

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 iron) 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 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 range from 10.12 ± 3.5 to 20.60 ± 6.6 ppm; and the iron values range from 14.6 ± 0.12 to 40.10 ± 2.5 ppm ($P < 0.05$). The chronic daily index for zinc and iron are lower than 1, the hazard quotient for zinc is very elevated, and that of iron is lower than 1. However, the hazard index values for the micronutrients is of concern, because they are 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, egg, micronutrient, zinc, iron, hazard, microbes

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INTRODUCTION

Living organisms are distinguished from non-living things due to their high level of organization displayed in their ability to perform basic functions of life such as reproduction, growth, movement, 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 provides 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, poultry meat is 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 remains 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 at comparatively lower price and accessible to consumers [5–7]. Eggs from poultry are widely used as a source of carbohydrate, lipids, minerals, and vitamins source, playing a major role in the diet of the populace [5]. 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. 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 feed lifecycle, micronutrients such as zinc and iron are incorporated deliberately for nutrition reason or accidentally 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]. Nevertheless, due to 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 iron) 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 eight 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, and they were immediately taken to the Microbiology Laboratory of Umaru Ali Shinkafi for further analysis.

Preparation of Media

Nutrient Agar

Twenty-eight grams of nutrient agar powder was weighed using weighing balance and poured into a conical flask. It was dissolved in 1000 mL of distilled water and was placed on a hot plate to dissolve properly. Then, autoclave aided in sterilization (at a temperature of 121°C) for 15 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 (6.75 g) in 225 mL distilled H₂O contained in 250 mL 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 15 minutes. The flasks were

removed from the autoclave and were kept at room temperature. Twenty-five grams were weighed from each feed sample and inoculated into the flasks containing 225 mL 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 10-fold serial dilution was made for samples in appropriate dilution tubes which were done until the fifth 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 hours for visible growth. The colonies were counted as the total viable count (TVC) according to the Clinical and Laboratory Standards Institute guidelines [17].

For sub-culturing, the colonies on the nutrient agar 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 was performed as per Clinical and Laboratory Standards Institute guidelines [17].

Characterization Tests for Bacteria

Colonies to be identified were picked from each plate and kept on slants of nutrient agar media 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, and Oxidase test [16].

Bacteriological Analysis

The inoculation of study specimens into the blood agar was done. Then, plates were placed in inverted format and incubated in the presence of oxygen at 37°C and allowed to stay for 24 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 3 cm 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 dilutions 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].

Gram Staining

This method used to differentiate bacteria into Gram-negative (pink) and Gram-positive (purple) bacteria. 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 counterstain (carbol fuchsin) was used to impart a pink colour to the decolorized Gram-negative bacteria [16].

Biochemical Tests

Catalase Test

Catalase test was done by using a broomstick to pick the bacteria, then a drop of hydrogen peroxide was placed 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 it is positive (+ve) and if bubbles do not form, it means it is negative (–ve) [18].

Coagulase Test

Coagulase is done using a clean tile also and a broomstick. The sample is picked using the broomstick and a drop of the serum is placed on the tile and the broomstick containing the sample is used to mix the serum together with the sample and if there are bubbles, it means it is positive (+ve) and if there are no bubbles it means it is negative (–ve)[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 and AT = 2700 days.

Hazard Quotient (HQ) = CDI / RfD

where RfD = chronic oral reference dose, Zn = 0.003 mg/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 characterization of the bacteria isolated from the study area.

S.N.	Gram Reaction	Morphology	Cat	Coag.	Slant	Butt	Gas	H ₂ S	Isolated organisms
1.	–ve	Rod	+ve	–ve	A	A	+	–	<i>Klebsiella</i> spp.
2.	–ve	Rod	+ve	–ve	K	A	+	+	<i>Salmonella</i> spp.
3.	–ve	Rod	+ve	–ve	A	A	+	–	<i>Klebsiella</i> spp.
4.	–ve	Rod	+ve	–ve	A	A	+	+	<i>Proteus</i> spp.
5.	–ve	Rod	+ve	–ve	K	A	+	+	<i>Salmonella</i> spp.
6.	–ve	Rod	+ve	–ve	K	A	+	+	<i>Salmonella</i> spp.
7.	-	-	-	-	-	-	-	-	-
8.	-	-	-	-	-	-	-	-	-
9.	-	-	-	-	-	-	-	-	-
10.	-	-	-	-	-	-	-	-	-

A, acidic; K, alkaline; Coag, coagulase test; Cat, catalase test.

The different characteristics regarding the diverse bacteria studied in work are 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
<i>Salmonella spp.</i>	3	50.0
<i>Klebsiella</i>	2	33.33
<i>Proeius spp.</i>	1	16.67
Total	6	100

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 spp.*, and lastly the *Proeius spp.*

Table 3. Concentrations of micronutrients zinc and iron different types of poultry feed and whole eggs 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		

Results are expressed as mean ± standard deviation.

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

Table 4. 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

CDI, chronic daily intake; HI, hazard index; HQ, hazard quotient.

Table 4 shows the CDI, hazard quotients, and hazard index of metals determined in eggs 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 they are 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 (eggs 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 [23]. In this study, an analysis

of possible microbial contamination shows that, the most frequent bacteria were *Salmonella* spp., then *Klebsiella* spp., and lastly *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* spp., 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 that the microbes in feed can be relayed to the humans and other animals. Thus, the presence of microbes in feed calls for more proper preparation and handling from the sides of manufacturers, sellers and handlers [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 feed, 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]. Table 3 presents 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. [25]. The lower levels of Fe (in Table 3) are also lower than the finding of Okoye et al. [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 affect the consumers [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 the parameters of chronic daily intake (CDI), hazard index (HI), and hazard quotient (HQ) pertaining to the elements zinc and iron present in egg (that might be due to the feed of the poultry). The CDIs for 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 these are more likely to cause effects on consumers. This was similar to finding of Igwemmar and Kakulu [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 iron) in poultry feed in Sokoto, Nigeria. It has found the presence of three microbes, namely, *Salmonella* spp., *Klebsiella* spp. and *Proteus* spp. These organisms may contaminate poultry products and adversely affect 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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