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PHYSICAL AND MICROBIAL CONTAMINANT LEVELS IN FEED INGREDIENTS AND FEEDS FROM OKO-OBA AND IKORODU FEED MARKETS OF LAGOS STATE, NIGERIA

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ABSTRACT
The contaminants and microbial flora ofsixbrandedfeeds and feed ingredientspurchased from Agege and Ikorodu feed markets in Lagos state, Nigeriawere investigated.Hundred gram of each feed and ingredient was weighed in triplicate and spread on a clean sheet of paper to pick the contaminants which were weighed and percentage determined.Maize had the highest percentage (28%), among the ingredients obtained at Ikorodu while blood meal had the highest percentage (27.3%) at Agege. The full fat soybean had no visible contaminant at all, while 72% fishmeal had 0.1% at the two locations. The contaminants in 65% fish meal was significantly difference ($P<0.05$) compared to other feed ingredients at both markets.The type of contaminants found in individual ingredient varies with the type of feed ingredients which include sand particles, nylon, husk and maize cob. One gram each of the fish feeds and ingredients was separately dissolved in 9ml sterile distilled water. Samples from the serially diluted solution were cultured in agar for identification of microbes.Isolated bacteria were *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*while fungi were *Aspergillus niger*, *A.flavus*, *Mucor* sp. and *Rhizopus stolonifer*. The *B. subtilis*, was present in all the samples while the percentage frequency distribution revealed the prevalence of *B. subtilis* and *Aspergillus* sp. at the two markets. The level of contaminants found in fish ingredients and feeds at the two locations were low with the exception of *Aspergillus* sp.however, good hygiene is required to avoid or lessen feed contaminations.

KEYWORDS: Physical, Microbial, Contaminants, feed ingredients, fish feed

INTRODUCTION

The role of animal feed in the production of safe food is recognized worldwide, and recent events have underlined its impacts on public health, feed trade and food security. Common feed problems associated with *Salmonella* sp.,*E. coli* and other micro-organisms, have encouraged health professionals and the feed industry to scrutinize more closely the causes of these diseases and methods for their control. Animal feed is an important route by which hazards can enter the human food chain, its safety must be assessed prior to its feeding to animals. Safety assessments are often multifaceted and usually considerboth the safety of animals as the primary consumers of the feed, and safety of humans as the indirect consumers of any residues that may remain in food of animal origin. In some cases, risk to people working with and mixing feed, and risk to the environment are also assessed (FAO/WHO) [14].

Codex alimentarius an inter-governmental body founded in 1962 to implement the joint FAO/WHO food and feed standard programme indicated that Food hazards associated with animal feed can be biological, chemical or physical;each hazard is associated with particular sources and routes of contamination and exposure. Hazards may be introduced with source materials or via carryover or contamination of products during handling, storage and transportation. The presence of a hazard may also result from accidental or deliberate (e.g. fraud or bioterrorism) human intervention (FAO/WHO) [14]. Thus, a contaminant is any biological or chemical agent, foreign matter or other substances which are present in and/or on feed and feed ingredient that may compromise food safety or suitability and which constitute a risk to consumers' health(ANZFA) [3].The potentially hazardous physical contaminants include: bone, glass, metal and plastic while the potentially harzardous microbial contaminants include fungi(mycotoxins) and bacteria.Feed materials may be inoculated at any time during growing, harvesting, processing, storage and dispersal of the finished feed.

Fish production involves good nutrition and adequate feeding; poor feed composition may result in reduced feed intake with a resultant poor growth and eventual death. The efficiency of feed is dependent upon the quality of the feed which in turn depends on the quality of the feed constituents. Hence the aquaculture feed compounder and/or farmer must ensure that the fish feeds used on the farm should have undergone, quality and biological evaluations to ensurenutritionally balanced, good quality and economically viable feed ingredients and feeds(FAO/WHO) [12] [13].

This study therefore evaluates the physical and microbial contaminants of fish feed ingredients and feeds sold in two major local feed markets of Lagos State.

MATERIALS AND METHODS

Procurement of fish feed ingredients and feeds

Commercially available floating fish feeds and feed ingredients were procured from randomly selected sales outlets atAgege and Ikorodu marketsbecause they are the major animal feed markets in Lagos State.
Six branded floating feeds (Coppens, Aqua feed, Raanan,Multifeed,Zieglar and Durante)were purchased from Agege and Ikorodu feed markets.

The following feed ingredients of energy source (maize and indomie waste) and protein source (soybean meal, full fat soya, groundnut cake, fish meal (65% and72%) and blood meal) were purchased from both markets.

Analysis of fish feed ingredient and feeds

Percentage contaminant level

Hundred grams of each feed and ingredient was weighed in triplicate and spread on a clean sheet of paper; a magnet was run on the samples to see if they contain any metallic material. Both the metallic and non-metallic contaminants were handpickedand weighed. The percentage contaminants were determined thus;

$$\text{Percentage contaminants} = \frac{\text{weight of contaminants}}{\text{Total weight of sample taken}} \times 100$$

The average weight of the triplicate samples was calculated per sample.

Microbial analysis of feeds ingredients andfeeds

One gram each of the fish feeds and feed ingredients were separately dissolved in 9ml sterile distilled water. Serial dilution was carried out to get isolated colonies, for the twelve (12) samples of the different fishfeeds and forty two (42) samples of the various feed ingredients. Samples from the serially diluted solution were cultured in the following agar for possible identification of microbes. Nutrient agar (NA) was used to isolate bacteria, Potato dextrose agar (PDA) was used to isolate fungi/yeast, Mac Conkey agar (MAC) was used to isolate Coliforms, Monitor Salt agar (MSA) was used to isolate *Staphylococcus aureus*. The different plates were then incubated thus; NA plates were incubated at 37^0C for 24hrs, PDA plates were incubated at room temperature for 3-5 days, MAC plates were incubated at 37^0C for 24/48hrs, MSA plates were incubated at 37^0C for 24hrs. At the end of incubation period, the culture plates were observed, counted and screened. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbon) [7]. Gram staining was carried out to distinguish between gram-positive and gram-negative bacteria. Bacterial count was determined using spread plate count according to APHA [4].Fungal isolates were identified based on their morphological and cultural characteristics.The total viable counts, total fungi count, total coliform count, total staphylococci counts and the predominant species of microorganisms were isolated.

STATISTICAL ANALYSIS

The data collected during the course of the investigation were subjected to statistical analysis and T test was used to compare the mean values for the parameters. All computations were performed using the statistical package SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The contaminants and the average level of contaminants present in feed ingredients obtained at Agege and Ikorodu Markets are shown in Table 1while Table 2 is a comparative analysis among the two locations studied.

Maize had the highest level of percentage contaminants (28%), among the ingredients obtained at Ikorodu while bloodmeal had the highest percentage contaminant (27.3%) from Agege. At the two stations studied, full fat soybean had no visible contaminant at all, while 72% fishmeal had only 0.1% at the two locations. The contaminants in 65% fish meal is significantly difference ($P<0.05$) compared to other feed ingredients at both markets. The type of contaminants found in individual ingredient varies with the type of feed ingredients and these include sand particles, nylon, husk and maize cob. Generally the presence of contaminants in the feed ingredients may be due to poor handling, processing and storage (Tacon *et al.*) [19]. The occurrence of these contaminants limits the digestibility of the feeds and reduces their nutritional values because; feed physical properties affect its intake and digestibility (Cruywagen and Calitz) [9].

The comparative average level of microbial load isolated in feeds ingredients at Agege and Ikorodu markets areas of Lagos state is shown in Table 3. All ingredients except full fat soybean at Agege had fungi contamination however, there was a significant difference ($P<0.05$) in the fungi load of Indomie when the two locations were compared. There was no*S. aureus* found in maize and SBM from Agege and, the GNC from Ikorodu. Values of *S. aureus* in other ingredients were not significantly different ($P>0.05$) among locations.

The viable count among ingredients studied at the two locations was only significant for the 72% fish meal (2.75 ± 0.63 and 1.36 ± 0.20) and 65% fish meal (2.30 ± 0.14 and 1.43 ± 0.25)for the two locations respectively.
The level of microbial load isolated in floating (Branded) feeds in Agege and Ikorodu marketsis shown in Table 4. The fungi load is low except for Durantee feed at the two locations. Also, *S. aureus*was present in all the floating feeds except Durantee and Biomarfeeds while Coliform, was present in Zeigler, Durantee and Biomar feeds.

Fish feeds and their ingredients are constantly in contact with environmental organisms and become readily colonized by various microbial species. Animal feed, due to its composition, provides a favourable environment for the micro organisms' growth (Čabarkapa) [8].

Though, there was a negligible difference in the microbial load of the samplesbetween the two locationsnevertheless, all the samples had Bacillus *subtilis*.The occurrence of *B.subtilis* can be said to be as a result of prevalence of their spores in the environment (Adebayo-Tayo *et al.*) [1]. *Bacillus* species are spore formers whose spores could survive high temperatures of processing (Adebayo-Tayo*et al.*) [2]. The organisms are present in most raw materials used in food manufacturing at concentration of 10^3/g or less butthe infectious dose has been estimated to be 10^5/g (Adebayo-Tayo*et al.*) [1].

The value of S. *aureus* ranges between $0.36\text{x } 10^1$ and $0.43\text{x } 10^1$per gram in both locations which did not exceed the permissible limit ($<10^5$ per gram)recommended by Food and Agricultural Organisationfor bacterial count for both fish and humans(FAO) [11].

To produce detectable levels of toxin, the number of organisms must be over $10^{5\text{-}6}$ per gram of product, which is higher than the level detected in the present study.

The presence of coliforms such as *E. coli* isolated from the feed/ingredient samples reveals the contaminations of the environment by faecal matters [2], though coliform was absent in sinking feedsat the two locations. According to Nester*et al.* [16] the presence of *E. coli* can cause dysentery in human if the product is consumed.

The attendance of species of *Aspergillus* sp. could be attributed to the prevalence of their spores in the atmosphere (Adebayo-Tayo*et al.*) [1]. This organism is easily trapped during the post-harvest processing and handling of feed ingredients. Since most fungal spores are found in the air, the spores must have contaminated the feeds/ingredients during storage, transportation and displaying of the feeds and ingredients at the market. The liberated spore can easily settle on food and ceilings of room and then germinate (Okhuoya and Ayanlola) [17]. Dongo and Ayodele [10] have shown that *Aspergillus* sp. occurred highest in the number of colonies identified from air spora of some localities. In the present study the *A. niger* was the most prevalent, this finding is in agreement with Pitt and Hocking [18] and Zimmerli and Dick [20], who had earlier established *Aspergillus* genera predominance over other genera in tropical environment. Also, Battilani *et al.*[6] attributed the predominance of *Aspergillus* to be due to their high temperature tolerance character.Thus, the high frequency of *Aspergillus* fungi in the two sales outlets might be due tothe high temperature condition of the studied locations.

Mould infestation in stored feeds reduces nutritional value owing to the loss of dietary lipids, amino acids and vitamins by enzymatic digestion (Lim and Ibrahim) [15]. The occurrence of *Aspergillus* species in fish feed is particularly of concern because they are known as the most toxigenic among the fungi (Azarakhsh *et al*.) [5]. Most studies indicate that there is no correlation between the presence of a toxin and the producing fungus in the same substrate, but the presence of toxigenic fungi in feeds may be an indicative of their potentiality to produce mycotoxins (Azarakhsh *et al*.) [5]. Hence, Mucor and Rhizopus which occurred in almost all the samples in this study corroborate the findings of Čabarkapa *et al*.[8], that saprophytic fungi are frequently found in analysed samples, but are rarely the cause of microbiological unwholesomeness. Their presence, however, should not be neglected since they utilize nutrients from the substrate to sustain their metabolic demands and in that way decrease nutritional value of substrate. Furthermore, if adequate moisture and temperature requirements are met, they can proliferate and convert nutrients into metabolic products that alter sensory properties of feed (Cabarkapa *et al*.) [8]. consequently, regardless of maximum permissible limit values, they can indirectly affect the quality of feedstuffs and feeds.

CONCLUSION

The results of this study showed that the level of contaminants found in fish ingredients and feedsat the two locations were very low with the exception of*Aspergillus* sp. nevertheless, very high standard of hygiene is necessary to avoid or reduce feed contaminationsin order to achieve a profitable fish production ventures.

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Table 1: Types and Mean Percentage Contaminants in Feed Ingredients at Agege and Ikorodu Markets, Lagos State.

Feed Ingredients	Agege		Ikorodu	
	Type of Contaminants	% Contaminants	Type of Contaminants	% Contaminants
Maize	Sand,maize cob	28.06	Sand, maize cob	21.0
Indomie	Sand,nylon	0.46	nylon	1.86
Soybean meal	Husk	8.83	Husk	7.76
Full fat soya		0.00		0.00
Groundnut cake	Glass-like material	0.36		0.00
72% fishmeal	Black solid particles	0.10	Black solid particles	0.10
65% fishmeal	Sand	0.35	Bone, scale, sand	2.00
Blood meal		24.30	Hair, sand, hoof, remnants of burnt tyres, paper, wood shavings	27.30

Table 2: Comparative Analysis of Mean Contaminants in Fish Feed Ingredients at Agege and Ikorodu Markets, Lagos State.

Locations	Maize	Indomie	soybean meal	Full fat soya	Groundnut cake	72% fishmeal	65% fishmeal	Blood meal
Agege	2.81±0.79	0.05±0.01	0.61±0.28	0.00	0.04±0.04	0.01±0.00	0.04±.02*	2.43±0.77
Ikorodu	2.10±0.17	0.56±0.37	0.78±0.26	0.00	0.00	0.01±0.00	0.20±0.00*	2.73±0.99

(*) Showing level of significant difference (P<0.05)

Table 3: Levels of Microbial load in Fish Feed Ingredients at Agege and Ikorodu Markets, Lagos State.

		Fungi($x10^2$)					
LOCATIONS	MAIZE	INDOMIE	SBM	FFS	GNC	72% FM	65% FM
Agege	1.33±0.57	1.00±0.00*	0.67±0.57	0.00	2.67±2.51	1.50±0.70	1.00±1.41
Ikorodu	1.33±0.57	3.00±1.00*	1.00±0.00	0.30±0.70	1.00±0.00	2.33±2.30	0.67±0.57
		Staphylococcus($x10^1$)					
LOCATIONS	MAIZE	INDOMIE	SBM	FFS	GNC	72% FM	65% FM
Agege	0.00	2.00±2.64	0.00	3.33±5.77	2.33±2.08	1.00±1.41	5.00±2.82
Ikorodu	2.33±2.51	1.33±2.30	1.33±2.30	1.50±2.12	0.00	2.67±2.51	4.67±4.16
		Viable Counts($x10^4$)					
LOCATIONS	MAIZE	INDOMIE	SBM	FFS	GNC	72% FM	65% FM
Agege	1.70±0.36	1.80±0.80	1.43±0.30	1.76±0.47	2.40±1.15	2.75±0.63*	2.30±0.1*4
Ikorodu	1.43±0.15	1.60±0.20	1.46±0.05	1.50±0.00	1.43±0.57	1.36±0.20*	1.43±0.25*
		Coliform($x10^2$)					
LOCATIONS	MAIZE	INDOMIE	SBM	FFS	GNC	72% FM	65% FM
Agege	0.36±0.63	0.00	1.06±1.00	1.33±0.05*	0.36±0.63	0.65±0.91	0.60±0.84
Ikorodu	0.43±0.75	0.36±0.63	0.36±0.63	0.00*	0.43±0.75	0.00	0.40±0.69

(*) Showing level of significant difference (P<0.05)

Table 4: Levels of Microbial load in Floating Fish Feed at Agege and Ikorodu Markets, Lagos State.

		Fungi($x10^2$)					
LOCATIONS	AQUAFEED	ZIEGLER	MULTIFEED	COPPENS	RAANAN	DURANTE	BIOMAR
Agege	0.00±0	2.00±0.94	0.00±0	1.00±0.11	0.00±0	2.00±0.79	0.00±0
Ikorodu	0.00±0	1.00±0.32	0.00±0	0.00±0	0.00±0	2.14±0.78	0.00±0
		Staphylococcus($x10^1$)					
LOCATIONS	AQUAFEED	ZIEGLER	MULTIFEED	COPPENS	RAANAN	DURANTE	BIOMAR
Agege	6.00±2.67	3.14±0.87	3.00±1.09	4.00±1.67	5.00±2.17	0.00±0	0.00±0
Ikorodu	5.87±3.14	3.00±1.11	5.00±2.09	2.00±1.06	6.07±2.34	0.00±0	0.00±0
		Viable Counts ($x10^4$)					
LOCATIONS	AQUAFEED	ZIEGLER	MULTIFEED	COPPENS	RAANAN	DURANTE	BIOMAR
Agege	3.10±1.45	1.70±0.78	1.40±0.46	1.90±0.89	3.40±0	2.10±0.87	2.07±0.63
Ikorodu	3.25±1.23	2.30±1.19	1.30±0.53	1.10±0.67	2.94±1.13	2.63±0.58	1.90±0.76
		Coliform($x10^2$)					
LOCATIONS	AQUAFEED	ZIEGLER	MULTIFEED	COPPENS	RAANAN	DURANTE	BIOMAR
Agege	0.00±0	1.10±0.43	0.00±0	0.00±0	0.00±0	1.20±0.54	1.53±0.86
Ikorodu	0.00±0	0.97±0.54	0.00±0	0.00±0	0.00±0	1.33±0.72	1.30±0.54