

Microbial and Biochemical Characterization of Poultry Feeds from farms of Abidjan district

ABSTRACT

This study aims to evaluate the biochemical and microbial quality of poultry feeds in farms of the Abidjan district. Thus, 164 samples of industrial feeds and farmers formulated feeds were collected in Bingerville, Yopougon, Port-Bouet, Anyama and Songon areas for microbiological and biochemical analysis. The microbiological analysis consisted of isolation and identify of bacterial and fongical flora potentially pathogenic for the animal. While, the biochemical analysis was aimed to evaluate essentially the nutritional quality of these feeds. Results showed that among these samples, 15 (10.33 %), 91 (55.48 %) and 48 (29.26 %) were positive respectively for *Salmonella* sp., *E. coli* and *Aspergillus* sp. Moreover, biochemical analysis showed low feeds moisture rate levels ranging from $7.52 \pm 0.16\%$ to $12.14 \pm 0.05\%$. Feeds formulated by farmers had low content in ash, protein and lipid with proportions ranging from $4.30 \pm 0.15\%$ to $11.61 \pm 0.24\%$, $9.30 \pm 0.24\%$ to $15.81 \pm 0.20\%$ and $2.07 \pm 0.02\%$ to $3.68 \pm 0.11\%$ respectively comparatively to commercial feeds. In conclusion, this study shows a poor hygienic quality of the feeds used in modern poultry husbandry in the Abidjan district. However, the analyzed feeds content are mostly in conformity with the recommended standards.

Keywords: [Poultry diseases, feeds quality, chemical content, Abidjan]

1. INTRODUCTION

In Côte d'Ivoire poultry farming has an important place in national meat production and for the Ivorian economy. Indeed, it covers about 44 % of this production [1] and combines modern broiler and layer chicken farming with traditional local chicken farming.

Moreover, later report by **Koffi-Koumi** [2] showed that total incoming in 2015 was estimated about 412.8 millions US Dollar. Because of their lowest prices than other animal meat, poultry products are consumed by all population. According **Bakayoko** [3], this sector would cover slightly more than 91% of poultry meat needs and 100 % of egg needs. Poultry sector also accounts for 4.5 % of agricultural Gross domestic product (GPD) and 2 % of total GPD. In addition, Côte d'Ivoire's government intends to increase this performance by reaching 200 000 tons of poultry meat and more than 1.678 billion of eggs / year in 2030 to fully cover population needs of animal proteins [2]. Thus, poultry meat could be the main source of animal protein for the Ivorian population.

Despite, its rapid growth and the diversity of its products, the development of the poultry sector remains rather fragile, due to the sanitary problems that leading to economic losses [2]. The most common pathologies are Gumboro and Newcastle diseases, salmonellosis, collibacillosis and avian coccidiosis [4]. In 2017, the loss of production causes by these diseases each year was estimated to 39.45% corresponding more than 240 000 USD. Occurrence of these pathologies is generally due to poor knowlegment of hygiene practices conditions during production process including poor application of biosecurity measures [5]. Other factors are implicated in the outbreak of diseases in farmed chickens, such as the poor hygienic and nutritional quality of animal feeds.

In fact, farmers formulate their own feedstuffs because of high input costs. However, feeds production is carry out generally in poor hygienic conditions. This fact could favor the development of pathogenic microorganisms in the feeds. Moreover, the formulation of these feeds does not always take into account the nutritional needs of farm animals.

These nutritional imbalances could have the serious consequences on the zootechnical performance of animals. Therefore, the aim of this work was to assess the biochemical and microbial quality of feedstuffs intended for farmed chickens in the district of Abidjan.

2. MATERIAL AND METHODS

2.1 Study area and farm selection criteria

The study was carried out on poultry farms located in district of Abidjan (in the south of Côte d'Ivoire). The subdivisions Bingerville, port-Bouet, Ayama and Yopougon were chosen due to the importance of poultry farming in these locations (**figure 1**). The district of Abidjan lies between longitude 4° 1' 59.999" W of the Greenwich meridian and latitude 5° 19' 0.001" N of the equator. This region is characterized by a typical humid tropical climate with temperature ranges between 22 ° C and 32 ° C and a precipitation evaluated to 787 mm and 1500 mm throughout the year.

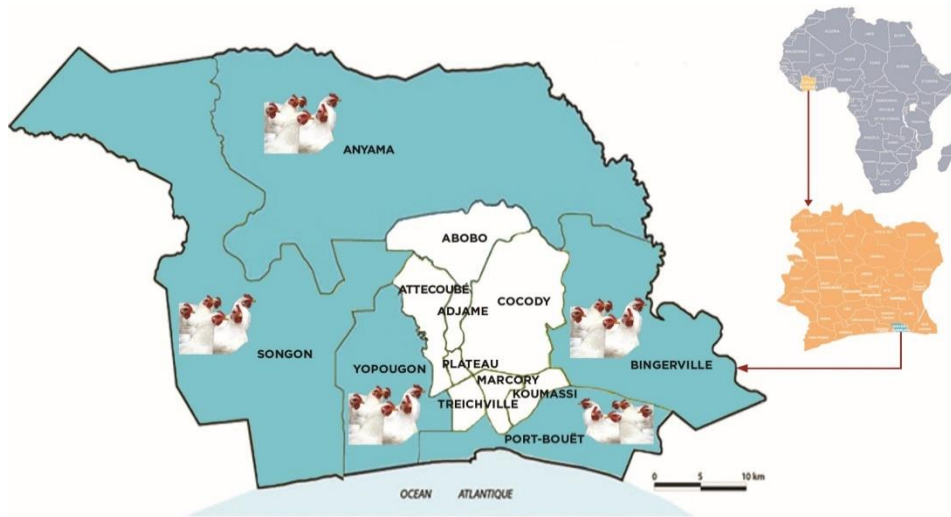


Figure 1 : Map of the district of Abidjan (in the south of Côte d'Ivoire) indicating the study area

2.2 Sample collections

From September 2018 to May 2019, 164 poultry feed samples were collected in 6 areas of Abidjan district. At each visit, one (1) sample is taken per farm depending on the origin of the feed (industrial or formulated). Each sample taken directly from the feed bins have put in a sterile jar and stocked in a cooler before quickly transported to the laboratory for microbiological and physico-chemical analysis.

2.3 Microbiological analyses

In this study, three microorganisms involved in pathologies in poultry have researched on agar medium from the collected samples. Thus, each samples was prepared in the proportions 10 : 90 (g/mL) in buffered peptone water (NF EN ISO 6887-V08-010-6, 2013) and from these stock suspensions, successive decimal dilutions (10^{-1} to 10^{-7}) were made in sterile salt buffer. At last, 0.1 mL of the stock solutions or dilutions were spread on selectifs agar culture media [6].

- OGA (Oxytetracycline-Glucose-Agar) agar was inoculated and incubated at 30°C for 3 to 7 days for the detection of moulds (**AFNOR, NF ISO 21527-2, 2008**).
- TBX (Tryptone Bile X-glucuronide) agar was used for the isolation of *E. coli* and incubation was performed at 42 °C for 24 hours at aerobic conditions.
- *Salmonella* isolation was performed according to NF ISO 6579 on Hektoen agar after pre-enrichment in buffered peptone water and selective enrichment in Rappaport Vassiliadis (RV10).

The presumptive colonies of *Salmonella* and *E. coli* were identified from morphological observations (Gram stain) and from biochemical criteria on the following media: Kligler-Hajna (AES laboratory, Combourg, France), Simmons citrate, mannitol-mobility, urea-indole, Clark and Lubs, malonate, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase, arabinose, inositol and adonitol. Concerning moulds, the identification of the genus *Aspergillus* was carried out using morphological tests including the appearance of colonies on the agar after 7 days of incubation and microscopic observation in the fresh state.

2.4 Biochemical composition determination

Twenty (20) samples including 10 industrial feeds and 10 feeds which were formulated by farmers were used in this study. Then moisture content, protein content of feeds were determined as described by Mweta et al. [7]. Fat content was determined by Soxhlet extraction of 3 g of dried feeds using hexane for 3 h. Ash content was determined by weight difference after ashing 2–3 g of dried feeds samples in a muffle furnace at 525°C for 5 h. Carbohydrates and crude fibre content were determined as previously described by FAO [8].

2.5 Data analysis

The data was subjected to analysis of variance (ANOVA) using STATISTICA 7.1 for Windows (Analytical Software, Tallahassee, USA). The averages of the values measured for each parameter were calculated and the significant differences between these averages were also determined.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Microbial characteristics of poultry feeds

A total of 164 poultry feeds samples were analyzed for microbiological and physico-chemical quality. Among these samples, 15 (9.14%) were positive for Salmonella, 91 (55.48%) for E. coli and 48 (29.26%) for Aspergillus sp. The distribution of positive samples had presented in Tables 1. Results show that Songon area had a higher rate sample positive for Salmonella than Port-Bouet and Anyama areas and none sample were positive for Salmonella in Yopougon and Bingerville. At last, among the feedstuffs from industrial production tested in this study, three (3) were positive for Salmonella.

Concerning E. coli, high prevalence rates were obtained in Anyama, Songon and Bingerville with 100 %, 53.48 % and 53.48 % positive samples respectively. On the other hand, the rate of E. coli is similar for all samples from industrial origin collected in the different areas visited.

Aspergillus genus was isolated in the five (5) breeding areas visited with a high prevalence in Bingerville (43.33%). Moreover, among the 63 samples of industrial origin analyzed, 45.33% were positive for Aspergillus and Bingerville had the highest prevalence with 31.23%. At last, 25.13% of the industrial origin samples from Songon were positive for Aspergillus.

Table 1. The distribution of positive feed samples

Microorganism	Areas					
	Songon	Yopougou	Bingerville	Port-bouet	Anyama	Total
<i>Salmonella sp.</i>	11 (25.58 %)	0	0	2 (15.38 %)	2 (9.09 %)	15 (9.14 %)
<i>Escherichia coli</i>	23 (53.48 %)	5 (19.23 %)	38 (63.33 %)	3 (23.07 %)	22 (100 %)	91 (55.48 %)
<i>Aspergillus sp.</i>	9 (20.93 %)	7 (26.92 %)	26 (43.33 %)	3 (23.07 %)	3 (13.63 %)	48 (29.26 %)
Positives samples by area	43	26	60	13	22	154 (93.90 %)

3.2 Biochemical composition of poultry feeds

Among 164 samples collected, 20 were tested for the determination of biochemical composition. Results show that the moisture content of feeds analyzed in this study range between 7.52 ± 0.35 % and 12.15 ± 0.05 % and the lowest values were obtained for industrial origin samples (Table 2 and 3). Regarding the ash contents, a significant difference were observed between the industrial origin samples and those formulated by the farmers.

In general, the ash contents varied between 4.30 ± 0.33 % and 11.61 ± 0.13 % for industrial origin feeds and between 6.30 ± 0.19 to 8.13 ± 0.06 % for formulated samples.

The carbohydrate contents ranged from 62.19 ± 0.26 % and 65.64 ± 0.41 % for industrial origin feeds and 63.88 ± 0.20 % and 71.61 ± 0.29 % for farmer's formulated feeds (Table 2).

On the other hand, farmer's formulated feeds exhibited high levels of protein comparatively to those of industrial origin with values respectively ranged from 7.64 ± 0.21 % to 17.45 ± 0.25 % and 10.30 ± 0.35 % to 19.99 ± 0.20 %.

However, two classes of feeds were observed on base of proteins contents. The first class concerns the feeds with levels above 16 % and the second the second class included feeds with protein levels below 16%. The first group concern four (4) industrial origin feeds and two (2) of those formulated by farmers. While, the second group include six (6) industrial origin feeds and eight (8) farmer's formulated feeds.

In addition, results show that the fiber contents values range from 25.21 ± 0.05 % to 8.90 ± 0.11 % within the formulated samples (Table 2).and from 22.81 ± 0.11 % to 9.60 ± 0.22 % within the industrial origin feeds samples (Table 3).

Table 2. Biochemical properties of farmers formulated feeds from different farms of Abidjan district

	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8	FF9	FF10
Moisture	8.52±0.35	7.92±0.33	10.07±0.01	9.21 ±0.22	9.19±0.03	9.15± 0.02	12.14±0.01	9.62±0.03	9.07±0.06	10.17±0.03
Carbohydrates	69.66±0.35	71.61±0.11	63.88±0.22	64.19±0.32	71.06±0.25	65.70±0.02	64.23±0.66	69.63±0.22	63.87±0.03	62.23±0.11
Fat	2.07±0.06	2.71 ±0.33	2.99 ±0.11	3.25±0.22	3.68±0.25	2,71±0.22	2,92±0.06	3.53±0.13	2.49±0.22	3.01±0.22
Proteins	14.23±0.22	9.30±0.25	16.81±0.05	11.71±0.33	9.88±0.4	17.45±0.33	7.64 ±0.08	14.61±0.33	14.32±0.12	9.58±0.36
Ash	4.90 ±0.11	5.47±0.28	5.14±0.23	11,61±0.22	5,14±0.07	11,61±0.5	4,30±0.05	5.89±0.14	8.71±0.04	9.20±0.06
Fiber	10.45±0.06	10.18±0.09	25.21±0.01	11.46±0.03	9.62 ±0.14	8.30 ±0.01	8.90±0.01	18.23±0.22	14.41±0.01	23.05±0.03

FF: Farmer's formulation

Table 3. Biochemical properties of industrial origin feeds from different farms of Abidjan district

	IF1	IF2	IF3	IF4	IF5	IF6	IF7	IF8	IF9	IF10
Moisture	7.52±0.35	7.65±0.22	9.16±0.11	10.37±0.34	10.49±0.06	9.76±0.35	7.24±0.06	12.11±0.01	10.25±0.21	9.36±0.03
Carbohydrates	62.19±0.65	65.64±0.55	69.66±0.19	59.11 ±0.22	68.68±0.25	61.69±0.37	64.65±0.08	60.59±0.25	61.62±0.11	59.60±0.45
Fat	2.99±0.22	4.45±0.14	4.17±0.09	3.55±0.11	3.43±0.15	2.91±0.1	3.17±0.25	3.43±0.35	3.68±0.22	4.40±0.35
Proteins	16.70±0.08	12.64±0.26	19.59±0.78	13.11 ±0.26	13.55 ±0.22	10.30 ±0.08	17.64±0.35	18.330.22	13.15±0.11	10.55±0.06
Ash	8.13±0.12	6.36±0.22	5.40±0.66	7.17±0.11	7.4±0.18	6.8±0.04	6.21±0.31	7.14±0.03	6.31±0.03	8.03±0.08
Fiber	9.90±0.06	10.71±0.08	15.70±0.14	5.86±0.47	13.11±0.25	22.81±0.07	9.60±0.12	17.51±0.02	13.22±0.05	10.47±0.02

IF: Industrial formulation

3.2 Discussion

Pathologies affecting poultry farms are consist of one of the major constraints of this sector and therefore the main cause of economic losses registered each year. In this study, we isolated and identified, in poultry feeds samples, three microorganisms including *Salmonella*, *E. coli* and *Aspergillus* involved in the diseases that frequently reported in poultry sector.

E. coli and *Salmonella* are normal hosts of the animals' digestive tract. Therefore, the presence of these bacteria in poultry feed could be due to contamination from their droppings. Indeed, most of the samples analyzed in this study were collected directly from the feed bunks to better assess the animals' exposure risk to these pathologies. This contamination could also be due to poor hygiene conditions during the formulation of these feeds. Indeed, our results indicate that the *Salmonella* and *E. coli* contamination level of formulated feeds was higher than those of industrial origin.

Colibacillosis due to *E. coli* pathogen for avian is probably the most frequent and most important bacterial infection in poultry disease. They can lead to mortality and reduced performance. In birds population, the APEC group including O1K1, O2K1 and O78K80 and other non-typeable pathogenic serotypes are usually implicating in respiratory infections, septicaemia and diarrhea diseases cases.

Concerning *Salmonella*, over 2400 different serotypes have been identified. Among these, *S. gallinarum* and *S. pullorum* are known to be producing severe systemic disease in chickens. *Salmonella* infection is normally via the oral route. The organisms rapidly invade the host through lymphoid tissue, including the Peyer's patches, the caecal tonsil in chickens and possibly also the enterocytes of the intestinal mucosa. In some cases, bacterial multiplication may result in host death. *Salmonellosis* due to these serotypes continue to cause economic losses in those parts of the world where the poultry industries are continuing to intensify [9].

In other hand, *Salmonella* and *E. coli* are also potentially pathogenic for humans so the presence of these bacteria in animal feeds could induce health problems for farmers because of the risk of farmer contamination.

In this study, *Aspergillus* genus were detected in the five rearing areas visited. Many surveys also indicated the presence of *Aspergillus* in different animal feeds [10, 11]. These moulds which contaminate these feeds could have several origins. According to the existing studies, fungi constitute a significant part of the airborne microflora in this sector and their concentrations in stationary measurements usually range from 10^2 to 10^4 cfu/m³. The presence of *Aspergillus* in these feeds could also be due to contamination of the raw material used in the feeds formulation by farmers or in industry. Indeed, poultry feeds contain, among other ingredients, mixtures of home grown cereals and imported commodities. The use of such ingredients inevitably leads to the contamination of the final mixed feed with molds [12]. *Aspergillus*, is usually reported as contaminants on a wide range of organic substrates, especially in stored grains, maize and feedstuffs [13].

Moreover, *Aspergillus* genus such as *A. fumigatus* is implicating in aspergillosis diseases cases in poultry sector. *Aspergillosis* is acute pneumonia in chicks and respiratory distress in adults. Other signs such as weight loss, anorexia and febrile state are very often associated with this pathology. In some cases, acute aspergillosis can cause lesions in the respiratory tract, particularly in lung tissue [14]. In addition, Rosa et al. [11] were reported that some *Aspergillus* species isolated from animals feeds ability to produce OTA and aflatoxins.

The aspergillosis disease implicated according to Richard et al. [15] in 5-10% of mortality rate in the youngest birds in poultry sector.

Moreover, both viable forms of these fungi and their products (mycotoxins) as well as fungal spores may cause a number of disorders in poultry breeding workers, concerning mainly the respiratory tract (mucous membrane irritation, invasive mycoses of lungs, allergic rhinitis, allergic pulmonary alveolitis, asthma) and the skin (dermatomycoses and onychomycosis [16]). The aspergillosis diseases implicated, according to Richard et al. [15], in 5-10% of mortality rate in the youngest birds in poultry sector. The presence of *Aspergillus* in the feeds analyzed in this study would constitute a danger not only for the animals but also for the farmers. However, further studies will need to identify the species isolated and to evaluate their mycotoxin production capacity.

In general, the majority of microorganisms needs a relative humidity (RH) ranged from 20 % to 60 % and lower (RH) humidity than 20 % creates an unfavorable environment for their growth [17]. Thus, low feeds moisture levels ($7.52 \pm 0.16\%$ to $12.14 \pm 0.05\%$) observed in this study could limit the survival time of microorganisms in both industrial origin feeds and those formulated by farmers.

In present work, the analysis of the chemical content of feeds was carried out according to standards recommended for broilers chickens, pullets and layers feed. As the ash amount obtained after incineration is proportional to the total mineral amount of feed analyzed, our results indicate that the industrial origin samples with higher ash content (8.36% -) could be richer in mineral content than those formulated by farmer. indeed, mineral content fulfil different functions such as maintaining osmotic pressure (sodium), maintaining ionic balance (chlorine) or the constitution of the skeleton and/or the eggshell (calcium, phosphorus). In this context, minerals must be provided in sufficient quantities by the feed to avoid deficiencies detrimental to the proper physiological functioning of the animal body. Even if further characterization studies are need to identify the types of minerals these feeds, industrial origin feeds are better indicate for obtaining good zotechnical performance.

The fiber correspond to undigested or unassimilated carbohydrates, doesn't provide energy but make the feeds more digestible. Moreover, fiber are known to increase the volume of stools and reduce the discomfort associated with constipation [18]. Therefore, feeds formulated by farmers with the highest level ($25.21 \pm 0.05\%$) of fiber would be more digestible for poultry and thus contribute to the maintenance of animal health.

According to **Nesello et al. [19]**, the nutritive content of chicken meat, and in particular its fatty acid profile, depends on composition of the feeds during farming. These feeds must provide nutrients in sufficient quantities to cover the animal's needs. In animal farming, the needs shall be defined in relation animal (age, stage of production, sex, strain, individual variability), the environment (ambient temperature, quality and use of the grazing area), and the production objectives set (age at slaughter, piece yield, meat quality for broilers, number and quality of eggs for laying hens).

Regardless of the breeding conditions, the standards recommended nutritive content for chickens **Nesello et al. [19]** for protein and fat range respectively from 16 to 21% and 2 to 7%. Thus, the second class which including feeds with levels below 16 % would be considered low in protein unlike samples in the first class. This deficiency in protein of these feeds could reduce zootechnical performance and impact the final quality of the meat, as protein affects muscle yields and the shape of the animal.

However, characterization studies of these proteins should make it possible to verify the presence of essential amino acids such as lysine, methionine, arginine, tryptophan, threonine, glycine and cysteine, which are recommended for good zootechnical performance and for obtaining meat of better nutritional quality.

Concerning the lipid rate in the feedstuffs analyzed, values are approximately similar to the recommended standards indicating a good nutritional lipid intake from feeds formulated and of industrial origin even if further research must be done to determine the fatty acid profile of analyzed feeds.

4. Conclusion

The present study shows that the poultry feeds used in Abidjan district are poor microbial quality. In addition, potential pathogenic microorganisms such as *E. coli*, *Salmonella* and *Aspergillus* generally involved in poultry diseases during rearing were detected in these feeds. However, further studies of isolated strains are needs to confirm involvement of these microorganisms in poultry diseases. This study also indicates that the analyzed feeds content are mostly in conformity with the recommended standards.

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