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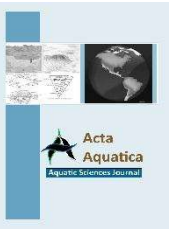

Nutritional and microbial safety of common commercial aquafeeds sold in Ogun State, Nigeria

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Abstract

The nutritional and microbial safety of fish feeds were investigated in this study. Commercially available fish feed brands (Eco-float, Skretting, Top-Feeds, Blue Crown) were procured from sales outlets in Ogun State, Nigeria. Moisture ranged from 8.15 to 8.82 and these values were not significantly different ($p>0.05$) except for Topfeed. Skretting had the highest crude protein value (48.49±0.03) while Ecofloat recorded the least value of 45.30±0.06 ($p<0.05$). The values obtained for crude fibre, crude fat, ash and carbohydrate were significantly different ($p<0.05$) across the sampled feeds. Crude fibre ranged from 4.31±0.04 in Ecofloat to 3.20±0.03 in Skretting. Skretting had the highest value recorded for crude fat (6.27±0.11) and ash (10.06±0.18) while Ecofloat had the least value of 1.29±0.00 and 8.01±0.17 respectively. Ecofloat had the highest value for carbohydrate (32.95±0.07), marginally followed by Topfeed (28.79±0.13) and Bluecrown (28.65±0.06) with Skretting recording the lowest value (23.55±0.38). The values for all the minerals analyzed were significantly different ($p<0.05$). Calcium had the highest values across the sampled feeds, followed by sodium while potassium recorded the lowest values. Tannin recorded the highest value amongs all the phytochemicals, followed by flavonoids and the least value was recorded for glycosides. Total aerobic count, total coliform count and total fungal count ranged from 1.61±0.01 to 3.53±0.0, 0.00±0.00 to 3.10±0.01 and 0.00±0.00 to 2.34±0.05 respectively. Isolated bacteria include *Staphylococcus aureus*, *S. saprophyticus*, *Klebsiella spp*, *Bacillus spp* and *Escherichia coli*. Fungi identified were *Aspergillus niger, Rhizopus spp, Aspergillus flavus, Geotrichum spp* and *Saccharomyces spp*. Hence, there is need for quality monitoring.

Keywords: aquafeed safety, microbial risk, Nigeria

1. Introduction

Fish like other animals have a requirement for essential nutrients in order to grow properly. In the wild, natural feeds are available and as the fish forage for these, they are able to meet their body needs. When fish is removed from its natural environment to an artificial one, enough food must be supplied in order for them grow (Eyo, 2003). Fish nutrition has advanced in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture industry as it expands, to satisfy increasing demand for affordable, safe, high-quality fish and seafood products. Thus, fish feeds play a key role in the development of fish farming as they provide nutrients for optimum fish growth and bring higher economic return to farmers. However, feed cost is one of the major constraints against the greater expansion of aquaculture, especially in developing nations (Teves and Ragaza, 2016; Rana *et al*., 2009; Agbo, 2008). According to Babalola (2010), fish feed accounts for 60–75 % of the total cost of fish production in many African countries such as Nigeria. Hence, for aquaculture to be highly successful, there is need for good quality and affordable feed, which can also encourage small scale farmers in the field of aquaculture for sustainable production and also meet the demand for fish (Robinson *et al*., 2001). In addition, when fish is fed with feed that is of low quality, it affects the nutrient composition which might lead to low residual protein in the flesh of the fish. Consequently, this is passed unto the consumer that consumes the fish and this makes the fish a poor source of animal protein. Therefore, good nutrition in fish production

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system is essential to economically produce healthy, high quality fish products. At present, there are different commercial fish feed industries in Nigeria. Farmers depend only on the existing information about the feed composition that is given by the industry. In addition, there is paucity of information on the mineral and anti-nutritional components of commonly sold fish feeds in Nigeria. Even, there is a possibility to use unauthorized feed ingredients in manufacturing feed by the feed producers. In addition, there have been reports concerning the contamination of aquaculture feeds and farmed produce with banned substances which has placed aquaculture feed and food safety in the spotlight. Also, owing to the recent lockdown due to COVID -19, there have been concerns on the microbial quality of fish feed due to long periods in storage without much sales being recorded. Microbial contamination of feed could lead to diseases in fish and also present public health problems to human population who consume infected fish or fish that habour pathogenic and multiple antibiotic resistant bacteria. Consumption of such contaminated fish can stimulate multiple antibiotic resistant problems in human population. The presence of pathogen contamination in fish feed may also reduce quality, hence, making it difficult for the fish to maximally utilize the feed.

Therefore, there is an urgent need to assess the actual nutritive value of the commercial fish feeds available in the market and compare with the fish feed manufactures' declared composition. In addition, the study went further to assess the minerals, anti-nutritional factors and microbial compositions of the selected fish feeds in order to verify their quality and safety.

2. Materials and Methods

2.1. Collection and preparation of sample

A quick survey was done to know the various commercial fish feeds used by fish farmers in Abeokuta, Ogun state. The feeds identified at the time of the study included Eco float®, Top®, Blue Crown®, and Skretting®. Samples of the commercial feeds were bought from different sales outlet within Abeokuta, Ogun state. The samples were then milled into powder using a burr mill. It was packaged, labeled, sealed and stored at room temperature for further analysis.

2.2. Proximate analysis

2.2.1. Determination of moisture content

Empty porcelain crucibles were dried in the oven at 105 ± 5^0C for 30 minutes in other to get rid of moisture present on the dishes. The porcelain crucibles were transferred into a desiccator and allowed to cool at room temperature for about 20 minutes. The weight of the empty porcelain crucibles was taken and recorded as $W_{0.}$ 1.00g of sample was weighed into the porcelain crucibles (record as $W_{1)}$ and dried in the oven at 105 ± 5^0C till constant weight or preferably for 4 hours. After drying the porcelain crucibles were transferred into the desiccator and allowed to cool at room temperature for about 30 minutes. The final weight of the porcelain crucibles and content was taken and recorded as W_2 (AOAC, 1990).

$$\% \text{ Moisture content} = \frac{(W0+W1)-(W0+W2)}{W1} \times 100$$

2.2.2. Determination of crude protein content

The powdered sample was tested for crude protein content according to the Kjeldahl's method as described in AOAC (1990). 1.00 gram of the powdered sample was weighed into a 250ml digestion tube, 2 kjeltabs Cu 3.5 and 12ml of concentrated H_2SO_4 (Sulphuric acid) was added. The whole mixture was subjected to heating at 1h at 420^{o}C in the digestion chamber until transparent residue contents was obtained. The rack of tubes was removed and placed in a stand and allowed to cool for 10 – 20 minutes. Tubes were inserted into the distillation unit and the safety door was closed. 80ml deionized water was added into the tubes. 25 – 30 ml receiver solution was then added into the conical flask and placed into the distillation unit and the platform was placed so that the distillate outlet was submerged in the receiver solution. 50ml of 40% NaOH (Sodium hydroxide) was dispensed into the tube and distilled for about 5 minutes. The distillate was titrated with standardized HCl (Hydrochloric acid) (usually 0.1 or 0.2N) until the blue grey end point was achieved. The volume of acid consumed in the titration was then noted.

$$\% \text{ Protein} = \frac{(T-B)x\ N\ x\ 14.007\ x\ 100}{W1\ (mg)}\ x\ F$$

$$\text{gN/L} = \frac{(T-B)x\ N\ x\ 14.007}{Sample\ Volume\ (ml}$$

W_1 = Sample weight (mg)
T = Titration volume of sample (ml)
B = Titration volume of blank (ml)
N = Normality of acid to 4 decimal places
F = Conversion factor for nitrogen to protein = 6.25 for food & feeds
gN/l = Gram Nitrogen per Liter

2.2.3. Determination of crude fibre

1.00g of well-prepared sample was weighed into the crucible containing the celite. 1.25% H_2SO_4 was prepared and heated on hot plate. The crucibles were inserted using the holder and locked into position in front of the radiator in the Fibertec® hot extraction unit ensuring that the safety latch engaged. The reflector was placed in front of the crucibles. All the valves were placed to closed position. Cold-water tap (1 – 2L/min) was opened for reflux system. 150ml of preheated 1.25% H_2SO_4 was added into each column. When the reagents started to boil, it was adjusted to moderate boiling using the 'heater' control. The solution was then boiled for 30minutes. Reversed pressure was used to wash the sample. It was washed three times with hot deionized water. 30ml portions of water was used and it was sucked dry as possible between washings. 150ml of preheated 1.25% NaOH solution was added into each column. Using the crucible holder, the crucibles was transferred to the Fibertec® cold extraction unit. At the end of the Fibretec® cold extraction procedure, crucibles were

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cooled slowly to room temperature in a desiccator and weighed accurately to 0.1mg.

$$\% \text{ Crude fibre} = \frac{W2-(W3+C)}{W1} X\ 100$$

W_1 = Sample weight (g)
W_2 = Crucible + residue weight after drying (g)
W_3 = Crucible + residue weight after ashing (g)
C = Blank

2.2.4. Determination of crude fat

5.00g of well blended sample was weighed into the thimbles and cotton wool was placed on the sample inside the thimble to prevent pouring out of the sample during extraction. The round bottom flask was dried in the oven at 60^0C and the initial empty weight recorded. 80ml of hexane was poured into the flask, the thimble containing sample was also fitted/placed into the extractor. The heating mantle was switched on and water was set running through the condenser for cooling. The extraction was allowed to continue its reflux for 2hrs after which it was discontinued. The flask was then dried again in the oven to eliminate all hexane present. The amount (%) of crude fat or oil present in the sample was calculated by subtracting the weight of the empty flask from the final weight (AOAC, 2008)

$$\% \text{ Fat} = \frac{weigh\ \ of\ flask\ after\ extraction\ and\ drying - weight\ of\ empty\ flask}{\text{Sample weight}} \times 100$$

2.2.5. Determination of ash

Ash is an inorganic residue remaining after the material has been completely burnt at a temperature of 550 °C in a muffle furnace. It is the aggregate of all non-volatile inorganic elements. Empty crucibles were dried in the oven at 130 ± 15^0C for 30 minutes to get rid of moisture present on the crucibles. The weight of the empty crucibles was taken and recorded as W_0. Thereafter, 1.00g of sample was weighed into the crucibles (recorded as W_1) and ashed in the furnace at 500 ± 15^0C for 5 - 6 hours. The ash was cooled in desiccators and reweighed as W_2.

$$\text{Ash content} = \frac{(W2 - W0)}{W1} \times 100$$

2.2.6. Determination of nitrogen free extract (NFE)

The nitrogen free extract (NFE) of the samples was calculated according to the following equation:

$$\text{NFE} = 100 - (\%\ moisture + \%\ ash + \%\ crudefat + \%\ crudefibre + \%\ crudeprotein)$$

2.2.7. Determination of mineral content

The mineral content of the feed samples was determined using the methods of the AOAC (2010). Calcium, potassium, magnesium, sodium and phosphorus were determined by Atomic Absorption Spectrometry. 2g of dry sample was weighed into a porcelain crucible and ashed at 550°C for 3hours. Crucibles were allowed to cool and the ash was dissolved with 100ml of 3N HCl and then stored in a plastic bottle with a plastic cap and taken to AAS for readings. In the Atomic Absorption Spectrophotometer (AAS), the corresponding lamp was placed for corresponding mineral and the wavelength was set specific for the minerals to be determined. The AAS siphoning hose was placed into the digested sample after running the standards for the mineral determined. The concentration of the mineral in the solution was displayed on the screen of the AAS machine.

2.3. Phytochemical analysis

2.3.1. Determination of tannin

Tannin was determined using Folin-Ceocalteu method. 1g of dry well blended sample was weighed into a flask. 10ml of distilled water was added. The content was centrifuged at 2500rpm for 15minutes. At the end of the procedure the absorbance's of the tannic acid concentrations at a wavelength of 725nm was read off. A calibration curve was drawn for the tannic acid standards (absorbance against concentration). The absorbance of the sample was traced down the concentration axis to obtain the tannic acid concentration of the sample (Rajeev *et al*., 2012).

$$\text{TAC (mg/kg)} = \frac{Conc.\ obtain\ (mg\ per\ l) x\ sample\ volume\ x\ DF}{Sample\ weight}$$

2.3.2. Determination of phenol

1.00g of powdered sample was weighed into a conical flask. 10ml of ethanol was added and it was plugged with aluminium foil. The content was shaken vigorously and left to stand for 30min for proper extraction. The content was filtered to obtain clear supernatant. The supernatant was used for total phenolics assay (Lee and Intan, 2012).

$$\text{TAC (mg/kg)} = \frac{Conc.\ obtain\ (mg\ per\ l) x\ sample\ volume\ x\ DF}{Sample\ weight}$$

DF: Dilution factor. If not diluted, then DF = 1

2.3.3. Determination of total flavonoids

1.0g of sample was weighed into a conical flask. 50ml of 80% methanol was then added. The content was extracted by placing on a hot plate at low temperature for 30min while stirring. It was then allowed to cool and filtered into a 100ml volumetric flask and made up to mark of 100ml with 80% methanol. 3ml of extract was pipetted into a test tube. 0.1ml of 10% $AlCl_3$ (Aluminium chloride) was then added together with 0.1ml Na and 3ml of distilled water. Absorbance of the solution was read at 415nm. The concentration of the samples was determined by extrapolating the absorbances down the concentration axis (Mahajan and Badujar, 2008).

$$\text{Total Flavonoid (mgRE/Kg)} = \frac{Conc.\ obtain\ (mg\ per\ l) x\ total\ volume\ of\ extract\ x\ DF}{Sample\ weight}$$

DF**:** Dilution factor. If not diluted, then DF = 1

2.3.4. Determination of alkaloids

This was determined using the method of Harbone (1973). 5g of sample (w0) was weighed into a conical flask and 200ml of 10% acetic acid in ethanol was added. The flask was shaken and left to stand for 4hours. The content was filtered and the filtrate evaporated to about a quarter of its original volume. Few drops of ammonium hydroxide were added to precipitate (ppt) the alkaloid. The ppt was trapped by filtering through a previously weighed filter

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paper (w1). The filter paper was dried at 60°C and final weight recorded as w2.

The % alkaloid was calculated as (W2 – W1) x 100/W0

2.3.5. Determination of total saponin

20g (W0) of well blended sample was weighed into a conical flask and 100ml of 20% aqueous ethanol was added. The content was heated in a hot water bath for 4hrs with continuous stirring at 50^{0}C. The content was filtered and then re-extracted using 200ml of 20% ethanol. The volume of extract was reduced to 40ml by evaporating in a water bath at 90^{0}C and then the concentrate was then transferred into a 250ml separating funnel. 20ml of diethyl ether (petroleum ether) was added and the content was shaken. 60ml of n-butanol was added to the aqueous layer in the separating funnel. the combined butanol layer was washed twice with 10ml of 5% aqueous NaCl (Sodium chloride). The remaining solution was collected in a weighed petri dish (w1). The petri dish was dried in an oven at about 90^{0}C. The final weight of the petri dish was recorded as W2 (Obdoni and Ochuko, 2001).

$$\% \text{ Saponin content} = \frac{W2-W1}{Wo}\ x\ 100$$

2.3.6. Determination of cyanogenic glycosides

5g of sample was weighed into a conical flask, 50ml of distilled water was added and the content was made to stand overnight. The content was filtered and 4ml of alkaline picrate solution was added to 2ml of filtrate in a test tube. The content was incubated in a water bath for 5min at 80°C (colour change from yellow to reddish brown after incubation was observed). Absorbance was read at 510nm. Blank was 2ml distilled water containing 4ml alkaline picrate solution. Cyanide standard curve with 1, 2, 4, 6, 8, 10mg/l cyanide standard was prepared. The concentration of sample in mg/l was determined by extrapolating from the prepared graph or from an existing graph by tracing down the absorbance of the sample down to concentration axis (Onwuka, 2005).

$$\text{mg/kg HCN} = \frac{\text{conc. obtained in mg/l x Vol.of sample x dil.Factor (if any)}}{Sample\ weigh\ \ x\ 1000}$$

2.4. Microbial analysis

Serial dilutions of the samples were done and 1ml from each sample were pipetted into sterile petri dish using pour plate method. The media used (mannitol salt agar, nutrient agar, euosin methylene blue agar, rose Bengal chloramphenicol agar and potato dextrose agar) were prepared according to manufacturer's instructions. Upon solidifying, after gentle clockwise and anticlockwise agitation, the plates containing different media were inverted and incubated at 37^{O}C for 18-24hrs with exception to Potato Dextrose Agar medium which were incubated at room temperature for 3-5days. Colonies were counted with the aid of colony counter and sub-cultured for biochemical characterization.

3. Results

In Table 1, the result for proximate composition of the sampled feeds is presented. Moisture ranged from 8.15 to 8.82 and these values were not significantly different ($p>0.05$) except for Topfeed. Skretting had the highest crude protein value (48.49±0.03), followed by Bluecrown (47.99±0.13) while Ecofloat recorded the least value (45.30±0.06). These values were statistically significant ($p<0.05$). The values obtained for crude fibre, crude fat, ash and carbohydrate were significantly different ($p<0.05$) across the sampled feeds. Crude fibre ranged from 4.31±0.04 in Ecofloat to 3.20±0.03 in Skretting. 6.27±0.11(Skretting) was the highest value recorded for crude fat while Ecofloat had the least value (1.29±0.00). Skretting also had the highest ash content (10.06±0.18), followed by Bluecrown (8.72±0.03) and the lowest value observed in Ecofloat (8.01±0.17). Ecofloat had the highest value for carbohydrate (32.95±0.07), marginally followed by Topfeed (28.79±0.13) and Bluecrown (28.65±0.06) with Skretting recording the lowest value (23.55±0.38).

3.1. Mineral composition of sampled feeds

The mineral composition of the sampled feed is shown in Table 2. The values for all the minerals analyzed were significantly different ($p<0.05$). Calcium had the highest values across the sampled feeds, followed by sodium while potassium recorded the lowest values. The value for calcium observed ranged from 983.82±2.92 in Ecofloat to 1545.38±2.46 in Skretting. Bluecrown recorded the lowest value for potassium while Ecofloat had the highest value of 100.10±1.60. Skretting recorded the highest values for magnesium (248.65±6.10) and phosphorus (115.84±0.42), followed by Topfeed (250.24±1.58 and 102.65±0.87) while Ecofloat recorded the lowest values (244.86±1.24 and 97.52±0.56) for the two minerals respectively.

3.2. Phytochemical composition of sampled feeds

Tannin recorded the highest value amongst all the phytochemicals, followed by flavonoids and the least value was recorded for glycosides (Table 3). Topfeed recorded the lowest values for tannin (90.99±0.14) and flavonoids (80.01±0.01) while Skretting recorded the lowest values, 131.17±0.31 and 107.32±0.36 respectively. Phenol values observed ranged from 51.85±0.20 in Skretting to 85.44±0.07 in Bluecrown. 1.70±0.08 and 0.25±0.00 were recorded in Skretting for saponin and glycosides respectively while Ecofloat recorded 1.15±0.02 and 0.84±0.00 respectively the same phytochemicals. All the values observed for the phytochemicals varied significantly across the sampled feeds except for Bluecrown and Skretting that had the same value for alkaloids, 0.80±0.01 and 0.80±0.02, hence, were not significantly different ($p>0.05$).

3.3. Proximate composition of sampled feeds and the manufacturer's declared values

The analyzed values for Topfeed are 46.92±0.06, 3.23±0.06, 3.77±0.06 and 8.49±0.05 for crude protein, lipid, fibre and ash respectively (Table 4). These values were different from the manufacturer's declared values of 42, 3.5, 12 and 7 respectively. Skretting had 48.49±0.03 for

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crude protein, 6.27±0.11 for lipid, 3.20±0.03 for fibre and 10.06±0.08 for ash while the manufacturer declared 45, 2.9, 14 and 7 respectively for the same parameters. Crude protein, lipid, fibre and ash values declared by the manufacturer of Ecofloat were 36, 3, 8 and 9 which were different from the analysed values of 48.30±0.06, 1.29±0.00, 4.31±0.04 and 8.01±0.17 respectively for the same parameters. Bluecrown declared 47.99±0.13 for crude protein, 2.64±0.06 for lipid, 3.60±0.06 for fibre and 8.72±0.03 for ash. These values were different from the analysed values of 47.99±0.13, 2.64±0.06, 3.60±0.06 and 8.72±0.03 respectively for the same value. All the values obtained from the sampled feeds were significantly different ($p<0.05$) from the manufacturers' declared values.

3.4. Microbial count and diversity of sampled feeds

The highest microbial counts were observed in Skretting with values of 3.52±0.03, 3.10±0.01 and 2.35±0.05 recorded for total aerobic count, total coliform count and total fungal count respectively. This was followed by Ecofloat with total aerobic count of 3.12±0.02 and total coliform count of 3.00±0.01. Topfeed had total aerobic count of 2.35±0.05, total coliform count of 1.61±0.01 and total fungal count of 1.75±0.05. The lowest values were observed in Bluecrown with total aerobic count of 1.62±0.01 and total fungal count of 2.09±0.01. No microbial count was recorded in Bluecrown and Ecofloat for total coliform count and total fungal count respectively. The differences in the microbial counts were significantly different ($p<0.05$) across all the feeds sampled (Table 5).

Bacteria isolated from the feeds include *Staphylococcus aureus*, *S. saprophyticus*, *Klebsiella spp*, *Bacillus spp* and *Escherichia coli* (Table 6). *S. saprophyticus* was observed in all the feeds while *S. aureus* occurred in Topfeed and Bluecrown. *Klebsiella spp* was isolated from Topfeed and Ecofloat while *Bacillus spp* was recorded in Ecofloat and Skretting. *Escherichia coli* was observed only in Skretting. *Geotrichum spp, Aspergillus flavus, Aspegillus niger, Rhizopus spp* and *Saccharomyces sp.* were the fungi isolated from the sampled feeds (Table 6). No fungus was isolated from Ecofloat. *Geotrichum spp* is the only fungus recorded in Topfeed. *Aspegillus niger* and *Rhizopus spp were found in Bluecrown* while *Aspergillus flavus* and *Saccharomyces sp* were found in Skretting.

4. Discussion

A balanced fish feed should contain carbohydrates, proteins, fats, minerals and vitamins in the right proportion. Commercial fish feeds are designed to be "complete", that is, to provide all the nutrients required for proper growth, reproduction and health of fish. The appropriate percentage composition of these constituents should not be overlooked in fish feed formulation. The growth health and reproduction of commercial fish and other aquatic animals are primarily dependent upon adequate supply of nutrients both in terms of quality and quantity irrespective of the culture system they are grown (Hassan, 2001). Commercial feeds are widely used to get higher aquaculture production. Protein is the major growth promoting factor in feed. The protein requirement of commercial fish is influenced by various factors such as commercial fish size, water temperature, feeding rate, availability and quality of natural foods and overall digestible energy content of diet (Satoh, 2000; Wilson, 2000). The present study was undertaken to know the actual proximate composition and compare with the nutrient content declared by the different companies.

From the proximate composition the analyzed, crude protein contents in all the feed samples were higher than that declared by the company. It is believed that this could be due to failure of the feed production companies to update the nutritional information on their package bags. In addition, the discrepancies in the analyzed values and the company declared values could be as a result of irregular nutritional assessment of raw ingredients used in compounding the feeds. Crude protein of 35% has been recommended for catfish production, hence the feeds sampled could be said to be suitable for catfish production as the crude protein values recorded in the feeds were all above 40%. However, excess protein in the feed could lead to protein wastage and excessive fouling of pond water.

Lipids are primarily included in formulated diet to maximize their protein sparing effect (Hassan, 2001) being a source of energy. The analyzed lipid content of the different commercial feed had lesser value compared to the company declared value. This could have been caused by lipid rancidity arising from long storage periods as induced by the COVID-19 lockdown. 10-20% of lipid in most freshwater fish diets gives optimal growth rates without producing excessively fatty carcass (Cowey and Sargent, 1979). On the other hand, Wilson (2000) reported that lipid level in catfish feeds should be 5.0 to 6.0%. Luquet (2000) also stated that dietary lipid levels of 5.0 to 6.0% are often used in Tilapia diet. However, the values analyzed and company declared values recorded for lipid were lower than the recommended values stated above.

All plant ingredients contain a certain amount of fibre. Fibre provides physical bulk to the feeds. A certain amount of fibre in feed permits better binding and moderates the passage of feed through the alimentary canal. However, De Silva and Anderson (1995) noted that it was not desirable to have a fibre content above 8-12% in diets for fish, as the increase in fibre content would consequently result in the decrease of the quality of an unusable nutrient in the diet. When the fibre content is excessive, it results to lower digestibility of nutrients. The analyzed crude fibre content of all the diets under study, although higher (1%-2%) than the company declared value, were within the safe dietary limit for fish.

Phytochemical screening of the fish feed samples indicates the presence of tannin, phenol, flavonoids, alkaloids, saponin and cyanogenic glycosides in different commercial fish feeds. Becker and Makkar (1999) opined that tannins interfere with the digestive processes by inhibiting protease and also forming indigestible complexes with dietary protein at inclusion rate of 2 g/100 g. All the analyzed fish feed samples consisted of tannins below this inclusion rate. Dietary saponins above a level of 0.15 g/100

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g can retard growth and damage intestinal mucosa in fish (Francis *et al.,* 2001), however, all the fish feeds tested contained saponins above this level. All the fish feeds sampled contained trace amount of *C. glycosides*. Cyanides derived from hydrolysis of cyanogens can suppress natural respiration and cause cardiac arrest (Davies, 1991). No exact limit of toxicity of cyanide for fish has been recorded yet and more studies are required to determine the cyanide level of tolerance for fish (Francis *et al.,* 2001).

Microbiological evaluation of fish feeds gives qualitative and quantitative indication of organisms present and microbial load associated with them. Ogbulie (1998) reported that bacteria genera may have originated from nitrogenous waste products in compounding animal feeds while fungi species may have resulted from carry-over of over seasoned fungal species from the field and from unhygienic handling of feed during sales and storage. Pathogenic fungi and bacteria are capable of causing diseases in fish and this can have negative effect on the growth (Effiong and Alatise, 2009) when fed to fish causing significant economic losses (Adejumo and Adejoro, 2014; Bankole, 1994; Bayman and Baker, 2006; Richard, 2007).

The bacteria found in the sampled feeds include *Statphylococcus aereus, S. saprophyticus*, *Klebsiella spp*., *Bacillus spp* and *Escherichia coli*. *Staphylococcus aureus* is a pathogenic organism which causes food poisoning (Bennett and Lancette, 1998). The presence of this organism in fish feeds can cause death to fish when fed to them which is also harmful to human when being consumed. Unhygienic and improper handling of feed and or feed ingredients could have resulted in the presence of this organism in the sampled feeds. *Staphylococcus saprophyticus* is a primary pathogen and its presence of the organism in fish feed can cause ocular proptosis in fish, which induces continuous mortality in the fish and leads to economic losses. This coagulase-negative microorganism has been reported to cause urinary tract infection in sexually active young female when affected fish is consumed. *E. coli* has been implicated in disease condition, and with *Staphylococcus aureus*, are capable of producing acute and chronic infections in all or most types of animals (Mallinson, 1984). It is shown that Skretting fish feed had the highest count of both fungi and bacteria counts, this is probably because of lack of proper handling of feed and through the means of unhygienic environmental storage from the sales outlets.

Fungi have been found in this study, namely *A. niger*, and *A. flavus*. Aspergillus species produce metabolites called mycotoxin and aflatoxin which are toxic substances capable of having carcinogenic effects on human consumers of contaminated fish (Brown, 2009). This fungus has been implicated in skin ulcers in fish (Yagoub, 2004; Sharma *et al*., 2013). In addition, Iqbal and Saleemi (2013) opined that fungal infection in fish might occur as a result of the use of contaminated feed or alternatively decomposed feed in the aquatic environment of the fish. Presence of this fungus in the sampled feeds may be due to defective processing conditions of the feed or poor storage conditions, hence, this may cause problems for fish.

5. Conclusion

The study revealed that the manufacturers declared nutrient compositions were quite different from the values obtained when analyzed; but even at that, the crude protein contents of all the feeds were above 40% and therefore suitable for catfish culture as recommended by the manufacturer. However, fungi and bacteria were present in all the fish feeds sampled. Although it is impossible that microbes are absent from the feeds, however, the presence of certain microbial contaminants, especially Aspergillus species, are capable of producing toxic metabolites that can result in health impairment in fish. Hence, the fish feeds may be considered unsafe to the health of fish. Furthermore, contaminated fish can be a potential source of food-borne diseases when consumed by humans.

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Table 1
Proximate composition of sampled feeds

Parameters	Top Feed	Skretting	Ecofloat	Blue Crown
Moisture %	8.82±0.15 [a]	8.46±0.10 [b]	8.15±0.01 [b]	8.41±0.04 [b]
Crude Protein %	46.92±0.06 [c]	48.49±0.03 [a]	45.30±0.06 [d]	47.99±0.13 [b]
Crude Fibre %	3.77±0.06 [b]	3.20±0.03 [c]	4.31±0.04 [a]	3.60±0.06 [b]
Crude Fat %	3.23±0.06 [b]	6.27±0.11 [a]	1.29±0.00 [d]	2.64±0.06 [c]
Total Ash %	8.49±0.05 [bc]	10.06±0.18 [a]	8.01±0.17 [c]	8.72±0.03 [b]
NFE %	28.79±0.13 [b]	23.55±0.38 [c]	32.95±0.07 [a]	28.65±0.06 [b]

Means with different superscript in the same row are significantly different (p<0.05)

Table 2
Mineral composition of sampled feeds

Parameters	Top Feed	Skretting	Ecofloat	Blue Crown
Calcium Ca (mg/100g)	1212.77±2.06 [c]	1545.38±2.46 [a]	983.82±2.92 [d]	1326.93±1.26 [b]
Potassium K (mg/100g)	98.01±0.36 [a]	91.00±0.35 [b]	100.16±1.60 [a]	89.32±0.12 [b]
Magnesium Mg (mg/100g)	250.24±1.58 [c]	348.65±6.10 [a]	244.86±1.24 [c]	331.20±0.37 [b]
Sodium Na (mg/100g)	391.53±0.89 [a]	377.21±0.64 [c]	385.19±0.03 [b]	368.40±0.46 [d]
Phosphorous P (%)	102.65±0.87 [c]	115.84±0.42 [a]	97.52±0.56 [d]	109.10±0.43 [b]

Means with different superscript in the same row are significantly different (p<0.05)

Table 3
Phytochemical composition of sampled feeds

Parameters	Top Feed	Skretting	Ecofloat	Blue Crown
Tannin (mg/100g)	90.99±0.14 [d]	131.17±0.31 [a]	110.26±0.44 [b]	103.51±0.13 [c]
Phenol (mg/100g)	64.09±0.34 [c]	51.85±0.20 [d]	78.21±0.43 [b]	85.44±0.07 [a]
Flavonoid (mg/100g)	80.01±0.01 [d]	107.32±0.36 [a]	95.70±0.02 [c]	103.64±0.46 [b]
Alkaloids (%)	0.97±0.01 [b]	0.80±0.02 [c]	1.15±0.02 [a]	0.80±0.01 [c]
Saponin (%)	1.87±0.02	1.70±0.08	1.81±0.01	1.71±0.02
C. Glycosides (mg/100g)	0.71±0.00 [b]	0.25±0.00 [d]	0.84±0.00 [a]	0.52±0.00 [c]

Means with different superscript in the same row are significantly different (p<0.05)

Table 4
Proximate composition of sampled feeds and the manufacturer's declared values

Feed	Proximate	Analyzed value	Manufacturer value	t-value	p-value
Top	Moisture	8.82±0.15	-	-	-
	Crude protein	46.92±0.06[a]	42[b]	82.00	0.00
	Lipid	3.23±0.06[b]	3.5[a]	-5.00	0.04
	Fibre	3.77±0.06[b]	12[a]	-149.73	0.00
	Ash	8.49±0.05[a]	7[b]	33.00	0.00
Skretting	Moisture	8.46±0.10	-	-	-
	Crude protein	48.49±0.03[a]	45[b]	139.40	0.00
	Lipid	6.27±0.11[a]	2.9[b]	32.05	0.00
	Fibre	3.20±0.03[b]	14[a]	-432.20	0.00
	Ash	10.06±0.08[a]	7[b]	17.46	0.00
Ecofloat	Moisture	8.15±0.01[a]	8[b]	15.00	0.00
	Crude protein	45.30±0.06[a]	36[b]	169.00	0.00
	Lipid	1.29±0.00[b]	3[a]	0.00	0.00
	Fibre	4.31±0.04[b]	8[a]	-92.25	0.00
	Ash	8.01±0.17[b]	9[a]	-5.82	0.03
Blue Crown	Moisture	8.14±0.04[a]	8[b]	10.25	0.01
	Crude protein	47.99±0.13[a]	45[b]	23.00	0.00
	Lipid	2.64±0.06[b]	4.5[a]	-33.91	0.00
	Fibre	3.60±0.06[b]	12[a]	-140.00	0.00
	Ash	8.72±0.03[a]	8[b]	24.00	0.00

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Means with different superscript in the same row are significantly different (p<0.05)

Table 5
Microbial count of sampled feeds

	TF	BC	EF	S
TAC	2.35±0.05 [c]	1.61±0.01 [d]	3.12±0.02 [b]	3.53±0.03 [a]
TCC	1.61±0.01 [c]	0.00±0.00 [d]	3.00±0.01 [b]	3.10±0.01 [a]
TFC	1.75±0.05 [c]	2.09±0.01 [b]	0.00±0.00 [d]	2.35±0.05 [a]

Means with different superscript in the same row are significantly different (p<0.05). TAC: Total Aerobic Count TCC: Total Coliform Count; TFC: Total Fungal count

Table 6
Microbial diversity in sampled feeds

Feed	Isolated bacteria	Isolated fungi
Topfeed	*Staphylococcus aureus, S. saprophyticus, Klebsiella spp*	*Geotrichum spp*
Bluecrown	*S. saprophyticus, S. aureus*	*Aspergillus niger, Rhizopus spp*
Ecofloat	*S. saprophyticus, Klebsiella spp, Bacillus spp*	-
Skretting	*S. saprophyticus, Bacillus spp, Escherichia coli*	*Aspergillus flavus, Saccharomyces sp*