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Bacteriological Study of the Fishmeal used in Feed for Imported Chicken in Atlantic Department

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Abstract: Bacteria are microorganisms involved in the digestion of foods by many animals. At the same time, some of them are pathogens causing serious diseases to the survival of animals that harbor them. It is therefore essential to identify the different species of bacteria in feed served to farm animals. The present study aims at identifying the bacteria in the fishmeal used for feeding imported chickens in Atlantic Department. Out of sixty-one (61) samples collected, twenty six (26) came from provender sites, sixteen (16) from wholesaling importers and nineteen (19) from imported chicken breeds. A total of twelve types of bacteria were identified: *Staphylococcus*, *Klebsiella*, *Serratia* spp, *Actinomyces piogenes*, *Erysipelothrix* spp, *Citrobacter* spp, *Escherichia coli*, *Lysteria* spp, *Levinea* spp, *Salmonella* spp, *Bacillus* spp, *Providencia* spp. Ten out of them, namely *Staphylococcus* spp, *Klebsiella* spp, *Serratia* spp, *Actinomyces piogenes*; *Erysipelothrix* spp, *Escherichia coli*, *Levinea* spp, *Bacillus* spp, *Lysteria* spp, *Providencia* spp were isolated from provender and five (5) bacteria: *Staphylococcus* spp, *Klebsiella* spp, *Actinomyces piogenes*; *Lysteria* spp; *Levinea* spp were found in fishmeal from wholesalers. Regarding the poultry farms, seven (7) bacteria out of twelve, *Staphylococcus* spp, *Actinomyces piogenes*, *Citrobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Levinea* spp and *Providencia* spp were isolated from fishmeal. As for the two varieties of fishmeal used, it is clear that *Klebsiella* spp was more present in the Extra variety at a rate of 27.27%. On the other hand, the Valcru variety was contaminated with Staphylococcus spp at a rate of 18.18%. Therefore, it is essential to take into consideration hygienic measures.

Key words: Bacteria, feed, fishmeal, imported chickens

INTRODUCTION

In most African countries activities concerning poultry represent a significant part of the rural economy particularly and of the national economy as a whole (Ouandaogo, 1990; Diambra, 1990; Kulube, 1990; Sonaiya, 1990). Although enhanced in West Africa the first and second decades after independence, intensive poultry production has not yet blossomed (Adeyeye, 1990). The real problem appears to be the unsustainable nature of intensive poultry production systems developed in the post-independence period. This non-sustainability is due to technical, biological, institutional and socio-economic problems.

In Benin Republic as in the rest of West African countries, poultry breeding has considerably evolved over the last ten years. There was a number of 800,000 livestock (laying hens, chickens, broiler fowls) in 2003

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compared to 14,700 in 1983. About 300 farms ranging from 500 to 20 000 livestock produce 1400 tons of meat and 7,000 tons of eggs, that is 112 million units of eggs (Aliou, 2001). However, breeding of imported chickens is faced with a lot of constraints which restrain the increase in livestock production. For instance in most African countries, the chickens have no regular health control programme, may or may not have shelter, and scavenge for most of their nutritional needs. Supporting data in the literature have been provided for Burkina Faso (Bourzat and Saunders, 1990), Ghana (Veluw, 1987), Mali (Kuit *et al*., 1986), the Niger (Abdou and Bell, 1992), Togo (Aklobessi, 1990) and the United Republic of Tanzania (Yongolo, 1996). Among other factors, there is lack of quality control of biological and anti-infectious products. In addition, the lack of knowledge in breeding techniques has been the subject of many research topics in poultry farming (Sonaiya, 1989). However no scientific research has been conducted on the microbiological quality of raw materials which make up poultry feed in Benin Republic. For proper monitoring of poultry, it is imperative to identify the bacterial flora present in fishmeal that represents the nutritional diet of imported chickens. Therefore the present research was carried out to investigate the bacteria contaminating the fishmeal used for feeding chickens imported in Atlantic department.

MATERIALS AND METHODS

Materials: The present investigation was carried out during September and October 2010 in Zou and Collines department located in the central part of Benin. Two varieties of fishmeal, namely Extra-fishmeal and Valcru-fishmeal imported from Dakar, Senegal and sterile bags were used to collect samples from 16 wholesalers, 26 provender sites and 19 poultry farming. The wrapped and labeled samples were stored in a refrigerator at +4ºC to be used the next day. Dehydrated media purchased from the "Pasteur Institut Production" made in France were used as media culture.

Sampling and labeling of bacteria: Two hundred grams of fishmeal samples were collected from 16 wholesalers, provender sites and poultry farming into sterile bags. To make the sampling representative, the fishmeal was collected from three different levels of the bags (top, middle and bottom). They were labeled and transported to the Microbiology laboratory for bacteria analyses. The isolation and identification of pathogenic bacteria were conducted at the Veterinary Laboratory in Bohicon (Labovet, BP 2069, Abomey, Benin Republic) using the method of Chantal (1973). The bacteriological analysis of fishmeal was centered on the search of germs on selective medium and the identification of Micrococci Gram-negative bacteria (Gram-) and Gram-positive bacteria) Gram+. The incubation was carried out for 24 h to isolate and identify Gram+. It was then followed by enrichment on selenite stock to isolate Enterobacteriaceae Gram-negative bacteria (GRAM-). After thorough examination at cool condition and staining of GRAM, the bacteria were cultured on agar Chapman. It was a selective medium for isolation and identification of Micrococcaceae, including *Staphylococcus aureus*. The bacteria were also cultured on agar nutrient. Biochemical characteristics (catalase, haemolysis, urease, indole, DNase, fermentation of glucose, lactose, mannitol) and mobility properties were used to identify the different species of bacteria present in the fishmeal.

Statistical analysis: Z-test of Fischer for comparison values was used. The test intended to compare two amounts or percentages from two independent populations to determine which was statistically higher (or lower) or equal. The null hypothesis test was H0: $p1 = p2 = p$, or H0: $p1-p2 = 0$ and the alternative hypothesis was H1: $p1 \neq p2$ or H1: $p1-p2 \neq 0$. It was a test of homogeneity of two frequencies (or proportions). The formula used was: $Z\ a = (p1-p2)/ [(p\ (1-p)\ /n1) + (p\ (1-p)\ /n2)]^{1/2}$, where p1 is the proportion in the first population, p2 that of the second population, p the average frequency of both populations, n1 the number of the first population and n2 that of the second population.

RESULTS

Content and frequency of pathogenic bacteria isolated in the samples: The results are shown on Table 1. In respect of samples from wholesalers, the highest frequency 43.33% was recorded with Klebsiella spp and the lowest frequency 0% was recorded with *Serratia* spp, *Erysipelothrix* spp, *Citrobacter* spp, *Escherichia coli*, *Salmonella* spp and *Bacillus* spp. Regarding the provender site, the highest frequency was 31.71% with *Actinomyces piogenes* and the lowest frequency of 0% was obtained with *Citrobacter* spp and *Providencia* spp. Finally, for poultry farming, *Staphylococcus* spp had the highest frequency about 33.33%. The lowest frequencies (0%) were obtained with *Serratia* spp, *Erysipelothrix* spp, *Listeria* spp, *Salmonella* spp, *Bacillus* spp.

The results showed significant difference at 5% among provender sites, poultry farming and wholesalers for Staphylococcus spp and *Listeria* spp on one hand. On the other hand, there was a statistically significant difference between provender sites and wholesalers for *Actinomyces piogenes*. However, there was no significant difference between the frequencies from provender sites and wholesalers for Staphylococcus spp and *Listeria* spp. The comparison between values from provender sites and poultry farming for *Klebsiella* spp, and those from Wholesalers and poultry farming for *Serratia* spp, *Erysipelothrix* spp showed no significant difference. Similarly, there was no statistically significant difference

Table 1:Frequency of germs isolated per pattern and Z-test for comparison of proportions (2x2): comparison of three patterns for each germ isolated

	Germs isolated											
Patterns	*Staphylococcus* spp	*Klebsiella* spp	*Serratia* spp	*Actinomyces* piogenes	*Erysipelothrix* spp	*Citrobacter* spp	*Escherichia coli*	*Listeria* spp	*Levinea* spp	*Salmonella* spp	*Bacillus* spp	*Providencia* spp
Provender	12.2A	19.51A	12.2B	31.71B	4.88A	0A	2.44A	9.76B	2.44A	2.44A	2.44A	0A
Wholesalers	13.33A	43.33B	0A	16.67A	0A	0A	0A	23.33B	3.33A	0A	0A	0A
Farms	33.33B	19.44A	0A	19.44A	0A	2.78A	11.11A	0A	8.33A	0A	0A	5.56A

Frequencies followed by the same letter are not significantly different at the probability of 5% but those followed by different letters are significantly different at the probability of 5% (Z test results for comparison of proportions)

Table 2: Frequency of microorganisms isolated per variety of fish meal and the Z-test comparison of proportions (2x2): Comparison of two varieties of fish meal

	Germs isolated											
Varieties	*Staphyloc* occus spp	*Klebsiella* spp	*Serratia* spp	*Actinomyces piogenes*	*Erysipeilothrix* spp	*Citrobacter* spp	*Escherichia coli*	*Listeria* spp	*Levinea* spp	*Salmonella* spp	*Bacillus* spp	*Providencia* spp
Fish meal extra	11.36A	27.27A	6.82A	20.45A	4.55A	0A	6.82B	18.18A	2.27A	2.27A	0A	0A
Fish meal valcru	25.81B	25.81A	3.23A	24.19A	0A	1.61A	3.23A	4.84A	6.45A	0A	1.61A	3.23A

Frequencies followed by the same letter are not significantly different at the probability of 5% but those followed by different letters are significantly different at the probability of 5% (Z test results for comparison of proportions)

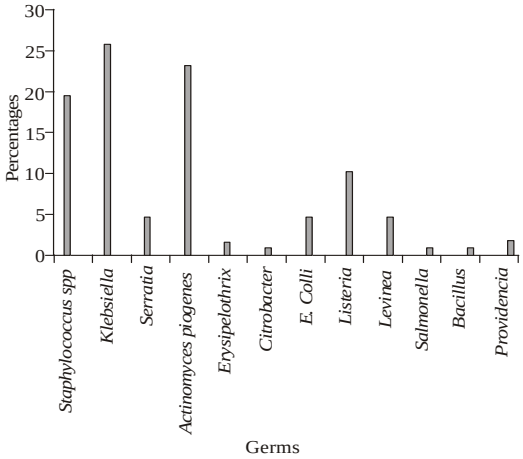

Fig. 1: Frequency distribution of germs isolated from fish meal in the republic of Benin

among the frequencies from provender sites, Wholesalers and poultry farming for *Citrobacter* spp, *Levine* spp, *Salmonella* spp, *Bacillus* spp; *Escherichia coli* on one hand and between provender sites, Wholesalers for *Listeria* spp on the other hand.

Comparison of frequencies of bacteria isolated from the two types (Extra and Valcry) fishmeal using Z-test: The trend from the comparison between the frequencies from Extra-fishmeal and Valcry-fishmeal is shown in Table 2. For Extra-fishmeal, the highest frequency 27.27% was obtained with *Klebsiella* spp. The lowest frequency (0%) was obtained with *Citrobacter* spp, *Bacillus* spp and *Providencia* spp. In respect of Valcry-fishmeal, the frequency of *Staphylococcus* spp was similar to that of *Klebsiella* spp. It was 25.81% and represents the highest value. The lowest (0%) was obtained with *Erystipelothrix* spp and *Salmonella* spp. However statistic test at 5% showed no significant difference between these values from the two types of fishmeal.

Frequency distribution of microorganisms isolated from the fishmeal in Atlantic department: Figure 1 depicts the trend reported in the present investigation.In the Department of Atlantic, the highest frequency of 26.17% was obtained with *Klebsiella* spp, followed by 23.36, 19.63, 10.8, 4.67 and 1.87%, respectively for *Actinomyces piogenes*, *Staphylococcus* spp, *Listeria* spp, *Escherichia coli*, *Levine* spp. *Erysipelothrix* spp and *Providencia* spp. *Citrobacter* spp, *Salmonella* spp and *Bacillus* showed the lowest frequency (Fig. 1).

DISCUSSION

The study was based on a bacteriological analysis of different types of fishmeal used in Atlantic Department to feed imported chickens. Five bacteria out of twelve commonly recorded were observed in fishmeal obtained from wholesalers. This result could be explained by the contamination of fishmeal at the origin or alternatively during transport or in the storage site after the purchase. According to Chantal (1973), the microbiology of aquatic environment determines significantly that of fish. Therefore, fishmeal would have been contaminated if the fish used in its making had been infected. In addition, the manufacturing conditions of fishmeal could increase the rate of contamination of the final product. It should be noticed that fishmeal was imported from Dakar (Senegal) where many manufacturers do not have the suitable industrial equipment to produce fishmeal exempt of harmful pathogens. Moreover, the pursuit of maximum profit through the purchase of raw materials at low price leads importers in Benin to buy fishmeal whose microbiological quality is doubtful. Above all, warehouse or storage of fish used could increase the amount of germs of the final product. Indeed it is in precarious and unhealthy places which do not meet hygienic and health standards that storage for fishmeal is to be found. In such cases, the risks of contamination are high especially when fishmeal is poorly sterilized and preserved (Bodin *et al*.,

1965; Guillaume *et al*., 1999). Pilet *et al*. (1983) explained that the contamination of food can be observed along the food chain including producers, processors, distributors and consumers. The presence of ten out of twelve bacteria in provender sites poses the problem of environmental conditions as a result of proliferation and spread of germs. The collective provender sites are places where many farmers come to buy feed where the exchange of microbes associated with avian diseases is promoted. It is also easy to notice that some of collective provender sites do not provide hygienic conditions such as a foot soak outside the building areas. Periodic disinfection is almost nonexistent in the practice of food sellers. These bad practices of food preservation for breeding contrast with the recommendations of Jourdain (1990) which stipulate that "breeding is nothing but hygiene".

With respect to poultry farming, the identification of seven different pathogenic bacteria out of a total of twelve could be explained either by the contamination that often occurs during the transport of food or lack of care during the various samplings leading to their usage or both factors. Polluted breeding materials associated with a poorly hygienic preparation of food on the ground could also contribute to the dissemination of germs affecting the quality of fishmeal. In many cases, raw materials used in the composition of food rations were stored directly on the ground, sometimes near feces of chickens which were kept in conditions that promote the spread and proliferation of microbes. Adeyeye (1990), Kulube (1990) and Aliou (2001) associated some of these aspects with poor farming practices.

The two types of fishmeal used in poultry areas in the Republic of Benin contain microbial germs: 9 types germs out of 12 from Extra-fishmeal and 10 out of 12 types from Valcru- fishmeal. The large number of microorganisms isolated and identified from different types of fishmeal could explain the emergence of various diseases such as fowl flu, fowl pest, etc. often recorded in poultry breeding centers and are responsible for significant economic losses through high mortality. Poultry farmers, faced with this situation, and in total absence of diagnostic laboratory, completely ignorant of the risks, still use antibiotics which were the main cause of poultry diseases. It should be noticed that no criterion for bacteriological analysis was taken into consideration by government-controlled institutions responsible for ensuring the promotion of poultry activities. These observations confirm the findings of, Adeyeye (1990), Kulube (1990), Ngoupayou(1990), Sonaiya (1989, 1990) and Chrysostome and Allard (1997) who pointed out that the difficulties of breeding poultry in Africa and particularly in Benin were mainly due to poor control of hygienic and sanitary aspects of raw materials used to feed chickens.

The high frequencies of bacteria such as *Klebsiella* spp, *Actinomyces piogenes*, *Staphylococcus* spp and to a lesser extent for others such as *Listeria* spp, *Levine* spp, *Erysipelothrix* spp, *Providencia* spp, *Citrobacter* spp, *Salmonella* spp and *Bacillus* spp in both types of fish meal constitute evidence in favor of questionable microbiological quality of some raw materials used for poultry feed in Atlantic Department. The absence or insufficiency of an appropriate program of training in poultry farming in this Department as in other parts of Benin Republic, where poultry has increased over the last ten years, is one of the factors causing the spread of microbial agents. The health and hygiene standards are virtually nonexistent; unfortunately we cannot avoid high risk of losses owing to dissemination and contamination. The setting up of aviculture in Benin is not subjected to authorization by the officials in charge of promoting farming. The modern poultry farms, regarded as a secondary activity are practiced by the majority of farmers without a minimum of basic knowledge in the field. The absence of organizations to plan the distribution of the rations for poultry gives the occasion for the excessive use of feed of questionable quality. In addition, as the major concern for farmers is feeding the poultries, nutritional and bacteriological control of fishmeal are neglected.

CONCLUSION

This study allowed us to analyze the bacteriological status of the different types of fishmeal used for feeding imported poultry in Atlantic Department. Our study indicates that fishmeal from provender sites are more contaminated with a large number of several microbial species: *Staphylococcus* spp, *Klebsiella* spp, *Serratia* spp, *Actinomyces piogenes*, *Erysipelothrix* spp, *Escherichia coli*, *Levine* spp, *Salmonella* spp and *Bacillus* spp. It should also be pointed out that among the imported fishmeal for poultry farming Valcru type was more contaminated than Extra type. It is then imperative to initiate a collaborative program involving the government and all stakeholders in aviculture which complies with health and hygiene standards of fishmeal.

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