A	+	+	*Salmonella spp.*
3.	-ve	Rod	+ve	-ve	A	A	+	-	*Klebsiella Spp.*
4.	-ve	Rod	+ve	-ve	A	A	+	+	*Proteus spp.*
5.	-ve	Rod	+ve	-ve	K	A	+	+	*Salmonella spp.*
6.	-ve	Rod	+ve	-ve	K	A	+	+	*Salmonella spp.*
7.	-	-	-	-	-	-	-	-	-
8.	-	-	-	-	-	-	-	-	-
9.	-	-	-	-	-	-	-	-	-
10.	-	-	-	-	-	-	-	-	-

Key: *A = Acidic. K = Alkaline. Coa = Coagulase test. Cat = Catalase test*

The different characteristics regarding the diverse bacteria studied in work were shown in Table 1. Therein, *Proteus spp, Salmonella spp., and Klebsiella spp* were identified to be present in poultry feeds

collected from Sokoto, Nigeria.

Table 2. Percentage frequency of occurrence.

Isolates	Frequency	Percentage (%) of occurrence
Salmonella spp.	3	50.0
Klebsiella	2	33.33
Proeus spp.	1	16.67
Total	**6**	**100**

The Table 2 shows the frequency and percentages of the three bacteria species found in poultry feeds in Sokoto, Nigeria. The most frequent bacteria were *Salmonella spp.,* then *Klebsiella,* and lastly the *Proeus spp.*

Table 3. Showing the concentrations of micronutrients zinc and iron different types of poultry feed and whole egg collected in Sokoto, Nigeria

Feed	Zinc (ppm)	Transfer factor for Zinc	Iron (ppm)	Transfer Factor for Fe
A	*10.12 ± 3.5*	0.6047	40.10 ± *2.5*	0.261
B	*15.20 ± 0.5*	0.4026	17.80 ± *2.6*	0.587
C	*21.60 ± 6.6*	0.297	18.80 ± 2.6	0.5559
D	*15.01 ± 0.1*	0.290	25.10 ± *0.12*	0.416
E	*12.60 ± 0.05*	0.408	35.01 ± 0.16	0.298
F	*17.8 ± 1.15*	0.488	14.6 ± *0.12*	0.716
G	*20.60 ± 6.6*	0.344	18.80 ± *1.5*	0.559
Whole egg	*6.12 ± 0.5*	10.45 ±*0.1*		

Key: Result is expressed as mean ± standard deviation

The Table 3 shows the concentrations of zinc, and iron micronutrients present in different poultry feeds and whole egg. The zinc values in different feeds ranges from 10.12 ± 3.5 to 20.60 ± 6.6 ppm; and likewise, the iron ranges from 14.6 ± 0.12 to 40.10 ± 2.5 ppm.

Table 4. Showing the estimated risks due to consumption of poultry that takes feed containing Zn and Fe in Sokoto, Nigeria

CDI	Zinc	Iron	HI	HQ for Zn	HQ for Fe
Adult	0.874	0.149	2913.543	2913.33	0.213
Children	0.044	0.116	146.836	146.67	0.166

Table 4 shows the CDI, hazard quotients, and hazard index of metals determined egg in Sokoto. The CDI for zinc and iron are lower than 1, the HQ for zinc is very elevated, and that of Fe is lower than 1. However, the HI for the micronutrients is of concern, because it is above 1.

Generally, poultry is an important aspect of our daily nutrition. Egg is a poultry product of versatile roles in the human nutrition. However, food safety is becoming a global concern, because of rising pattern of pollution of our environment, and food items [21, 22]. In turn, due to pollution of food items, like poultry (egg in particular) through the inappropriate release of chemicals (such as zinc and iron) and contamination through the release of microbes, the health of many consumers may be at stake [8]. Therefore, it is imperative and pertinent to monitor the quality of poultry food in our country. Poor quality feed due to chemicals contamination or microbial contamination affects the health of poultry and can easily transgress to affect humans in the course of the food chain (Hamad etal., 2012). In this study, an analysis of possible microbial contamination shows that, the most frequent bacteria were *Salmonella spp.,* then *Klebsiella,* and lastly the *Proeus spp.* This is in agreement with a study from Iraq, that shows the presence of *Klebsiella,* and *Proeus* spp in quail birds and can be a source of microbes to the humans consuming the poultry products [23]. Likewise, *Salmonella spp., Klebsiella,* and *Proeus*

spp were determined in chickens in an Indonesian study due to poor sanitation and poor hygiene [24]. The presence of microbes of public health importance in poultry feed might be due the original source of the products, poor manufacturing, poor processing, and poor handling [24]. However, the concern is, the microbes in feed can be relayed to the humans and other animals. Thus, the presence of microbes in feed call for more proper preparation and handling from the sides of manufacturers, sellers, and handlers as well [3].

Nevertheless, apart from the microbial content of feeds, it is also significant to measure the amount of micronutrients such as zinc and iron present in the fed, because uptake of excess amount of metals is possible considering the rising pollution trend across parts of the world. Excess intake of zinc and iron by poultry can harm the health of the poultry and can serve as source of excess metals to the humans and resultant effects can occur [12]. The Table 3 displays a varying concentrations of Zn and Fe in various feeds and egg collected in Sokoto, Nigeria. The zinc found in all the feeds is lower than the 500 mg/kg stipulated by European Union [12]. And is lower than the concentrations shown by a Southern Nigeria study [12]. Another similar outcome was related in a study done by Akter et al., (2020) [25]. The lower levels of Fe (in Table 3) is also lower than the finding of Okoye etal., (2011) [12]. Thus, the levels of zinc and iron are low and might indicate low quality of the feed being taken by the poultry and could in turn affects the consumer [12]. To estimate the possibility of transfer of Fe and Zn from the feed to egg, transfer factors were calculated and all the values in Table 3 show levels that are less than 1; therefore, probably, the metals do not come from the feed [26, 27]. Moreover, Table 4 shows, parameters of chronic daily intake (CDI), hazard index (HI), and hazard quotient (HQ) pertaining the elements zinc, and iron present in egg (that might be due to the feed of the poultry). The CDIs for all the two micronutrients are below 1, and thus, are less likely to elicit non-cancer negative effects to consumers. HQs for Fe are lower than 1 and therefore more likely unable to elicit health effects on consumers. However, the HI values for adult and children consuming the egg examined are above 1, and are more likely to cause effects on consumers. This was similar to finding of Igwemmar & Kakulu (2022) [28] in an Abuja study (Nigeria). It is now imperative to call on stakeholders to ensure proper quality assurance of poultry products to safeguard public health [25].

CONCLUSION

This study aimed at determining the incidence of some microbes and micronutrients (zinc and Fe) in poultry feed in Sokoto, Nigeria. It has found that the presence of three microbes, namely, *Salmonella spp., Klebsiella spp.* and *Proteus spp*. These organisms may contaminate poultry products and adversely affect the public health. The total aerobic count of bacteria was found to be high. However, the micronutrients determined in different feeds were lower than standard, but the presence of these metals in the examined poultry egg could pose hazard to the public like the microbes.

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