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Proximate and Heavy Metals Compositions of Some Commercial Fish Feeds available in Keffi Metropolis, Nasarawa State

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ABSTRACT

The nutrient balance of feed influences its utilization and growth in fish. Proximate and heavy metals examinations were carried out on three different fish feed samples obtained from commercially available fish feed shops in Keffi metropolis and were labeled A, B, and C. The sealed samples were taken to the laboratory, ground using a mortar and pestle and kept in an airtight container for subsequent chemical analysis. The results of the proximate analysis shows that the moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate content range from, 3.17% - 7.50%, 9.39% - 11.61%, 37.85% - 48.90%, 5.00% - 13.33%, 2.53% - 6.00% and 21.31% - 30.78% respectively. The estimated levels of heavy metals analysed ranges from 0.35mg/kg - 0.61 mg/kg, 0.27mg/kg - 0.44 mg/kg, 1.15 mg/kg - 1.47 mg/kg, 0.02 mg/kg - 0.44 mg/kg, 3.15 mg/kg - 3.25 mg/kg and 0.40 mg/kg - 0.50 mg/kg for Cu, Cr, Co, Cd, Ni and Pb respectively. The concentrations of the heavy metals determined are in order of Ni > Co > Cu > Pb > Cr > Cd. The results of the proximate analysis obtained are in close argument with that obtained in the literature which ranges from 10.06% - 10.38%, 5.33% - 9.45%, 8.512% - 24.40%, 3.20% - 12.73%, 5.00% - 13.00% and 25.89% - 52.65% respectively. For the heavy metals, it ranges from 0.0579mg/kg - 0.0023mg/kg, 0.0002mg/kg - 0.0005mg/kg, 0.01mg/kg - 0.37mg/kg, 1.22mg/kg - 4.71mg/kg and 0.06mg/kg - 0.68mg/kg for Cu, Cr, Co, Cd, Ni and Pb respectively. It is therefore concluded that all the fish feed samples analysed contain the required nutrients in required proportions declared by the manufacturers and the regulatory guide lines. The heavy metals concentrations and proximate compositions obtained are within the certified limits as regulated by WHO, SON and FAO.

Keywords: Concentration, Fish feeds, Heavy metals, Nutrient balance, Proximate analysis

INTRODUCTION

The nutrient balance of feed influence feed utilization and growth of fish. It is very essential to know the nutritional requirements particularly for protein, lipid and energy for optimum growth of the fish species as well as in formulating a balanced diet as this will promote fish farming and result in their availability as food for consumption and commercial purposes. Insufficient energy in diets caused protein waste due to the increase proportion of dietary protein use for energy and the produced ammonia can pollute the water and make it unfit for fish. However, Daniels and Robinsons (1986) and Van der Meer *et al.* (1997) reported that excessive energy in diets could lead to increased body lipid deposition and growth reduction because of lack of necessary nutrient for growth. From the economic stand point, feed cost appears to be one of the major constraints against the expansion of aquaculture.

Feed quality can profoundly affect farm production and economics in many different ways, directly and indirectly. For example, if a feed labelled as 25% protein and a FCR (feed conversion rate) of 2.0 actually has 5% less protein than labeled, it will result in either of the following two scenarios; if feeding scheme of 2.0 kg feed per kg fish is maintained, it will actually produce less fish due to lower protein content; or if feeding scheme is changed in order to meet the protein demand of 25% for the species, 25% kg feed will be needed as feed for producing one kg of fish. Clearly, the true FCR will be 2.5 instead of 2.0, which means a 25% increase in the cost of feeding per each kg of fish produced. If protein is even lower, production cost will be increased further. (White, 2013). Furthermore, feeding at higher rate will deteriorate water quality as reported Kosemani *et al.,* (2017). This should however attract additional mandatory regulations that feed manufactures accurately label their products with respect to the nutrient composition, energy content and expected growth performance of fish (particularly FCR). It is also necessary to evaluate and monitor the manufactured aqua-feed by comparing the labelled information with those obtained in laboratory and in actual farm situations. Such comparisons are necessary not only to facilitate the farmers choosing the right feeds but

also to enforce the manufacturers producing feeds of required quality.

Fish meal can be produced from whole fish or wastes from the use of fish prepared for human consumption (Rahman *et al.,* 2014), but currently, fish meal is mostly produced from smaller oily fish caught specifically for fish meal production (Leeson and Summers, 2005).

The chemical composition, mineral contents and protein quality of fish meal can vary greatly depending on the species of fish used, freshness of the raw materials, condition and length of storage, amount of residual oil, processing methods, handling conditions, drying methods, temperature and weather the meal is made from whole fish or the waste from some other processing operation (NRC, 1994; Leeson and Summers, 2001, 2005).Thus fish meal needs to be evaluated continuously, there is a paucity of information on the nutrient contents of fish feed produced by different feed manufactures in Nigeria. There is also no reliable published information on chemical compositions of commercial fish feeds and fish ingredient in Nigeria. The farmers have to depend only on the existing information about the feed composition and growth performance that is given by the feed manufactures. The government has no legal legislation and control over feed components and feed quality. Also, there are no guidelines for the establishment of a new feed company. So, there is a great possibility that the farmers will be deceived by the feed manufacturers.

There is no monitoring by the government on the quality and nutrient content of the feeds produced by different feed manufacturers. There is a possibility to use unauthorized feed ingredients. So far, there has been dearth of information on evaluation of the nutrient content of feeds produced by commercial industries (Shyong *et al.,* 1998). Therefore, the present study investigated the nutrient composition of some commercial fish feeds available in Nigerian market (both local made, domestic and foreign) and compares those value with those declared by the manufacturers.

Heavy metals are included in the group of trace element that have negative influence on human health, even at low concentration. Heavy metals are dangerous because they accumulate in living tissues and decrease or even block the intercellular biochemical processes. The absorption, accumulation and toxicity of each heavy metal are affected by diverse factors, including interaction with other metals, both essential and toxic. Hence, interactions between toxic and essential metals are central to mineral balance and the antioxidant defence system in mammals and birds (Lopez-Alanso *et al.,* 2007). The risks associated with the exposure to heavy metals present in feeds represent a concern in human health. Improvement in the feed production and processing technology increase the chances of contamination of feed with various environmental pollutants especially heavy metals (European Commission, 2006).

MATERIALS AND METHODS

Sample Collection and Preparation

Three commercially available fish feed samples were obtained in the month of September 2019, from fish feed shops in Keffi metropolis, Nasarawa State Nigeria, and were labelled A, B, and C. The sealed samples were taken to the laboratory, ground using a mortar and pestle and kept in an airtight container for subsequent chemical analysis.

Proximate Analysis

Collected commercial feed samples were analysed for proximate compositions such as the moisture, ash, crude fibre, crude lipid, crude protein and carbohydrate contents according to standard procedures as described below by (Ayuba and Iorkohol, 2013; Alam *et al.,* 2012; AOAC standard method, 2010; Shuaibu *et al*., 2021 and Bathiya *et al*., 2019).

Determination of Moisture Content

This is a measure of percentage moisture lost due to drying at a temperature of 105^{o}C in an oven. The moisture content determination was carried out following the method described by Ayuba and Iorkohol, (2013) and Alam *et al.,* (2012). The fish feed (2g) was weighed (W_1) into a pre-weighed crucible (W_0) and placed into a hot drying oven at 105^{0}C for about 24 hours. It was removed, cooled and reweighed. The heating and weighing were repeated until a constant weight was obtained. The final constant weight (W_2) was recorded and % moisture content was calculated using equation 1.

$$\% \text{ moisture} = \frac{(W_1 - W_2)}{W_2} \text{ x } 100 \qquad (1)$$

Where; W_1 =Weight of sample

$W_{2=}$ weight of dried sample

Determination of Crude Fibre Content

Percentage of crude fibre was determined by the method of Udo and Oguwele (1986), in which 3g of the fish feed sample was weighed (W_0) into a beaker. Water (100cm^3) and 20cm^3 of 20% H_2SO_4 were added and boiled gently for 30min.The content was filtered through Whatmann No.1 filter paper. The residue was scrapped back into the beaker with a spatula. Water (100cm^3) and 20cm^3 of 10% NaOH were added and allowed to boil gently for 30min. The content was filtered and the residue was washed thoroughly with hot distilled water, it was then rinsed once with 10% HCl and twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into a crucible and dried for 6hr at 105^{0}C in an oven. The dried sample was weighed (W_1) and

ashes at 600^0C for 90min in a muffle furnace. It was finally cooled in a desiccator and weighed again (W_2).The percentage crude fibre was calculated using equation 2.

$$\text{Crude fibre (\%)} = \frac{(W_1 - W_2)}{W_0} \text{ x } 100 \qquad (2)$$

Where; W_0 = Weight of sample (g)
W_1 = Weight of dried sample (g)
W_2 = Weight of ash sample (g)

Determination of Crude Lipids

The crude lipid content in the sample was extracted using Soxhlet extraction procedure, described by AOAC standard method (2010). The ground sample (3g) was weighed (W_0) into a porous thimble and covered with a clean white cotton wool. Petroleum ether (200cm^3) was poured into a 250cm^3 extraction flask, which was previously dried in the oven at 105^{o}C and weighed (W_2).The porous thimble was assembled. Extraction was carried out for 3hr. The thimble was removed carefully and the extraction flask placed in a water bath so as to evaporate the petroleum ether and then dried in the oven at a temperature of 105^0C to completely free the solvent and moisture. It was then cooled in a desiccator and reweighed (W_1).The percentage crude lipid was calculated using equation 3.

$$\text{Crude lipid (\%)} = \frac{(W_2 - W_1)}{W_0} \text{ x } 100 \qquad (3)$$

Where; W_0 =Weight of sample (g)
W_1 =Weight of empty beaker (g)
W_2 =Weight of beaker + lipid (g)

Determination of Ash Content

Ash is an inorganic residue obtained by burning off organic matter of the fish feed sample at 450-500^0C in muffle furnace for 6 hr. The method followed was described by Shuaibu *et al*. (2021) in which 2g of the fish feed sample was weighed (W_1) into a pre-weight empty crucible (W_0), and placed into a muffle furnace at 500^0C and allowed for 6 hr. It was then removed and cooled in a desiccator and weighed (W_2).The weight of the sample was determined by difference between the ash sample and pre-weighed crucible. The percentage ash calculated using equation 4.

$$\text{\% moisture} = \frac{(W_1 - W_2)}{W_2} \text{ x } 100 \qquad (4)$$

Where; W_1 = Weight of sample (g)
W_2 = Weight of ash sample (g)

Determination of Crude Protein Content

Crude protein is a measure of nitrogen in the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.60. This process involves three stages;

Digestion

The sample (2 g) was weighed into a digestion flask and 25cm^3 of concentrated sulphuric acid, 0.5 g of copper sulphate and 5 g of sodium sulphate and 2g of broken bottles as a catalyst were added and heated in a fume cupboard, digestion was carried out for about 45min until the digest become clear pale green. It was cooled and 100cm^3 of distilled water was added.

Distillation

Markham distillation apparatus was used for distillation. The distillation apparatus was steam up and 10 cm^3 of the digest was added and allowed to boil. 40% sodium hydroxide (20 cm^3) was added to avoid the loss of ammonia. It was later distilled into 50 cm^3 of 2% boric acid containing screened methyl red indicator in which alkaline ammonium borate is formed.

Titration

The alkaline ammonium borate formed was then titrated directly with 0.1N HCl. The titre value which is the volume of acid used was recorded. The crude protein was calculated from equation 5.

$$\text{Crude protein (\%)} = \text{\% N} \times 6.60 \qquad (5)$$

The nitrogen content of the sample is obtained from equation 6.

$$N\ (\%) = \frac{T_v\ x\ Na\ x\ 0.014\ x\ V_1}{G\ x\ V_2}\ x\ 100 \qquad (6)$$

Where; T_V = Titre value of acid (cm^3)
Na = Concentration or normality of acid
V_1 = Volume of distilled water used for distilling the digest (50cm^3)
V_2 = volume of aliquot used for distillation (10cm^3)
G = Original weight of sample used (2g)

Determination of Carbohydrate Content

The carbohydrate contents were calculated by subtracting the sum of moisture content, ash content, crude fibre, crude lipid and crude protein from 100 (Bathiya *et al*., 2019).

Heavy Metal Analysis

The samples were digested according to procedure describe by Bukar and Saeed, (2015) in which the residual ash from ash determination were dissolved using concentrated HNO_3 acid and then filtered using Whatman filter papers. The filtrates were poured into 50cm^3 standard flask and made up to mark with distilled water. The sample solutions were then kept in sample bottles for further AAS analysis.

RESULTS AND DISCUSSION

Proximate Analysis

The mean moisture content of the samples analysed ranged between 3.17 to 7.50%. The highest %moisture content was found in sample C and the lowest was in sample B. Samples A and B have %moisture contents which fall within the ranges declared by the companies. The results obtained for sample C was less than the literature values reported by Alam *et al.* (2012) in Mega and Nourish feed feeds with values of 10.06% and 10.38% respectively.

The ash contents of the samples analysed were found to be between 9.39-11.62%. All the analysed samples have 1-2% lower ash contents than the values declared by the companies as shown in Tables 1 and 2 but they are all within an acceptable limit.

The results of the ash contents reported were in agreement with the literature values reported by Ayuba and Iorkohol, (2013) and Alam *et al.*, (2012) who reported ash contents of fish feeds to be 5.33-9.45% and 8.51-24.40% respectively.

The analysed crude fibre content of sample A, Sample B and Sample C, were 6.00%, 2.77% and 3.00% respectively. The crude fibre in sample A and C were slightly higher than the 5% and 2.7% declared by the companies, while samples B with crude fibre content of 2.77% fall within the range of 2.70% to 2.00% declared by the companies. All the samples analysed have crude fibre contents that agreed with the work of Ayuba and Iorkohol, (2013) who reported 3.20 – 12.73% crude fibre in fish feeds.

Crude fibre provides physical bulk to the feeds. A certain amount of fibre permits better binding and moderates the passage of feed through the alimentary canal. However, it is undesirable to have a fibre content exceeding 8 – 12% in diets for fish, as the increase in fibre content would result in the decrease of the quality of a usable nutrient in the diet (De Silva and Anderson, 1995). When fibre content is excessive, it results to lower digestibility of nutrients. The analysed crude fibre contents of all the feeds under study were within the safe dietary limit for fish. So, fibre may not have any negative effect on fish (De Silver and Anderson, 1995).

The analysed crude lipid contents of different fish feeds varied considerably among the feed manufacturers. The crude lipid for samples A, B, and C, were recorded as 5.00%, 7.66%, and 13.33 % respectively. The analysed crude lipid for sample A was found to be less than the manufacturers' maximum value of 12% but it falls within the range but for sample C, it was slightly higher than the manufactures value of 13%.

Lipids are primarily included in formulated diet to maximize their protein sparing effect (Hassan, 2001). The analysed crude lipid content of different commercial fish feeds ranged from 5.00-13.33% which marched with the company's declared crude lipid content. This lipid values are lower than that of Cowey and Sargent (1979) who reported in general 10-20% of lipid in most fresh water fish diets. On the other hand, Wilson, (2000) reported that lipid level in catfish feed should be 5-6%. Also, Luquet (2000) stated that dietary lipid levels of 5-6% are often used in tilapia diet.

The crude protein contents were found to be between 37.88 to 48.77% in the samples analysed. These values nearly correspond to the findings of Ayuba and Iorkohol, (2013) who reported 25.89 to 52.65% in various fish feeds. From the chemical analysis, it was observed that most of the analysed data of crude protein were more or less similar to the company declared values. The crude protein content of most of the feeds of different commercial fish industries analysed was within the acceptable range recommended in commercial fish (NRC, 1983).

Table 1: Proximate Analysis of the Commercial Fish Feed

Parameter (%)	Sample			Literature values
	A	B	C	
Moisture Content	7.17±0.79	3.1 7±0.88	7.50 ± 0.71	6.87-8.10[a] 9.87-15.30[b]
Ash content	11.62±0.81	11.62±0.81	9.42±0.05	5.33-9.45[a]
Crude Protein	48.90±0.05	46.17±0.02	37.85±0.02	8.51-24.40[c]
Crude lipid	5.0 ± 0.00	7.66 ± 0.00	13.33±0.00	23.15-31.67[c]
Crude fibre	6.0 ± 0.57	2.77 ± 0.28	3.0 ± 0.00	6.93-11.13[c] 3.20-12.73[a]
Carbohydrate Content	21.31±0.0	28.61±0.00	28.90±0.01	26.24-38.29[c]

Results: mean ± std dev of triplicate determinations
a= Ayuba and Iorkohol (2013)
b= Rahman *et al*. (2010)
c= Alam *et al.* (2012)

Table 2: The Manufacturers' Proximate Compositions of the Fish Feeds

Parameter (%)	Sample		
	A	B	C
Moisture	8	12	N.D
Ash Content	10	10	8.5
Crude lipid	12	N.D	13
Crude fibre	5	2.8	2.7
Crude protein	35	45	42

KEY: N.D: Not detected

Heavy Metals

The concentrations of Cd in the three samples were found to be, 0.03mg/kg, 0.03mg/kg, 0.02mg/kg for samples A, B, and C, respectively. Which are almost closer to the findings of Islam *et al.* (2007) with values of 0.0579mg/kg and 0.0232mg/kg, and also lower than the 0.5 declared value by WHO/FAO, (2003).

Okacha and Adedeji, (2011) reported that the highest cadmium levels were detected in the kidneys and liver of fish. Fish exposed to cadmium above permissible levels are known to have problems ranging from mortality, reduced growth, inhibited reproduction, and other adverse effect.

The copper contents of the samples were found to be between 0.31 to 0.61mg/kg. These values were all higher than the finding of Benjamin and Kaana, (2012) of 0.0002mg/kg in Coppens feed and 0.0005mg/kg in Multi feed, and also lower than the 30mg/kg declared value by WHO/FAO, 2003. Studies have shown that Cu is highly toxic in aquatic environments and has effect on fish, invertebrate, and amphibians. Kamaruzzaman *et al.* (2010) observed that copper will bio-concentrate in many different organs in fish and mollusks. While mammals are not as sensitive as aquatic organism to copper toxicity. Toxicity in mammals include a wide range of animals and effect such as liver cirrhosis, necrosis in kidney and the brain, low blood pressure and fetal mortality (Ezeonyejiaku *et al*, 2012).

Nickel concentrations in the samples were observed to be 3.25, 3.13, 3.24mg/kg for samples A, B, and C respectively. The observed values where higher than the values 0.0003 in Multi fish feed and 0.0002mg/kg in Coppens feed reported by Benjamin and Kaana, (2012). The result is lower than the 30mg/kg declared by WHO/FAO, 2003. Ololade, (2012) reported that Nickel toxicity include skin rash (called nickel dermatitis), nausea, dizziness, diarrhea, headache, vomiting and chest pains.

The concentrations of lead (Pb) were found to be 0.4 to 0.5mg/kg in all the samples investigated. The observed values are all higher than the 0.01 to 0.37mg/kg reported by Suleiman *et al.,* (2015) who conducted an analysis on four feeds samples. Lead is known to cause the disease called plumbism and is also known to damage the brain, liver, kidney and reproductive system (Ekpo *et al.,* 2008).

The concentration of Cobalt was found to be 1.15 to 1.47mg/kg in the samples analyzed. The observed values are all in agreement with 1.22 to 4.71mg/kg reported by Oana *et al.* (2012) who conducted analysis on different poultry feeds. Cobalt is used as additives in animal's nutrition. (European Food Safety Authority, 2009).

The concentration of Cr was found to be 0.27 to 0.44mg/kg in the samples analysed. The observed values are all with the range of 0.06 to 0.68mg/kg reported by Abdullah *et al.* (2013) who conducted an analysis on chicken feeds. Toxicity of Chromium causes diseases such as asthma, chronic bronchitis and chronic irritation. (Lindberg and Hedenstierna, 1983; Dayan and Paine, 2001).

Table 3: Concentrations of Heavy Metals Investigated in the Feed Samples.

Metal	Samples			Literature Values
	A (mg/kg)	B (mg/kg)	C (mg/kg)	(mg/kg)
Cu	0.61	0.41	0.35	0.37-59a
Cr	0.27	0.44	0.30	0.06-0.68b
Co	1.47	1.25	1.15	1.22-4.71c
Cd	0.03	0.33	0.02	0.03-0.46a
Ni	3.25	3.13	3.24	2.25-4.87a
Pb	0.5	0.5	0.5	1.10-7.85a

a= Okoye *et al.,* (2011); b= Abdullahi *et al.,* (2013); c= Oana-Margarita *et al.,* (2012)

CONCLUSION

In conclusion, from the findings of this study, all the fish feed samples analysed contained the required nutrient in required proportions declared by the manufactures and the regulatory guidelines. The results also shows that all the fish feeds analysed contained some heavy metals within the certified limits as regulated WHO and FAO,

2003. However this findings revealed that all the fish feeds analysed are safe to be used as fish feed and consequently safe for human consuming the fish.

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