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Airborne Microflora and Aerial Pollutants of Poultry Intensive Housing Systems in Edo State, Nigeria

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Abstract

The intensive poultry housing system provides the ideal conditions for pathogen proliferation and transmission. The goal of this research was to study how standard, semi-standard and substandard intensive housing styles affected the air pollution and microflora of selected poultry farms in Edo State, Nigeria from December 2016 to November 2017. Air samples and aerial pollutants were collected monthly with the aid of Casella Cel 712 micro dust pro air sampler and Gasman gas detector respectively. Physicochemical characteristics of the air around the poultry farms varied depending on location and housing types. CO_2, N_2O, H_2S as well as particulate matter (PM_{10}) concentrations were below the World Health Organization's recommended limits (WHO). However, all substandard poultry farms exhibited significant increases in NH_3 and SO_2 levels. Regardless of location or poultry type, total bacterial counts ranged from 148.05 - 481.29 cfu/m^3. Bacterial counts in the standard poultry (209.02 cfu/m^3) deferred significantly from that in the substandard (383.22 cfu/m^3). The same was reported for fungal counts ($p<0,05$). The lowest microbial counts were obtained in the Standard poultry farms. *Staphylococcus aureus* had the greatest percentage frequency of occurrence (25%) among the airborne bacterial isolates tested, whereas *Aspergillus niger* was the most prevalent airborne fungal isolate (30%) among the sampled poultry farms. Poor poultry housing standardization resulted in poor air quality and deterioration of the microbiological quality of the poultry air in and around the poultry environment, according to the research.

1. Introduction

Thousands of birds congested in an enclosed, warm and dusty environment is ideal for disease transmission. Poultry facilities cause stench by attracting flies, rodents and other pests which can cause nuisances and infectious diseases in the local area [1]. Odor emissions caused by a large number of pollutants (including ammonia, hydrogen sulfide and volatile organic compounds) from poultry farms have a negative impact on the lives of nearby residents [2]. The emission of odor mainly depends on the frequency of feces removal and cleaning, the temperature and humidity of the feces, the type of feces storage and the main air flow. Although in general, this smell does not cause any public health problems, it may represent a serious local problem, especially when large numbers of poultry birds are kept in confinement areas. This is related to the frequent complaints of odor nuisances and other health

symptoms by poultry farm workers and people living near these sites including headaches, inflammation of the eyes, nose and throat, and lethargy [3].

In recent decades, due to the introduction of modern intensive production systems, poultry production has undergone rapid changes. These systems place great importance on health care, sanitation and management and have a small workforce but with skilled services [4]. Poultry production has grown rapidly with an annual production of 3.7% in the last ten years. Therefore, in industrialized countries, the vast majority of chickens and turkeys are now produced in houses with 15,000-50,000 birds [5].

More than fifty years ago, intensive poultry production was introduced in Nigeria and it developed rapidly, especially in recent decades as a major livestock enterprise in the country [4]. This is the commercial breeding of high-yielding exotic chicken breeds. The system is resource-based and requires operators to monitor the feed, nutrition and health needs of poultry [6]. In recent decades, successive governments have encouraged the development of modern poultry establishments and as a result, people of different backgrounds suddenly entered the industry. This has led to the development of unplanned and unregulated industries, with many consequent problems [7]. Commercial poultry production is appealing because poultry is simple to adapt, has a high economic value, has a quick breeding period and produces a lot of eggs. Meat can be generated in eight weeks, the first egg in eight weeks, and the first chick can be hatched in 10 weeks. [8]. Therefore, there is a continuing need to generate factual information related to poultry industries. This information is necessary for proper planning and regulation of Nigeria's fast growing livestock and poultry industries. However, available data on the characteristics of enterprises and current facts that restrict the development of good poultry enterprises in Nigeria are scarce and fragmented [7]. There are 3 common poultry house systems in Nigeria. They are intensive, semi-intensive and extensive systems [9]. The intensive system is the most efficient, convenient and economical system in modern large-scale poultry production. Commercial poultry farming is majorly carried out using this system. The intensive poultry house systems is further sub divided into standard, semi standard and substandard systems. The intensive system is a market-oriented production, with a large number of birds (>2000) and a high level of productivity of exotic poultry breeds. Approximately 21% of chickens in Nigeria are raised on commercial/integrated farms [5].

The poultry housing system used in Edo State is comparable to the poultry house system in other parts of the country or abroad. Breeders build poultry houses according to their taste or available resources and environmental impact [10, 11, 12]. The most common residential systems in this area are open buildings with zinc half-wire mesh (47.4%) and half-wire open buildings (21%) and completely enclosed residential systems [12]. For this reason, mechanization and automation are considered. Climate, material costs and the presence of pests (especially termites) have become decisive factors in the poultry house system in Edo State. The purpose of this study was to investigate how standard, semi-standard and substandard dense housing styles affected the air pollution and microflora of selected chicken farms in Edo State, Nigeria.

2. Materials and Methods

2.1 Study Area/ Study locations

The data for this study were collected from nine selected poultry farms in Edo State, Nigeria. The State is divided into three Senatorial districts, namely: Edo Central, Edo North and Edo South. The State lies between longitudes 05º 041 E and 06º 431E and latitudes 05º 441N and 07º 341N of the equator. The population of the study consists of commercial poultry farms housing domestic chickens in confinements identified by the Poultry Association of Nigeria, Edo State Branch and the Ministry of Agriculture and Natural Resources, Benin City, Edo State. Commercial flocks of rearing and laying birds with a capacity of 2,000-6,500 were considered for this study; Standard poultry farms with mechanical

ventilation, automated feeding and drinking systems and Standard practices (Plate 1a). The semi-standard-Poultry farms with mechanical ventilation, automated drinking system and traditional feeding methods (Plate 1b). The third category was the sub-standard poultry farms without mechanical ventilation systems, only natural ventilation with traditional feeding and drinking methods (Plate 1c).

(a) **(b)** **(c)**

Plate 1: Different standards of poultry environment with birds in confinements (a) standard Poultry in Auchi(b) Semi standard poultry in Benin and (c) Sub-standard poultry in Ekpoma.

2.2 Sample collection.

Air sampling

The air across all poultry farms was sampled for 12 months at different points in each farm in the three Senatorial districts of Edo State (Edo Central, Edo North and Edo South) between December 2017 and November 2018. The study sites were located in Ekpoma (Edo Central), Auchi (Edo North) and Benin (Edo South). Each of the Poultry farm sampled housed over 2,000 birds. The indoor and outdoor air was sampled using Casella cell 712 air sampler (Casella incorporated, U.S.A) consisting of a Casella pump and mixed cellulose ester filter paper gridded black with a pore size of 0.10µm and a diameter of 25mm. Measurements were in triplicates, collecting 0.1 m^3 of air in 1 minute at a time, depending on the expected level of contamination air volumes were 200 litres and the sampling rate was 100l/min. During the measurement the sampler was situated at a height of the human breathing zone of 1.5m. The emission level outside the poultry farms was determined simultaneously. In view of the expected high concentration of microflora in poultry facilities, a filtration method was used in this study. The measuring sets were calibrated before each sampling procedure using Gillibrator 2 calibrator (U.S.A). After sampling, the filters with collected biological materials were picked using sterile tweezers into tightly closed containers with Stuart-Ringertz medium (Sigma – Aldrich chemie GMLH Munich, Germany) and transported to the laboratory for microbiological analyses. The filters in the containers with the transport medium were immersed in 5ml of the phosphate buffer solution BTL, Lodz, Poland) and by shaking on a shaker at 420 revolutions per minute for 50mins, the biological material on the filters were eluded. A series of 3 fold dilution was prepared from the resultant elutes [13].

2.3 Media Preparation

The media used in this study to isolate bacteria and fungi were Nutrient Agar (NA), MacConkay Agar, Potato Dextrose Agar (PDA), Mannitol salt agar, Sabouraud Dextrose Agar (SDA) and Blood Agar. All media preparations were carried out in accordance with the Manufacturer's instructions. Antibiotics (Streptomycin and Chloramphenicol – 50mg/L each) were introduced into the dissolved

media after sterilization was carried out for the inhibition of bacteria. Sterilization of media was done by autoclaving for 15 mins at 121^{O}C and 15 pounds pressure [14].

2.4 Enumeration and isolation of airborne bacterial and fungal isolates

Qualitative and quantitative bacterial and fungal investigations were carried out using the collected air samples.

(a) Isolation of airborne bacteria from the air:

All media were aseptically prepared and allowed to stand overnight at 37°C. Thereafter 0.1ml of the 10^3 dilution was inoculated onto sterile plates of nutrient agar, mannitol salt agar, blood agar and MacConkay agar (Merck, Dermstadi, Germany). The plates, which were prepared in triplicates were covered and incubated for 18-24 hours at 37°C for the isolation of pathogenic bacteria. The airborne bacterial isolates were enumerated using the formular:

$$\frac{\text{Number of colonies x Dilution factor x Elute volume}}{\text{Serial dilution material plated x Volume of air sampled.}} \qquad \textbf{Eqn. 1}$$

The resultant concentration was expressed in terms of the number of colony forming unit per cubic meters (cfu/m^3). Thereafter, discrete colonies were sub-cultured for preliminary identification subjected to biochemical tests and characterized on the basis of their cultural, morphological and biochemical characteristics, according to [15].

(b) *Isolation of airborne fungi from the air*

Sterile plates PDA and SDA (Oxoid, England) incorporated with penicillin and streptomycin were used for the enumeration and isolation of airborne fungal isolates. The plates were incubated for 3-5 days at room temperature (28±2°C) and discrete colonies were sub cultured. The airborne fungal isolates were characterized based on their morphological appearances. The fungal colonies were sub cultured to obtain pure cultures which were identified [14, 16].

2.5 Microclimatic Parameters.

Air temperature and relative humidity were determined on monthly basis with the aid of a Testo Device 400 (Testo GmbH & Co. Lenzkirch, Germany) in the indoor and outdoor environment. Both indoor and outdoor measurements were performed from 9.00 a.m to 12.00 noon [17].

2.6 Measurement of Dust Concentration.

The concentration of dust of aerodynamic diameter of <10mn was determined electronically with the aid of a direct reading active personal sampler, Casella cell dust (Environmental Device co-operation, U.S.A). The active sampler uses a pump and a power source to move air through a collector. The sampler has a sampling flow rate of 1.0 l/mins and the instrument software allows direct reading of dust concentration. The sampler was placed 1.5m above the floor, the device switched on and dust concentration determined after 1 minute and measurements were taken on monthly basis in each of the poultry houses investigated. The results were expressed in mg/m$^{3.}$ [7].

2.7 Measurement of gases in poultry farms.

The concentrations of ammonia (NH_3), Hydrogen Sulphide (H_2S),Carbon dioxide (CO_2), Methane (CH_4) and Nitrous oxide (N_2O) in the sampled poultry farms were determined with the aid of potable direct reading instrument, the procedure involved taking representable reading at different locations in the poultry farm. The measurements were taken in triplicates inside the poultry house at three different locations (by the entrance, at the center and at the end wall). The representative readings from each confinement were pooled to obtain the mean for each farm. The concentration of Ammonia, hydrogen Sulphide, Carbon dioxide and Nitrous oxide were measured in Parts Per Million (PPM) while methane was measured in Lower Emissible Limits (LEL) as a flammable gas. The measurement was carried out with the aid of the Gasman Hand-held Personal Gas detector (Crowcon Instruments Ltd., England), which employs electrochemical sensors for Ammonia, hydrogen Sulphide, Carbon dioxide and Nitrous oxide and a catalytic bead sensor for methane measurements. During the gas measurements, the hand held equipment was held at about one foot above the litter level and the readings were recorded within 20 secs. All analyses were calibrated for zero and span before and after reading.[7].

2.8 DNA Extraction

DNA extraction was carried out using the standard protocol. Freshly grown culture was transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. The cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0), fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hr at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 mins at 65 ºC and kept in ice for 15 mins. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation in ice for 5 mins and centrifugation at 7200g for 20 mins. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 hrs. DNA was collected by centrifugation at 13000g for 10 mins, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately 3hrs and finally dissolved in 50 µl of TE buffer [18].

2.9 Statistical Analyies

The data obtained from this research is expressed as mean ± SEM (standard error or mean) or percentage. The t-test statistic was used to test the statistical difference between the treatment group and the control group under study. In most cases, the statistical software package SPSS 21.0 was used for data analysis in the evaluation version of Windows. Used measure of central tendency (mean ± standard deviation) to analyze triplicate values. [19]. One Way ANOVA was used to compare multiple variables while Duncan's multiple range test was used to check for significant differences between means. P value less than 0.05 were considered statistically significant. PAST software was used to calculate the diversity index of the microbial isolates. (version 2. 17c)

3 Results and Discussion

3.1. Airborne bacterial count

The mean indoor and outdoor airborne bacterial counts in poultry houses ranged between 148.05±46.24 cfu/m^3 to 481.29±148.99 cfu/m^3 (Table 1). The highest counts 481.29±148.99 cfu/m^3 was recorded in the sub-standard poultry in Auchi indoor environment while the lowest count was recorded

in the standard poultry farm in Ekpoma. The bacterial counts between indoor and outdoor air varied significantly only in standard and sub-standard poultry farms in Benin as well as in sub-standard poultry in Auchi. The bacterial counts were higher in all indoor environment than the outdoor environment. Between poultry types, bacteria counts in indoor air varied significantly between standard and sub-standard poultry farms as well as between semi-standard and sub-standard poultry farms in all three locations with sub-standard having the highest count. The outdoor bacterial counts were different between standard and sub-standard Poultry farms in all three locations as well as between semi-standard and sub-standard in Auchi with sub-standard having higher counts. Counts in Benin were also significantly different between standard and semi-standard poultry with the semi standard Poultry recording the highest concentration.

Table 1: Mean airborne microbial counts. Dec. 2016 - Nov. 2017

	Bacterial			Fungal		
	Indoor	Outdoor	p-value	Indoor	Outdoor	p-value
Auchi						
1	296.65±48.38 [b]	266.80±58.92 [b]	0.184	42.98±9.11 [b]	45.24±8.71[bc]	0.633
2	308.23±87.41 [d]	272.96±87.17 [d]	0.142	34.57±11.42[ad]	25.82±8.74[acd]	0.139
3	481.29±148.99[abd]	351.75±69.28[abd]	0.094	71.87±15.09[abd]	65.98±9.97[abd]	0.322
Ekpoma						
1	148.05±46.24	165.34±51.28 [b]	0.633	39.68±14.64 [b]	40.66±7.96 [ab d]	0.732
2	226.55±70.95	207.48±54.61 [d]	0.354	159.31±60.17 [d]	147.32±41.79 [c]	0.138
3	388.10±183.17	304.91±168.49 [bd]	0.535	150.32±39.89 [bd]	173.50±43.32 [b]	0.244
Benin city						
1	214.24±71.36[ab]	165.13±45.02[abc]	0.132	48.28±20	57.70±13.51 [c]	0.214
2	302.8967.80 [d]	277.00±75.46	0.325	50.99±8.49 [a]	42.27±8.23[acd]	0.093
3	441.30±130.46[abd]	332.86±108.01[ab]	0.174	39.97±7.22 [a]	32.86±108.01[ab]	0.112

Keys: 1-Standard Poultry, 2-Semi-standard Poultry, 3-Sub-standard Poultry

3.2. Airborne fungal counts

Table 1 also shows the mean airborne fungal counts which ranged between 25.82±8.74 cfu/m^3 to 159.31±60.17 cfu/m^3 with the highest count recorded in semi-standard poultry in Ekpoma (indoor) and the lowest count in the standard poultry farm in Benin City. Statistical significance between indoor and outdoor counts was only seen among semi-standard and sub-standard poultry farms in Auchi and Benin. Counts in indoor environments of poultry houses were significantly higher in standard poultry than semi-standard and sub-standard poultry in Auchi, on the contrary fungal counts in Ekpoma were higher in sub-standard poultry than standard and semi-standard poultry. There were no significant differences in fungal counts among poultry houses in Benin City. The fungal counts in all Poultry farms sampled were however below the 3000-5000 cfu/m^3 standard set by Polish authority for occupational exposure to airborne fungi.

Phenotypically, six airborne bacterial and five airborne fungal isolates were isolated and characterized.The airborne bacterial and fungal isolates were further characterized using culture dependent molecular characterization and identification technique which reveal the presence of the following; *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella pneumonia, Bacillus subtilis*, *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus stolonifer*, *Trichoderma polysporum* and *Aspergillus fumigatus*. The highest frequency of occurrence of the airborne bacterial isolates was recorded for *Staphylococcus aureus* (25.34%) while the

least was recorded for *Enterococcus faecalis* (13.25%) **(Table 2).** Among the airborne fungal isolates, *Aspergillus niger* (30.21%) recorded the highest frequency of occurrence while the least was recorded for *Trichoderma polysporium* (2.34%) **Table 3.** A comparison of types of poultry with respect to bacterial and fungal loads has been presented **Table 4**. Bacterial and fungal loads were significantly higher in the substandard poultry compared to the standard poultry.

Table 2: Frequency of Occurrence of Airborne Bacterial Isolates in Poultry Facility

Airborne Bacterial Isolates	Percentage frequency (%)
Staphylococcus aureus	40 (25.3)
Enterococcus faecalis	20 (12.5)
Bacillus subtilis	22(13.8)
Escherichia coli	24 (15.0)
Klebsiella pneumonia	30(19.0)
Pseudomonas aeruginosa	22 (14.4)
Total	158 (100)

Table 3: Frequency of Occurrence of Fungal Isolates in Poultry Facility

Airborne Fungal isolates	Percentage frequency (%)
Fusarium oxysporium	8(19.6)
Aspergillus niger	13 (29.8)
Rhizopus stolonifer	9 (21.1)
Trichoderma polysporium	1 (2.6)
Aspergillus fumigatus	11 (26.9)
Total	42 (100)

Table 4: Comparison of types of Poultry with respect to Bacterial and Fungal loads.

Poultry types	Bacterial load	Fungal load
Sub-standard Poultry	383.22a	93.94a
Semi-standard Poultry	266.01b	76.71b
Standard Poultry	209.02c	59.09c
P-value	<0.001	<0.001

Values within similar columns with the same alphabets do not differ from each other (p>0.05)

3.3 Gaseous pollutants

Mean hydrogen sulphide concentrations in the environment were recorded between 0.02 ppm and 13.10 ppm **Table 5**. There was statistically significant difference in concentrations in indoor and outdoor environments in all poultry farms studied ($P<0.05$). The H_2S levels were generally higher in the indoor environment for the poultry houses studied. Hydrogen sulphide concentrations in sub-standard poultry across all locations exceeded the W.H.O permissible limit (7.00ppm). Significant difference in H_2S concentrations between poultry types in all locations($P<0.05$) was also observed with the exception of standard and semi-standard poultry in Auchi and Ekpoma indoor and outdoor as well as semi-standard and sub-standard poultry outdoor in Benin City. The concentration of ammonia (NH_3) ranged between 0.004 ppm to 9.14 ppm. The concentration was recorded to also be above the W.H.O set limit 7ppm in sub-standard poultry in all sampled locations. There was also significant difference in mean values

between indoor and outdoor air with outdoor air generally higher. Relatively larger significant difference was also observed among poultry types in all locations
(indoor and outdoor). Levels between semi-standard and sub-standard poultry in Auchi (indoor and outdoor) and Ekpoma were similar statistically (P>0.05). The indoor levels of methane were higher in the indoor air of poultry houses with concentrations ranging from 0.22 LEL in Standard poultry (Auchi outdoor) to 7.54 LEL (sub-standard poultry Ekpoma indoor) **Table 5**. Significant difference in methane concentrations was also observed among poultry types and was higher in semi-standard poultry than standard and sub-standard poultry in Auchi indoor and outdoor and sub-standard poultry in all three locations indoor and outdoor.

PM_{10} levels in the indoor and outdoor environment of poultry houses were also measured, concentrations were significantly different statistically up to 89% in all Poultry types and ranged between 0.01±0.00 mg/m^3 to 1.75± 0.01 mg/m^3 **Table 5**. The highest reading 1.75 mg/m^3 was recorded in the Semi standard Poultry farm in Auchi while the lowest reading 0.01 mg/m^3 was recorded in the Standard poultry farm in Ekpoma. However, levels in Semi-standard poultry in Auchi were similar statistically, (P>0.05). Indoor CO_2 concentrations were significantly high in Semi-standard and Sub-standard Poultry farms in Ekpoma and Benin, there was however no significant difference in CO_2 concentrations among Poultry farms in Auchi. While outdoor concentrations of CO_2 were higher in Semi-standard poultry than Sub-standard in Auchi, there was however no difference in outdoor CO_2 levels in the three Poultry farms in Ekpoma and Benin.

3.4 Physicochemical parameters

Mean indoor temperature levels ranged from 24.60°C - 32.21°C, 25.5°C - 32.20°C and 25.10°C - 32.40°C in standard, semi-standard and sub-standard poultry farms respectively. The highest temperature reading 33.60°C was in the standard poultry farm in Auchi while the lowest temperature reading 24.60°C was recorded in the standard poultry farm in Ekpoma. The mean indoor relative humidity results ranged from 50% – 88%, 52%- 93% and 51% - 91%in standard, semi-standard and sub-standard poultry farms respectively with the highest reading 95% was recorded in semi standard poultry farm in Ekpoma while the lowest 50% was recorded in the standard poultry farm in Auchi **Table 5**.

This research showed that gaseous pollutants as well as airborne fungi and bacteria were relatively higher in indoor environment than in outdoor areas, this is similar to findings of [20]. The sources of these variations may be farm objects. There were however contrary findings in all poultry farms in Ekpoma and Benin as well as standard poultry farms in Auchi and Benin, where the fungi load in indoor and outdoor areas were not significantly different. This can be attributed to lack of good ventilation system. Similar trend was also observed for bacterial load in all poultry types in Ekpoma and Standard poultry in Auchi as well as semi- standard poultry in Auchi and Benin. The key reason for this uncommon occurrence in standard and semi-standard poultry farms may be as a result of non or improperly cleaned ventilation system. Mechanical ventilation systems not properly cleaned can be a source of microbial proliferation and spending of microorganisms as reported by [21].

Fungal load in Ekpoma were not significantly different in indoor and outdoor environment, this was contrary to the studies by [20], indoor fungal load was higher than outdoor fungal load, this may be a result of the relatively high humidity in the outdoor environment of these poultry farms, which is capable of supporting the proliferation of fungi [20, 22]. Indoor fungi load was highest in sub-standard poultry farms, this may be a confirmation of the assumption that poultry types play vital roles in determining fungal count as previous studies by Lonc and Plewa [22], showed that mechanical

ventilation systems coupled with increase air flow rate contribute significantly to reducing fungal loads inside poultry facilities. However, no significant difference was recorded among poultry types in Benin-City.

Table 5: Comparative mean annual physicochemical composition of air in and around poetry environments in Edo State

	Temp.	Humidity	CO_2	NH_3	CH_4	N_2O	H_2S	SO_2	Dust
Benchmark	**31°C**	**75%**	**1000 PPM**	**- 25PPM**	**25 LEL**	**- 25ppm**	**7 PPM**	**- 7PPM**	**- 150µg/m³**
A3I	29.28^{ab}	73.5^{a}	28.5^{de}	0.1^{a}	2.02^{bcd}	0.2^{bc}	1.34^{bc}	1.02^{de}	0.11^{ab}
A3O	30.79^{b}	77.92^{a}	13.1^{a}	0.04^{a}	0.84^{a}	0.09^{a}	0.56^{ab}	0.41^{ab}	0.03^{a}
A2I	29.09^{ab}	74.33^{a}	27.1^{d}	39.1^{d}	4.66^{h}	0.42^{e}	2.36^{de}	0.22^{ab}	0.87^{fg}
A2O	30.73^{b}	77.92^{a}	12.4^{a}	18.1^{b}	2.3^{de}	0.2^{bc}	1.18^{bc}	0.05^{a}	0.59^{de}
A1I	29.3^{ab}	73.33^{a}	30.5^{e}	40.5^{d}	6.12^{j}	0.49^{ef}	9.71^{h}	0.11^{a}	0.86^{fg}
A1O	30.73^{b}	76.33^{a}	16^{b}	18^{b}	2.81^{f}	0.22^{c}	4.1^{f}	0.04^{a}	0.44^{cd}
E3I	28.59^{a}	77.58^{a}	16.5^{b}	2.24^{a}	2.17^{cde}	0.32^{d}	1.65^{cd}	1.19^{de}	0.21^{ab}
E3O	29.23^{ab}	80.08^{a}	11.5^{a}	0.2^{a}	1.04^{a}	0.13^{ab}	0.64^{ab}	0.51^{bc}	0.05^{a}
E2I	29.18^{ab}	76.58^{a}	20.5^{c}	51.3^{e}	3.41^{g}	0.6^{g}	2.33^{de}	0.511^{bc}	0.99^{fg}
E2O	29.5^{ab}	77.75^{a}	10.5^{a}	22.8^{bc}	1.56^{b}	0.23^{c}	1.09^{bc}	0.19^{ab}	0.47^{cd}
E1I	28.73^{a}	77.92^{a}	21.1^{c}	61^{f}	6.15^{j}	0.45^{e}	11^{i}	1.61^{f}	1.06^{g}
E1O	29.76^{ab}	80.08^{a}	10.7^{a}	23.2^{bc}	2.93^{f}	0.2^{bc}	5.16^{g}	0.86^{cd}	0.47^{cd}
B3I	28.18^{a}	74.83^{a}	22.7^{c}	3.38^{a}	1.93^{bcd}	0.41^{e}	0.21^{a}	1.38^{ef}	0.29^{bc}
B3O	28.91^{a}	79.17^{a}	10.9^{a}	1.65^{a}	0.93^{a}	0.18^{bc}	0.05^{a}	0.5^{1bc}	0.09^{ab}
B2I	28.55^{a}	74.5^{a}	29.2^{de}	47.7^{e}	3.78^{g}	0.56^{fg}	2.49^{e}	1.21^{de}	0.64^{de}
B2O	29.57^{ab}	79.25^{a}	12.5^{a}	22.8^{bc}	1.75^{bc}	0.25^{cd}	1.23^{bc}	0.34^{ab}	0.17^{ab}
B1I	28.03^{a}	72.5^{a}	30.6^{e}	57.3^{f}	5.36^{i}	0.6^{g}	9.36^{h}	1.99^{g}	0.78^{ef}
B1O	28.69^{a}	76.58^{a}	12.2^{a}	25.7^{c}	2.56^{ef}	0.27^{cd}	4.3^{f}	1.03^{de}	0.16^{ab}
p-values	0.854	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.002

Means with similar alphabetic superscripts within same columns do not differ from each other (p>0.05)
A is Auchi, B is Benin City, E is Ekpoma, 1 is substandard, 2 semi-standard, 3 standard poultry, I is inside and O is outside poultry environment

High fungal load recorded in standard and semi-standard poultry farms in Benin- City may be as a result of improper cleaning of the ventilation systems in these facilities as suggested by [21], who reported high fungal counts in poultry with mechanical ventilators when compared to those adopting gravity ventilation.

Performed analysis of variance demonstrated significant impact of poultry types on indoor airborne bacterial loads, owing to the fact that high bacterial loads were recorded in all sub-standard poultry farms sampled. However, similar to fungal, there were no significant difference between indoor and outdoor bacterial loads in all poultry farms in Ekpoma as well as in standard and semi-standard poultry farms in Auchi and Benin City. This result did however not agree with findings by Lonc and Plewa [20] and may possibly be as a result of improper hygiene and practices that encourage the growth and abundance of bacteria outside poultry environment.

The isolated bacteria in this study are of both veterinary and public health importance. *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Klebsiella pnemoniae* and *Pseudomonas aeruginosa* were identified culturally and biochemically with *S.aureus*

being the most frequently isolated. *Eschericia coli* and other enteric bacteria such as *Klebsiella* and *Pseudomonas* species isolated from the sampled poultry houses in this study are members of normal intestinal flora. These bacteria become pathogenic when they reach tissues outside their normal intestinal or other normal flora sites. The anatomic sites of clinical importance in humans are urinary tracts, biliary tract, lung, bone, meninges, prostate gland and blood. The presence of these bacteria in Poultry facilities is in conformity with previous studies [21-23]. Two of the bacteria, *E.coli* and *K.pnuemoniae* isolated during this study belong to the risk group 2 bacteria according the Polish Ordinace, which is a risk classification for occupational exposure to bioaerosols. *E.coli* is an opportunistic pathogen which can cause urinary tract infections*, K.pnuemoniae* on the other hand which is also an opportunistic pathogen is capable of causing respiratory tract infection.

S.aureus, though not a spore producing bacteria has been proven to survive longer in air than any other bacteria meaning its airborne spread is a possible potential to cause serious infection. This coupled with its high frequency in aerosols makes it a probable candidate for bioaerosol airborne pollution. The high prevalence of *Staphylococcus aureus* in the sampled Poultry farms could be of serious concern considering its high pathogenicity and virulence. The organism has been reported in several human diseases such as cellulitis, local abscess formation (furuncles and carbuncles) and lymphadenitis. Infection can extend to bones and joints leading to primary osteomyelitis and septic arthritis [23]. Inhalation of *Pseudomonas aeruginosa* may cause necrotizing pneumonia and the involvement of ear and eye may result in otitis externa and rapid destruction of the eye respectively [24].

All fungi isolated in this study were in the mould group and include; *Fusarium oxysporium, Trichoderma polysporum, Aspergillus niger, Aspergillus fumigatus* and *Rhizopus stolonifer.* They are referred to as opportunistic fungi. They do not usually induce diseases, but do so when the body host defense is compromised [24]. Similar to studies by Musa and Abalaka [22] who isolated moulds as the major group of fungi from Poultry facilities. Moulds are associated with humid environments and are capable of causing respiratory tract infections as well as allergic effects, more worrisome is that *A. fumigatus* is among moulds isolated during this study and classified as risk group two biological agent. *A. fumigatus* is closely associated with humid environment and is frequently isolated from surface of ventilators and settled dust [22].

Results from this study indicate that hydrogen sulphide concentrations were relatively higher in sub-standard poultry farms across all locations, however the high concentrations recorded in some standard poultry farms is an indication of unhygienic state and hydrogen sulphide is released from manure decay. Highest indoor level in this study recorded in sub-standard poultry was far above the > 7 ppm concentration in poultry confinement [25].

Nitrous oxide concentrations in the indoor areas of poultry houses were generally higher in sub-standard poultry farms, however concentrations in semi-standard and sub-standard poultry farms in Auchi and Benin were similar statistically, this maybe as a result improper heating systems coupled with the relatively higher levels recorded in these areas during the months of May through July which are the peak wet seasons in Nigeria, as higher concentrations of N_2O were recorded by [26] during winter periods when compared with values record in summer. Another possible reason may be the bird feed compositions in the poultry farms as well as the stage of maturity of the birds, as suggested in separate studies by [27, 28] who reported that nature of feed and age of birds respectively determines the N_2O compositions of Poultry manure.

Indoor levels of methane was below the 25 LEL permissible limit by the World Health Organization similar to findings of previous authors [26] who also stated that amount of methane emitted from Poultry houses depends on management and condition of the Poultry. Concentrations were however higher in Sub-standard Poultry farms in all locations except that levels in standard and Semi-standard Poultry farms in Benin were not significantly different, this may be attributed to the number of birds in the Poultry [26].

Sulphur dioxide concentrations inside poultry facilities were higher in sub-standard poultry in Benin City, relatively higher concentrations were recorded in standard poultry farms in Ekpoma and Benin than in semi-standard and sub-standard poultry farms. This result thus gives a clue into the fact that ventilation system has little role to play in the amount of SO_2 in poultry confinement.

Ammonia concentrations in semi-standard poultry in Ekpoma was above the W.H.O permissible limit, this may be attributed to the feed sources as previously reported by [29], undigested proteins in poultry manure are potential sources of ammonia polarization.

Considering poultry types the concentrations of ammonia was significantly higher in substandard poultry farms than in other types of poultry farms. There was however no significant difference between concentrations in semi-standard and sub-standard poultry farms in Auchi and Ekpoma. The feed types used in these Poultry farms maybe a probable explanation for this.

Carbon dioxide levels observed indoor during this research was higher in semi and sub-standard poultry with no difference between concentrations in all three poultry farms in Auchi. As reported from earlier studies high CO_2 levels may be as a result of type of heating system used [30] these authors reported that the use of natural gas as source of heating system could contribute to the amount of CO_2 emitted in an animal farm. These authors also suggested that CO_2 amount in the indoor air of poultry should be considered in the operation of ventilation systems.

Dust levels in the poultry houses were highest in semi and sub-standard poultry farms with similar levels statistically recorded. Dust in the range of PM_{10} were however the only form sampled in this study, as this is the maximum level that is respirable and is capable of lodging in the lungs. Statistically, similar levels were recorded in the indoor and outdoor areas of the semi standard Poultry in Auchi, this can be attributed to the presence of several quarries around the sampling areas and thus the Poultry facility may not be the only contributor to the outdoor levels of the PM_{10} observed.

Highest PM_{10} level recorded in this study (1.75mg/m^3 in sub-standard poultry) was higher than 0.02 mg/m^3 level recorded by [25], though the poultry type studied was not defined. In poultry facilities PM_{10} originate from feed particles, bedding material, manure particles and feather particles blown from poultry fans [29], thus ventilation system maybe a major factor in the distribution of PM_{10} inside a poultry building.

Conclusion

The study shows that poor standardization of poultry housing means poor air quality and deterioration of the microbial quality of the poultry air in and around the poultry environment. It is obvious from this study that poultry farms are important reservoirs and sources of emission of microorganisms and gaseous pollutants that enter the environment. Poultry standards play a significant impact on the air quality of the poultry environment. The development of Nigeria's poultry industry requires a holistic approach to providing birds with the best environment, nutrition and health, while minimizing occupational and environmental health risks. To achieve this goal, adequate standardization mechanisms are needed

during the establishment of poultry farms. Operations in the poultry house must be mechanized, as automatic feed and drink devices reduce exposure rather than manual operations.

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