Proximate Composition and Heavy Metal Content of Commercial Tilapia Starter Feeds Sold at Asuogyaman Municipality, Ghana

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Abstract

One of the main requirements for sustained aquaculture is high-quality fish feed. The quality of fish flesh, including shelf life, appearance, flavour, colour, and nutritional value, is determined by the feed quality. Consumers today are highly concerned about several issues related to fish farming practices and the different materials used in producing fish feed. The primary objective of the present investigation was to evaluate the proximate composition (protein, lipid, moisture, ash, carbohydrate, and crude fibre) and levels of heavy metals (Cr, Pb, Cd, Zn, and Cu) in selected commercial tilapia starter feeds available in Ghana. There were no significant variations between the nutritional content offered by feed firms and the values identified in this analysis. However, the derived proximate values for the study were lower compared to the feed manufacturers. The findings revealed the presence of different heavy metals in the feeds at varying levels. Chromium (Cr), lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) were the heavy metals with the highest levels (mg/kg), with values of 0.544, 0.141, 0.020, 1.821, and 7.758, respectively. The concentration of chromium (Cr) in the current study exceeds the maximum allowable limits of 0.05 mg/kg set by international organisations, which may be detrimental to the fish's health. The study suggests that commercial fish feed producers take the necessary precautions to guarantee the nutritional integrity of their products and prevent heavy metal contamination. Otherwise, the final consumers—humans and fish—might be more susceptible to accumulating and assimilating the heavy metals, which could have health implications.

Keywords: aproximate, starter feed, Ghana, heavy metals, aquaculture

Introduction

Aquaculture is rapidly expanding globally and is expected to play a crucial role in addressing the growing demand for aquatic food supplies. It is also seen as a means to enhance fishery resources and restore ecosystems. In Ghana, aquaculture is intensifying due to increasing demand for fish and declining capture fishery outputs (Hasselberg et al., 2020). The shift from traditional to intensive or semi-intensive systems has led to a surge in demand for aquafeeds, surpassing even the demand for fertilizers (Waite et al., 2014). Farmers are transitioning from no feed or farm-made feeds to commercially produced feeds, highlighting the importance of aquafeeds in boosting production. Ghana's feed sector, particularly fish feed production, is growing rapidly, with four major commercial tilapia starter feed producers: Rannan, Koudjis, Aller Aqua, and Enam Pa.

In semi-intensive systems, where fish stocking density exceeds natural water productivity, commercial feeds are essential (Heriksson et al., 2021). They increase production system capacity, improve yields, and ensure optimal fish growth by providing balanced nutrition (Nazish & Mateen, 2011). Studies show that commercial feeds can increase fish production by 7.7 times compared to systems without feed supplementation (Uddin et al., 2012). However, feed quality and nutritional balance are critical. Proper energy, fat, and protein levels are vital for fish growth, while imbalances can lead to higher production costs, water quality issues, and stunted growth (Kim et al., 2012; Rahimnejad et al., 2021; Lassi et al., 2017).

Feed costs remain a significant barrier to aquaculture expansion, accounting for 60–80% of production expenses. Research has focused on finding alternatives to expensive fishmeal to improve profitability and sustainability (Ragasa et al., 2022). The global demand for aquafeeds has surged, reaching 41 million tonnes in 2019 (Alltech, 2020). Despite Ghana's growing demand for high-quality feeds, there is limited information on the nutritional value of locally produced and imported feeds.

While feeds are crucial for productivity, contaminated feeds pose risks to fish and consumers. Pollutants, including heavy metals like lead, cadmium, chromium, mercury, and arsenic, can enter feeds during production or from environmental sources (Sarkar et al., 2022). These contaminants can bioaccumulate in fish and transfer to humans, posing serious health risks. Heavy metals are particularly concerning due to their toxicity, persistence in food chains, and lack of biological benefits (Lopez-Alonso, 2012). Studies have identified harmful levels of these metals in fish feeds, linking them to kidney damage, cancer, cardiovascular diseases, and neurological disorders (Saha et al., 2018; Macomber et al., 2011; Sabbir et al., 2018).

In Ghana, data on the nutritional composition and heavy metal content of tilapia feeds is scarce. Farmers rely on proximate composition details provided on feed sacks, as there is no comprehensive regulatory oversight of feed quality or ingredients. Although fisheries and standards regulators exist, enforcement is often lax, leaving room for misleading practices by feed producers. This lack of regulation raises concerns about the use of contaminated ingredients and the overall safety of aquafeeds. In Ghana, the lack of regulation in the aquafeed industry has led to significant concerns regarding feed quality and safety. One notable incident that highlights this issue occurred in 2021 when several tilapia farmers reported uneven fish growth, poor feed conversion ratios, and increased susceptibility to diseases, leading to higher mortality rates and lower yields (Dimado, 2024). Investigations revealed that some of the feeds used contained unapproved ingredients and potentially harmful additives.

Given these challenges, this study aims to analyse the nutritional composition and heavy metal content of commercial starter feeds for tilapia in Ghana. By addressing the gaps in data and regulatory oversight, the findings will contribute to improving feed quality, ensuring food safety, and supporting the sustainable growth of Ghana's aquaculture sector.

Materials and methods

Sample Collection Procedure

A comprehensive list of commercial feed suppliers within the municipality was compiled, including the sale points of four starter feed companies. Engaging these companies in open discussions allowed for the collection of relevant information about their products. To ensure a representative sample of the tilapia starter feed market, a stratified random sampling method was employed, targeting feeds from at least five different sellers. This approach ensured diversity across various brands and formulations.

During sample collection, gloves were worn to maintain hygiene. A total of twenty-eight (28) samples, each weighing 1 kg, were collected in polythene bags. The decision to collect 28 samples was influenced by the limited number of starter feed producers in Ghana. With only four producers available, the total population of potential suppliers/retailers was inherently small. Thus, a sample size of 28 provides adequate representation of the feeds offered by these producers, effectively capturing variability among different batches and brands. This sample size is also optimal for assessing the overall quality and safety of the aquafeeds available to farmers.

Samples were taken from different batches to account for potential variability. Samples were collected whenever feeds were delivered to the shops, with each delivery constituting a batch. Each sample was carefully labelled with the brand name, batch number, and date of collection. To document the approximate composition of the feeds, photographs were taken of the labels on the feed sacks, accompanying leaflets, and any manufacturer-provided instructions. This meticulous documentation facilitated a detailed understanding of the nutritional content and formulation of the collected samples.

To preserve the integrity of the samples, they were stored in clean, dry, airtight containers to prevent contamination and degradation. The samples were then transported to the laboratory in insulated coolers, ensuring they remained in optimal condition for subsequent analysis. Finally, all starter feed samples were taken to the University of Ghana's Ecological Laboratory for analysis.

Proximate Analysis

The obtained samples were removed from the refrigerator and left at room temperature for a full hour. Every batch of samples collected were stored in a sealed container for further analysis. According to AOAC International's recommended methods, the gathered commercial starter feed samples were examined for their proximate composition, including moisture, crude fat, crude protein, ash, carbohydrate, and crude fibre. The chemical contents were determined for three duplicate samples of each commercial feed.

Moisture Content: The feeds' moisture content was calculated following AOAC (2000). After being cooled in a desiccant and reweighed, samples were dried in an air oven at 101° C for 10 hours to achieve uniform weights. The difference between dry and fresh weights was assumed to be the moisture content.

Ash Content: To determine the ash content, the samples were first dried to remove any moisture. The dried samples were then subjected to calcination in a muffle furnace at 550 °C for 20 hours until all the organic components of the samples had been converted to ash.

Crude Lipid Content: Using the Soxhlet extraction technique, as stated by AOAC standard procedure (AOAC 2000), the crude lipid composition in the material was determined. The ground sample (3 g) was placed into a permeable thimble and weighed (W_0) before being wrapped with fresh, white cotton wool. In a 250cm^3 extraction flask that had previously been dried at 105 ^{o}C and weighed (W_2), petroleum ether (200 cm^3) was added. The pore-filled thimble was put together. The extraction process took three hours. To thoroughly liberate the extraction flask of solvent and moisture, the thimble was gently taken away, and the flask was subsequently immersed in a water bath to evaporate the ether. After cooling in a desiccator, it was once again weighed (W_1). Using an equation, the percentage of crude lipids was determined.

$$Crude\ lipid\ (\%) = \frac{(w_2 - w_1)}{w_0} \times 100\%$$

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

Crude Protein Content and Carbohydrate: The Kjeldahl technique was used to analyse crude protein (AOAC 2000). Each feed sample underwent the three crucial procedures of digestion, distillation, and titration in order to convert total nitrogen to crude protein using a conversion rate of 6.25. Following that, the samples' crude protein content was calculated. The total carbohydrate amount was estimated (Onyeike et al., 2000) by deducting the sum of the contents of protein, fat, moisture, and ash from 100.

Heavy Metals Analysis

Following the technique described by AOAC (2000), samples were subjected to digestion for heavy metal analysis. A mixture of about 0.5- 0.9 g of the starter feed samples and 10 mL of HNO_3 (Merck, Darmstadt, Germany) was prepared and left overnight. After that, the digested samples were filtered with 125 µm filter paper (Whatman, Kyoto, Japan) and stored for analysis. A flame atomic absorption spectrophotometer (AA-7000 Shimadzu, Kyoto, Japan) was used to analyse heavy metals. A pure standard was used to produce a normal calibration curve for Pb, Zn, Cd, Cu and Cr. The following equation was used to determine the level of heavy metals.

$$Concentration\ of\ metal\ in\ feed\ sample = R \times Dilution\ factor$$

where,

$$Dilution\ factor = \frac{final\ volume\ of\ digest\ sample\ in\ mL}{wet\ weight\ of\ digested\ feed\ sample}$$

R = AAS, reading to digest

The amount of heavy metals present was given as mg/kg.

For the detection of metal level, the standard calibration graphs were established with a suitable coefficient of regression ($R^2 > 0.9997$). Certified reference material DORM- 4 Fish protein was used to verify the analytical process for heavy metals. As shown in Table 1, the certified materials' standard deviations of the means were within the range of 0.062–0.64%, and the recovery was within the range of 84 –115 %. The certified and observed values showed good concordance, according to the results.

Statistical Analysis

Following the sample analyses, estimated means were computed for the heavy metal and proximate content values for the four tilapia starter feeds. Significant differences in proximate compositions and heavy metal

TABLE 1

Concentrations of metals found in CRM (DORM-4) by AAS

Metals	CRM Values (mg/kg)	Obtained values (mg/kg)	Recovery (%)
Pb	0.404 ± 0.062	0.388	96
Zn	51.60 ± 2.80	43.344	84
Cd	0.299 ± 0.018	0.293	98
Cu	15.700 ± 0.460	18.100	115
Cr	1.870 ± 0.180	1.660	88

levels among feeds were found using a one-way ANOVA. The Tukey post hoc test was employed to compare means.

Results and Discussions

For fish reproduction, growth, and other aquatic species to reach their full potential in terms of quantity and quality, proper nutritional supplementation is crucial. To ensure that the species' optimal nutritional and energy requirements are met as well as the system's production objectives, it is necessary to ensure the availability of feeds in the proper quantity and quality. Laboratory analysis was done on the feed samples that were collected to determine their proximate composition, including crude protein, moisture, carbohydrate, crude fat, crude fibre, and ash. The nutritional values reported by companies and the values examined in a lab had no discernible differences (see Table 1).

Proximate Composition

The crude protein content reported by the feed companies ranged from 42.0% (Feed B) to 60.0% (Feed C). Feed A and D reported 48.0% and 47.0%, respectively. The laboratory results were close to the reported levels, with Feed C having the highest protein content (59.82%) and Feed B the lowest (39.98%). Feed A and D had protein contents of 46.25% and 46.59%, respectively. Crude protein is a critical nutrient for fish growth, especially in starter feeds. The laboratory results confirm that the feeds generally meet the protein requirements for starter fish feeds. However, Feed B's protein content was slightly lower than reported, which could affect its nutritional value. Discrepancies, especially significant reported values in Feed B, may lead to inadequate protein intake for fish. This may result in stunted growth, poor health, and increased mortality rates among fish, particularly in the starter stages when protein needs are critical. Additionally, consistent discrepancies can erode trust in feed brands, pushing farmers to seek alternatives, destabilizing market dynamics.

Fishmeal is often the primary protein source in the diets of both shrimp and fish, and it is widely recommended as an animal protein supplement for livestock. Nutritionally, high-quality fishmeal contains between 60% and 72% crude protein by weight (Ween et al., 2017). This places samples (A), (B), (C) and (D) as medium-quality fish feed. Several factors may influence the variation in protein content among the fishmeal samples analysed in this study, including the species of fish utilized, the freshness of the fish at the time of processing, storage conditions and duration, residual oil content, processing techniques, handling practices, and drying temperatures (Bernard & Adetola, 2022).

Ayssiwede et al. (2011), categorized fish feed produced in Senegal into high-quality fish feed containing 58-75% crude protein. Bernard & Adetola (2022) reported crude protein levels ranging from 52.13 - 81.30% in South-Western Nigeria. These levels were higher than those reported for this study. The analysed protein content in all feeds was higher than that Kader et al. (2005) declared in Bangladesh. Mohanty et al. (2019) revealed that the ideal protein ranges for carp cultivation were 35–45 % and 40 %, respectively. For rohu brood stock, 25 % of the recommended daily protein intake had the highest reproductive results (Afzal et al., 2005).

The laboratory results were close to the reported levels, with Feed D having the highest ash content (13.88%) and Feed B the lowest (10.12%). Feed A and C had ash contents of

12.12% and 12.40%, respectively. Ash content represents the mineral content in the feed. The laboratory results confirm that the feeds contain adequate mineral levels, which are essential for fish bone development and overall health. These typically encompass essential minerals such as calcium, phosphorus, potassium, and magnesium. For scaling and teething, the amount of minerals or ash content is crucial (Bernard et al., 2010). The phosphorus and calcium requirements for mrigal and rohu fingerlings are 0.75% and 0.19%, respectively (Paul et al., 2006). Insufficient phosphorus from the diet in Catla results in several kinds of organ-specific disorders (Sukumaran et al., 2008). Rohu fingerlings require 30 mg Zn/kg of feed, according to Meena et al. (2010). The study's ash content findings were greater than the 5.33–9.45 % reported by Ayuba & Lorkohol (2010), but they were consistent with the literature values (8.5–24.40 %) published by Alam et al. (2012). In the study, the reported level of ash significantly exceeded the range of 7.41% to 10.81% documented by Hasan et al. (2022) for feed sourced from Bangladesh.

According to the findings of Cho and Kim (2011), lipids in fish can be categorized into liquid fish oils and solid fats. Although fishmeal is typically processed to remove most of the extracted oil, the residual lipids generally constitute approximately 6–10% of the total weight, with variations ranging from 4% to 20%. For this study, the laboratory results were slightly lower than the reported levels, with Feed C having the highest fat content (13.96%) and Feed A the lowest (6.84%). Feed B and D had fat contents of 7.64% and 7.12%, respectively. Discrepancies between reported and laboratory values of fat content can significantly affect the nutritional profile of the feeds. Lower fat content than expected may lead to inadequate energy supply for fish, potentially affecting growth rates and overall health. Furthermore, if the laboratory results are consistently lower than reported values, this may necessitate adjustments in feed formulation. Producers might need to increase the inclusion of lipid sources to meet the energy requirements of the fish. The laboratory results indicate that the fat content in the feeds is generally consistent with the reported levels, although slightly lower. Feed C, with the highest fat content, may provide more energy, which could be beneficial for fast-growing fish species. The values obtained in this study are higher than those reported by Ayssiwede et al. (2011) in Senegal and by Bernard and Adetola (2022) in South-Western Nigeria. Lipids are largely employed in the specially formulated feed as an alternative source of energy to maximise the feed's ability to spare proteins, according to Hasan (2001). Liu et al. (2022) recommended a dietary fat level of 5 to 6 % to be frequently employed in tilapia diets. Luquet (2017) also stated that dietary lipid levels of 5 to 6% are often used in tilapia diet. For fish larvae to grow and survive, their diets must contain phospholipids (PL), especially phosphatidylcholine (Jaxion-Harm, 2021). Carp diets need a crude fat content of 6 % for broodstock and grow-out and 8 % for fries and spawn (FAD 2013). Fish suffer adverse effects when their lipid levels are too high. In trout, feeding them four times the recommended quantity reduces feed efficiency and inhibits growth (Nayeem et al., 2019). The present tendency in fish nutrition, and specifically salmonid diets, is to enhance the lipid content of the meal. Numerous investigations done on other species have supported this. According to estimates, salmonids can reduce their protein content from 48 % to 35 % without observing any changes in their ability to grow by increasing their lipid levels from 10 % to 20 % (Nayeem et al., 2019).

The feed gets its physical mass from crude fibre. A certain quantity of fibre in feed aids in better binding and is crucial to the feed's effortless movement through the digestive tract. Excessive dietary fibre may decrease the efficacy and digestion of nutrients, whereas low dietary fibre levels may promote fish development. For spawn, crude fibre demands for carp feeds are 6 %, and for fry, broodstock, and grow-out feed, they are 8 % (FAD 2013). However, too much fibre reduces the ability of nutrients to be absorbed, since it lowers the quality of a usable nutrient in the feed. Therefore, feed with a fibre concentration of more than 8–12% is not acceptable for fish (Adamidou et al., 2011). The inclusion of fibre up to 5% in the diet was found to have no effect on Sharpsnout sea bream growth rate or nutritional assimilation (Bou et al., 2014). With the inclusion of fibre in the diet up to 18%, gilthead sea bream growth performance was unaffected (Altan & Korkut, 2011). For this study, the companies' results were slightly lower than the reported levels, with Feed D having the highest fibre content (3.92%) and Feed C the lowest (0.66%). Feed A and B had fibre contents of 2.00% and 2.45%, respectively. Crude fibre is generally less important in fish feeds compared to other nutrients, as fish have limited ability to digest fibre. The laboratory results indicate that the fibre content is within acceptable limits for starter fish feeds.

The carbohydrate content reported by the feed companies ranged from 12.4% (Feed C) to 32.5% (Feed A). Feed B and D reported 28.0% and 21.0%, respectively. The laboratory results were close to the reported levels, with Feed A having the highest carbohydrate content (32.97%) and Feed C the lowest (11.59%). Feed B and D had carbohydrate contents of 30.42% and 23.49%, respectively. Carbohydrates provide energy and can be a cost-effective ingredient in fish feeds. The laboratory results confirm that the carbohydrate content is consistent with the reported levels, although Feed C has a significantly lower carbohydrate content, which may be due to its higher protein and fat content. The majority of prawns, shrimp, and fish appear to grow and utilise protein more effectively as a result of the necessary carbohydrates providing energy. A 26% carbohydrate treatment was found to promote the fastest growth of carp fingerlings, fry, and spawn (Kathane et al., 2017). Rohu, *Labeo Rohita*, effectively utilised a diet that contained 45% gelatinized carbohydrates (Mohapatra et al., 2003).

Feed companies B and D reported moisture levels of 12.0% and 11.0%, respectively. Companies A and C did not report moisture levels. The laboratory results show moisture levels ranging from 1.82% to 11.84%. Feed A had the lowest moisture content (1.82%), while Feed B had the highest (11.84%). The moisture content in Feed C was significantly lower (2.23%) compared to the reported levels, while Feed D's moisture content was slightly lower than reported (8.92% vs. 11.0%). Moisture content directly affects the nutritional quality of fish feeds. Lower moisture levels generally indicate a higher concentration of nutrients, which can enhance feed efficiency. However, excessively low moisture may lead to palatability issues. High moisture levels can promote microbial growth and spoilage, reducing the shelf life of the feeds. Discrepancies in reported moisture levels may lead to unexpected spoilage rates, affecting inventory management and profitability. Moisture content influences feed

TABLE 1
Proximate composition of selected commercial Starter fish feeds available in Ghana

	Feed Companies Reported Levels				Estimated Levels from the Laboratory			
Parameters (%)	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Moisture	-	12.0	-	11.0	1.82 ± 0.01	11.84 ± 1.40	2.23 ± 0.11	8.92 ± 0.04
Crude protein	48.0	42.0	60.0	47.0	46.25 ± 0.02	39.98 ± 0.06	59.82 ± 0.02	46.59 ± 1.22
Crude fat	7.0	8.0	15.0	7.0	6.84 ± 0.03	7.64 ± 0.00	13.96 ± 0.01	7.12 ± 0.21
Ash	12.5	10.0	12.6	14.0	12.12 ± 0.02	10.12 ± 0.05	12.40 ± 0.00	13.88 ± 0.15
Crude fibre	2.5	2.5	0.7	4.0	2.00 ± 0.04	2.45 ± 0.01	0.66 ± 0.00	3.92 ± 0.05
Carbohydrate	32.5	28.0	12.4	21.0	32.97 ± 0.08	30.42 ± 1.51	11.59 ± 0.01	23.49 ± 1.62

conversion ratios and growth performance. Feeds with high moisture may lead to lower energy densities, impacting fish growth rates and overall health. The discrepancy between reported and laboratory-estimated moisture levels could be due to differences in sampling, analytical methods, inadequate storage facilities, poor feed ingredients, or prolonged exposure to extremely humid weather. In contrast to wet feed, which has a water content of at least 40 % and typically ranges from 17 to 40 % (Biswas et al., 2018; Zaman et al., 2017), dry feeds have an 8–10 % moisture level.

Heavy Metals

As a result of various human and natural activities, environments can become polluted with heavy metals, which can then accumulate in fish and other invertebrates (Bhowmik et al., 2023). A build-up of heavy metals in fish may also be due to contaminated sediments, water, biomagnifications, and the food chain (Javed & Usmani, 2019). According to Hamada et al. (2018), impairment in the function of critical biological products like proteins, enzymes, and DNA is the result of heavy metal toxicity. Metals are carcinogenic to both humans and animals and disrupt DNA via the use of metal ions.

According to Javed and Usmani (2019), the build-up of heavy metals in fish also results in oxidative stress, genotoxicity, and tissue damage, which stunt fish growth and eventually result in mortality. The parts of the body most frequently impacted by anomalies are the swim bladder, vertebral column, lateral line, and cephalic region. According to Sharifuzzaman et al. (2016), excessive exposure to heavy metals has been associated with adverse effects on fish, such as reduced fertility, reproduction problems, prolonged hatching, damage to kidneys, poorer development and growth, and organ deformities. Fish may become contaminated with heavy metals by ingesting polluted water or via epithelia such as the epidermis, alimentary canal, and gills (Bhowmik et al., 2023). Additionally, as demonstrated by Gao et al. (2021), the mechanisms of biomagnification and bioaccumulation are what transfer heavy metals from one fish species to another across the food chain. In the end, as fish make up a large amount of our daily diets, such heavy metal assimilation puts people at serious risk. As a result, Table 2 provides an evaluation of the quantitative presence of different heavy metals (Pb, Cd, Cr, Zn, and Cu) in four starter feeds from different producers. Statistically significant differences exist for copper (Cu) and lead (Pb) among the starter feed companies, but not for cadmium (Cd), zinc (Zn), or chromium (Cr). Post-hoc tests reveal that Company C has the highest Cu, while Company D has the highest Pb.

Lead (Pb)

Only a very high level of 0.141±0.003 mg/kg of lead was found in feed D. This obtained value

TABLE 2
Heavy metal concentrations (mg/kg) of starter fish feeds of different companies

	Heavy metals				
Company name	**Cd**	**Cu**	**Zn**	**Pb**	**Cr**
A	BDL	2.445±0.003	1.243±0.211	0.006±0.001	0.154±0.000
B	0.018±0.002	5.194±0.004	1.821±0.410	0.082±0.004	0.321±0.020
C	BDL	7.758±0.004	0.694±0.003	0.032±0.000	0.238±0.012
D	0.020±0.003	3.128±0.003	1.468±0.019	0.141±0.003	0.544±0.002
World Standard	**1.00**	**10.00**	**150.00**	**2.00**	**0.05**

World standard- Bhowmik et al., 2023, BDL-Below detection limit

is several times lower than the values reported by Sarkar et al. (2022) and Bhowmik et al. (2023) for all tested commercial nursery feeds in Bangladesh. Our results differ from those of some other researchers, whose findings ranged from 7.671 to 12.232 mg/kg (Kundu et al., 2017), 2.49 to 14.87 mg/kg (Sabbir et al., 2018), and 4.6 to 18.2 mg/kg (Murthy et al., 2013) for the level of lead in various fish feeds. The levels of lead were discovered to be much below the WHO's maximum permissible level of 2 mg/kg in each of the tested samples of fish feed. Because of the excessive formation of reactive oxygen species (ROS) caused by Pb build-up in fish tissues, the fish experience oxidative stress, which leads to synaptic impairment and neurotoxicity (Lee et al., 2019). Pb exposure also alters the immune system's reactions in fish by acting as an immunological toxicity.

However, exposure to lead in humans results in reduced bone growth, which reduces the size and area of the head in foetuses and children (Hamada et al., 2018). Along with preventing some enzyme functions, it also has some adverse consequences on the human body, including malfunction of the central nervous system, kidneys, and liver.

Cadmium (Cd)

Cadmium was detected in feeds from Company B (0.018 mg/kg) and Company D (0.020 mg/kg), while it was below the detection limit (BDL) in feeds from Companies A and C.

Although the detected levels are below the World Standard limit of 1.00 mg/kg (Bhowmik et al., 2023), the presence of Cd in some feeds is concerning due to its high toxicity and potential to accumulate in fish tissues, posing risks to human health upon consumption. The mean Cd concentration in prawn feed employed in Bangladesh, according to Shamshad et al. (2009), was below 0.1 mg/kg. All of the Indian and Bangladeshi fish-meal-based tilapia feeds that were evaluated by Murthy et al. (2013) had a substantial Cd content, ranging from 0.22-4.4 mg/kg and 8.082 to 9.771 mg/kg, respectively. While Bhowmik et al. (2023) observed a Cd level in tilapia finisher feeds of 0.07 mg/kg in Bangladesh, Sarkar et al. (2022) documented a Cd content of feeds ranging from 0.006 to 0.027 mg/kg in Bangladesh. The amounts seen in this investigation fell below their conclusions. Despite this, our results were lower than the permitted maximum values based on the global standard (1 mg/kg).

It is thought that the non-essential element Cd, which accumulates more in fish internal organs than it does outside the body at levels above 1 mg/kg, is chronically harmful (Janbahkhsh et al., 2018). In fish, Cd causes substantial harm to the immunological system, reproductive control system, antioxidant defence system, and tissue function as well as structure stability (Liu et al., 2022). Kidney damage, severe bone pain, high blood pressure, a malfunctioning liver, tumours, and even human cancer are some of the harmful effects of Cd (Sarkar et al., 2022). Additionally, it can lead to biological abnormalities such as pancreatic necrosis, hepatocyte vacuole degradation, and enlarged arteries (Ayanda et al., 2018).

Chromium (Cr)

One of the most harmful environmental toxins is chromium (Cr). In theory, the trivalent form of Cr (Cr (III)) found in food is typically a necessary nutrient. In contrast, the hexavalent form (Cr (VI)) is more poisonous and typically absent from the food chain. Trivalent Cr is required for the proper metabolism of glucose, fat, and cholesterol (Mannan et al., 2018). Chromium is regarded as an essential nutrient for aquatic organisms and humans because a deficiency might result in delayed growth and an imbalance in glucose, lipid, and protein metabolism (Ayanda et al., 2018). However, when aquatic organisms raised in a Cr-polluted environment are ingested via the food chain, it appears to elevate the likelihood of damage to their lungs. The concentration of Cr in all four feed types ranges from 0.154 to 0.544 mg/kg, which is significantly higher than the 0.05 mg/kg maximum level imposed by international organisations. The results of this investigation are comparable to those of Adeniji and Okedeyi (2017), who conducted a preliminary evaluation of heavy metals in a few Nigerian feed ingredients, and Bernard and Adetola (2022) of a prominent fishmeal sample. The presence of different food additives with chemicals and colours in feeds may be the cause of the Cr pollution in feed samples. Additionally, the high level of Cr in the feed may also be due to Cr- contamination of solid tannery waste used in fish feed production (Hasan et al., 2016). Oxidation reactions caused by high levels of Cr in fish feed might harm the liver, kidneys, and blood cells (Suchana et al., 2021). Furthermore, fish erythrocytes exhibit a variety of cellular and nuclear abnormalities when exposed to high concentrations of Cr in aquatic media (Islam et al., 2020). Saha et al. (2018) reported that the concentration of chromium (Cr) varied between 2.1 and 16.49 mg/kg, while Dai et al. (2016) found the chromium content in fish feed to be 3.0 mg/kg. Notably, the levels identified in these studies exceed those observed in the current research. Humans are susceptible to cancer from Cr, and prolonged absorption in the body can impair cellular integrity and function by rupturing lipid and protein membranes (Sarkar et al., 2022)

Zinc (Zn)

Zinc is crucial for maintaining animal metabolism and growth at appropriate levels. The zinc level in the current study ranges from 0.694 mg/kg to 1.821 mg/kg, which is lower than the permissible limit established by the WHO (150 mg/kg) (FAO/WHO 2001). In cultured fish and shellfish, a Zn shortage has been seen to result in sluggish growth, skeletal deformities, cataracts, and significantly decreased activity of different Zn metalloenzymes (Lin et al., 2013). On the contrary, Zn is harmful when physiological demands are exceeded, which can induce generalised enfeeblement, growth inhibition, and pathological and metabolic abnormalities in fish (Abdel-Warith et al., 2011).

Copper (Cu)

Copper is required for the formation of haemoglobin and is a crucial component of numerous enzymes. Cu can, however, be poisonous in excess to fish, invertebrates, and amphibians. In different fish and molluscan organs, copper can bioaccumulate (Kamaruzzaman et al., 2010). Cu poisoning can cause an extensive variety of abnormalities, including necrosis, low blood pressure, gastrointestinal issues, foetal mortality and cirrhosis (Ezeonyejiaku et al., 2011). Additionally, long-term exposure to high concentrations of Cu can be harmful to people and result in symptoms such as localised cell death, kidney and liver dysfunction, and hypotension (Ezeonyejiaku et al., 2011). The current study's copper levels in fish feeds were much lower than the suggested FAO limit of 10 mg/kg (Kundu et al., 2017). Starter feed A had the lowest concentration of copper (2.445 mg/kg), while feed C had the greatest concentration (7.758 mg/kg). The results we obtained were significantly lower than the concentrations of fishmeal published by Murthy et al. (2013) (1.80-46.40 mg/kg) and by Kundu et al. (2017) (22.618-38.480 mg/kg) for tilapia feeds, but surpassing the findings of 30 tested feeds (0.008 0.303 mg/kg) conducted by Sarkar et al. (2022).

Limitations of the study

While this study provides valuable insights into the proximate composition and heavy metal content of commercial tilapia starter feeds in the Asuogyaman Municipality, Ghana, several limitations should be acknowledged:

Sample Size and Scope: It is clarified that while our research was conducted in the Eastern Region of Ghana, the feed manufacturers coded A, B, C, and D represent the only commercial tilapia starter feed manufacturers in the country. Therefore, the results obtained from the analysis of the samples can be reasonably generalised to the broader context of tilapia starter feeds available in Ghana rather than being limited to the Eastern Region alone. However, a wider geographical scope would have enhanced the generality

Additionally, we want to highlight that the authors entirely funded this research, and the costs associated with analysing the samples are significantly high. This financial constraint contributed to the selection of the samples for our study, which we believe is a representative number, given the limited number of manufacturers in the country.

Comparative Analysis: The study compares the proximate composition of the feeds with values reported by manufacturers but does not compare the heavy metal levels with those found in other countries or regions. Such a comparison could provide a better understanding of how Ghana's feed quality measures up to international standards.

Lack of Regulatory Data: The study highlights the absence of strict regulatory oversight in the feed industry in Ghana. However, it does not provide a detailed analysis of the regulatory framework or suggest specific policy recommendations. Further research is needed to explore how regulatory bodies can enforce feed safety standards effectively.

Temporal Variability: The study was conducted at a specific point in time, and the results may not account for seasonal variations in feed composition or heavy metal contamination. Feed quality and composition can vary depending on the source of raw materials, which may change over time.

Limited Heavy Metal Analysis: The study focused on five heavy metals (Cr, Pb, Cd, Zn, and Cu). However, other potentially harmful heavy metals, such as mercury (Hg) and arsenic (As), were not analysed. Including these metals could provide a more comprehensive assessment of feed safety.

Source of Contamination: The study identified the presence of heavy metals in the feeds but did not investigate the specific sources of contamination. Understanding whether the contamination originates from raw materials, processing, or environmental factors would be crucial for developing mitigation strategies.

Conclusions

The study provides a comprehensive evaluation of the proximate composition and heavy metal content in commercial tilapia starter feeds available in the Asuogyaman Municipality, Ghana. The findings reveal that while the nutritional content of the feeds generally aligns with the values reported by manufacturers, there are notable discrepancies, particularly in moisture and crude fat levels. The proximate analysis indicates that the feeds meet the basic nutritional requirements for tilapia growth, with crude protein, fat, ash, and carbohydrate contents within acceptable ranges. However, the presence of heavy metals such as chromium (Cr), lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) in the feeds raises significant concerns. Notably, the chromium levels in all feed samples exceeded the maximum permissible limits set by international standards, posing potential health risks to both fish and human consumers. The study underscores the importance of stringent quality control measures in the production and regulation of fish feeds to ensure their nutritional integrity and safety. The presence of heavy metals, even at low concentrations, highlights the need for regular monitoring and enforcement of feed quality standards to prevent bioaccumulation and subsequent health hazards. Feed manufacturers must adopt best practices to minimise contamination, particularly from heavy metals, and ensure that feeds are free from harmful pollutants. Furthermore, the findings emphasise the need for increased awareness and training among feed producers, distributors, and farmers regarding the importance of feed quality, proper handling, and storage practices. Regulatory bodies should also play a more active role in enforcing compliance with feed safety standards to safeguard public health and promote sustainable aquaculture practices. Future research should focus on identifying the sources of heavy metal contamination and developing strategies to mitigate their presence in fish feeds.

Reference

Abdel-Warith, A. A., Younis, E. M., Al-Asgah, N. A., & Wahbi, O. M. (2011). Effect of zinc toxicity on liver histology of Nile tilapia, Oreochromis niloticus. *Scientific Research and Essays*, **6(17)**, 3760-3769. https://10.5897/SRE11.883

Adamidou, S., Rigos, G., Mente, E., Nengas, I., & Fountoulaki, E. (2011). The effects of dietary lipid and fibre levels on digestibility of diet and on the growth performance of sharpsnout seabream (Diplodus puntazzo). *Mediterranean Marine Science*, **12(2)**, 401-412. https://doi.org/10.12681/mms.4

Adeniji, C. A., & Okedeyi, O. O. (2017). Preliminary assessment of heavy metal concentrations in selected fish feed ingredients in Nigeria. *Journal of Fisheries & Livestock Production*, **5(1)**, 1-4.

Afzal Khan, M., Jafri, A. K., & Chadha, N. K. (2005). Effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of rohu, Labeo rohita (Hamilton). *Aquaculture nutrition*, **11(1)**, 11-17. https://doi.org/10.1111/j.1365-2095.2004.00318.x

Alam, M. K., Habib, M. A. B., & Tahmid, M. S. (2012). A survey on commercial fish feed used at Fulpur area in Mymensingh distric. *Journal of the Bangladesh Agricultural University*, **10**(452-2016-35556), 175-178. http://dx.doi.org/10.22004/ag.econ.209315

Alltech (2020). Alltech global feed survey reveals first production decline in nine years. Alltech's e-Newsletter, Kentucky

Altan, Ö., & Korkut, A. Y. (2011). Appearent digestibility of plant protein based diets by European sea bass Dicentrarchus labrax L., 1758. *Turkish Journal of Fisheries and Aquatic Sciences*, **11(1)**.

AOAC (2000). Official Methods of Analysis of the Association of the Official Analytical Chemists, 17th edition, AOAC International, Washington, DC, USA,

Ayanda, I. O., Dedeke, G. A., Ekhator, U. I., & Etiebet, M. K. (2018). Proximate composition and heavy metal analysis of three aquatic foods in makoko river, Lagos, Nigeria. *Journal of food quality*, 2018. https://doi.org/10.1155/2018/2362843.

Ayuba, V. O., & Iorkohol, E. K. (2010). Proximate composition of some commercial fish feeds sold in Nigeria.

Ayssiwede, S. B., Zanmenou, J. C., Issa, Y., Hane, M. B., Dieng, A., Chrysostome, C. A. A. M., ... & Missohou, A. (2011). Nutrient composition of some unconventional and local feed resources available in Senegal and recoverable in indigenous chickens or animal feeding. *Pakistan Journal of Nutrition*, **10(8)**, 707-717

Bernard, E., & Adetola, O. (2022). Nutritional Composition and Heavy Metal Contamination of Prominent Fishmeal Samples. *J. Fish. Aquat. Sci*, **17**, 1-8.

Bernard,T., Wiryanta, W., Sunaryo, S.P., Astuti, S.P., Kurniawan, M.B. (2010). Budidaya dan bisnis ikan nila.

Bhowmik, S., Dewanjee, S., Islam, S., Saha, D., Banik, P., Hossain, M. K., ... & Mamun, A. A. (2023). Nutritional profile and heavy metal contamination of nursery, grower, and finisher feeds of tilapia (*Oreochromis niloticus*) in Bangladesh. *Food Chemistry Advances*, **2**, 100235. https://doi.org/10.1016/j.focha.2023.100235

Biswas, C., Hossain, M. M. M., Sarker, B., Billah, M. M., & Ali, M. A. (2018). Culture strategies, diseases and their mitigations in mono-sex Nile tilapia farming in Jessore sadar region, Bangladesh. Asian-Australasian *Journal of Bioscience and Biotechnology*, **3(3)**, 190-200. https://doi.org/10.3329/aajbb.v3i3.64823

Bou, M., Todorčević, M., Fontanillas, R., Capilla, E., Gutiérrez, J., & Navarro, I. (2014). Adipose tissue and liver metabolic responses to different levels of dietary carbohydrates in gilthead sea bream (*Sparus aurata*). Comparative Biochemistry and Physiology Part A: *Molecular & Integrative Physiology*, **175**, 72-81. https: // doi.org /10.1016 / j.cbpa.2014.05.014

Cho, J. H., & Kim, I. (2011). Fish meal–nutritive value. *Journal of Animal Physiology and Animal Nutrition*, **95(6)**, 685-692.

Dai, S. Y., Jones, B., Lee, K. M., Li, W., Post, L., & Herrman, T. J. (2016). Heavy metal contamination of animal feed in Texas. *Journal of Regulatory Science*, **4(1)**, 21-32.

Dimado, F. D. (2024). The challenges of fish feed costs in Ghana's aquaculture industry: Strategies for sustainable growth in 2024 and beyond. https://famerlio.org/fish-feed-costs-in-ghana-and-solutions

Ezeonyejiaku, C. D., Obiakor, M. O., & Ezenwelu, C. (2011). Toxicity of copper sulphate and behavioral locomotor response of tilapia (*Oreochromis niloticus*) and. Online *J Anim Feed Res*, **1**, 130-4.

FAO Aquaculture development (2011) Use of wild fish as feed in aquaculture. FAO Technical Guidelines for Responsible Fisheries, FAO, Rome

Fisheries and Aquaculture. Draft Indian Standard-Fish Feed, FAD 12 (2340) (2013). Freshwater Prawn (Macrobrachium rosenbergii), FAD 12 (2337), Carps, FAD 12 (2338), Catfish, Feed specification. Bureau of Indian Standards, Manak Bhavan, New Delhi-110 002.

Food additives and contaminants (2001). Joint FAO/WHO Food Standards Programme. ALINORM 01/12A

Gao, Y., Wang, R., Li, Y., Ding, X., Jiang, Y., Feng, J., & Zhu, L. (2021). Trophic transfer of heavy metals in the marine food web based on tissue residuals. *Science of the Total Environment*, 772, 145064. https://doi.org/10.1016/j.scitotenv.2021.145064

Hamada, M. G., Elbayoumi, Z. H., Khader, R. A., & M Elbagory, A. R. (2018). Assessment of Heavy Metal Concentration in Fish Meat of Wild and Farmed Nile Tilapia (*Oreochromis Niloticus*), Egypt. *Alexandria Journal for Veterinary Sciences*, **57(1)**. https://10.5455/ajvs.295019

Hasan, M.R. (2001). Nutrition and feeding for sustainable aquaculture development in the third millennium. InAquaculture in the third millennium. Technical proceedings of the conference on aquaculture in the third millennium (pp. 193-219). Rome: FAO.

Hasan, S., Rahman, L., & Chowdhury, S. (2016). Prevalence of chromium in fish feed and commercially cultivated tilapia. Available at SSRN 2816862.

Hasan, M. S., Al Bashera, M., Jahan, F., Hossain, A., Waliullah, M., & Islam, M. B. (2022). Proximate composition of some commercially available fish and poultry feeds sold in the market of Bangladesh. *Int. J. Biosci*, **20(4)**, 1-8.

Hasselberg, A. E., Aakre, I., Scholtens, J., Overå, R., Kolding, J., Bank, M. S., ... & Kjellevold, M. (2020). Fish for food and nutrition security in Ghana: Challenges and opportunities. *Global food security*, **26**, 100380. https://doi.org/10.1016/j.gfs.2020.100380

Henriksson, P. J. G., Troell, M., Banks, L. K., Belton, B., Beveridge, M. C. M., Klinger, D. H., ... & Tran, N. (2021). Interventions for improving the productivity and environmental performance of global aquaculture for future food security. *One Earth*, **4(9)**, 1220-1232.. https://doi.org/10.1016/j.oneear.2021.08.009

Islam, S. M., Rohani, M. F., Zabed, S. A., Islam, M. T., Jannat, R., Akter, Y., & Shahjahan, M. (2020). Acute effects of chromium on hemato-biochemical parameters and morphology of erythrocytes in striped catfish Pangasianodon hypophthalmus. T*oxicology Reports*, **7**, 664-670.

Janbakhsh, S., Hosseini Shekarabi, S. P., & Shamsaie Mergan, M. (2018). Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea. *Caspian Journal of Environmental Sciences*, **16(4)**, 307-317. https: // doi.org / 10.22124 /cjes. 2018.3200

Javed, M., & Usmani, N. (2019). An overview of the adverse effects of heavy metal contamination on fish health. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, **89**, 389-403.

Jaxion-Harm, J. (2021). Effects of dietary phospholipids on early stage Atlantic Salmon (Salmo salar) performance: A comparison among phospholipid sources. *Aquaculture*,

544, 737055

Kader, M. A., Hossain, M. A., & Hasan, M. R. (2005). A survey of the nutrient composition of some commercial fish feeds available in Bangladesh.

Kamaruzzaman, B. Y., Ong, M. C., Rina, S. Z., & Joseph, B. (2010). Levels of some heavy metals in fishes from Pahang river estuary, Pahang, Malaysia. *Journal of Biological Sciences*, **10(2)**, 157-161.

Kathane, Y. M., Sahu, B., Mohanta, K. N., Nanda, S., Patra, S. K., & Samantaray, K. (2017). Protein-sparing capability of carbohydrate in fringed-lipped carp Labeo fimbriatus (Bloch, 1795). *Indian Journal of Fisheries*, **64**.

Kim, K. D., Lim, S. G., Kang, Y. J., Kim, K. W., & Son, M. H. (2012). Effects of dietary protein and lipid levels on growth and body composition of juvenile far eastern catfish Silurus asotus. *Asian-Australasian journal of animal sciences*, **25(3)**, 369. https://doi.org/10.5713%2Fajas.2011.11089

Kundu, G. K., Alauddin, M., Akter, M. S., Khan, M. S., Islam, M. M., Mondal, G., ... & Huque, A. (2017). Metal contamination of commercial fish feed and quality aspects of farmed tilapia (*Oreochromis niloticus*) in Bangladesh. *Bioresearch Communications-(BRC)*, **3(1)**, 345-353.

Lassi, Z., Moin, A., & Bhutta, Z. (2017). Nutrition in middle childhood and adolescence. Disease Control Priorities, (Volume 8): Child and Adolescent Health and Development, 1896.

Lee, J. W., Choi, H., Hwang, U. K., Kang, J. C., Kang, Y. J., Kim, K. I., & Kim, J. H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental toxicology and pharmacology*, **68**, 101-108. https://doi.org/10.1016/j.etap.2019.03.010

Lin, S., Lin, X., Yang, Y., Li, F., & Luo, L. (2013). Comparison of chelated zinc and zinc sulfate as zinc sources for growth and immune response of shrimp (*Litopenaeus vannamei*). *Aquaculture*, **406**, 79-84. https://doi.org/10.1016/j.aquaculture.2013.04.026

Liu, Y., Wei, H., Li, S., Wang, G., Guo, T., & Han, H. (2022). Facile fabrication of semi-IPN hydrogel adsorbent based on quaternary cellulose via amino-anhydride click reaction in water. *International Journal of Biological Macromolecules*, **207**, 622-634. https://doi.org/10.1016/j.ijbiomac.2022.03.032

López-Alonso, M. (2012). Animal feed contamination by toxic metals. In Animal Feed Contamination (pp. 183-204). Woodhead Publishing.. https://doi.org/10.1533/9780857093615.2.183

Luquet, P. (2017). Tilapia, *Oreochromis* spp. In Handbook of Nutrient Requirements of Finfish (1991) (pp. 169-180). CRC Press.

Macomber, L., & Hausinger, R. P. (2011). Mechanisms of nickel toxicity in microorganisms. *Metallomics*, **3(11)**, 1153-1162. https://doi.org/10.1039/c1mt00063b

Mannan, M. A., Hossain, M. S., Sarker, M. A. A., Hossain, M. M., Chandra, L., Haque, A. H., & Kudrat-E-Zahan, M. (2018). Bioaccumulation of toxic heavy metals in fish after feeding with synthetic feed: a potential health risk in Bangladesh. *J Nutr*, **8(5)**, 1000728.

Meena, M. K., Aklakur, M. D., Siddaiah, G. M., Jadhao, B. S., Pal, A. K., Chouksey, M. K., & Gupta, S. (2010). Effect of dietary zinc levels on growth, metabolic responses and meat quality of Labeo rohita fingerlings. In Proc. Golden Jubilee National Seminar on diversification of Aquaculture through locally available fish species, held at CIFE, Kolkata Centre during (pp. 27-28).

Mohanty, B. P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D., & Anandan, R. (2019). Nutritional composition of food fishes and their importance in providing food and nutritional security. *Food chemistry*, **293**, 561-570.. https://doi.org/10.1016/j.foodchem.2017.11.039

Mohapatra, M., Sahu, N. P., & Chaudhari, A. (2003). Utilization of gelatinized carbohydrate in diets of Labeo rohita fry. *Aquaculture Nutrition*, **9(3)**, 189-196. https://doi.org/10.1046/j.1365-2095.2003.00243.x

Murthy, L. N., Mohan, C. O., Ravishankar, C. N., & Badonia, R. (2013, February). Biochemical quality and heavy metal content of fish meal and squid meal produced in Veraval, Gujarat. ICAR.

Nayeem, M. A., Hossain, A., Hannan, M. H., & Mondal, S. (2019). Comparative analysis of nutritional quality of different fish feed available in greater Noakhali region, Bangladesh. *Asian-Australasian Journal of Food Safety and Security*, **3(1)**, 1-14. https://doi.org/10.3329/aajfss.v3i1.55921

Nazish, N., & Mateen, A. (2011). Winter growth of carps under different semi-intensive culture conditions. *Pak. Vet. J*, **31(2)**, 134-136.

Onyeike, E. N., Ayalogu, E. O., & Ibegbulem, C. O. (2000). Evaluation of the nutritional value of some crude oil polluted freshwater fishes. *Global Journal of Pure and Applied Sciences*, **6(2)**, 227-233.

Paul, B. N., Sarkar, S., Giri, S. S., Mohanty, S. N., & Mukhopadhyay, P. K. (2006). Dietary calcium and phosphorus requirements of rohu Labeo rohita fry. *Animal Nutrition and Feed Technology*, **6(2)**, 257-263.

Ragasa, C., Osei-Mensah, Y. O., & Amewu, S. (2022). Impact of fish feed formulation training on feed use and farmers' income: Evidence from Ghana. *Aquaculture*, **558**, 738378.. https: / / doi.org /10.1016 / j.aquaculture. 2022 . 738378

Rahimnejad, S., Dabrowski, K., Izquierdo, M., Malinovskyi, O., Kolářová, J., & Policar, T. (2021). Effects of dietary protein and lipid levels on growth, body composition, blood biochemistry, antioxidant capacity and ammonia excretion of European grayling (*Thymallus thymallus*). *Frontiers in Marine Science*, **8**, 715636.https://doi.org/10.3389/fmars.2021.715636

Sarkar, M. M., Rohani, M. F., Hossain, M. A. R., & Shahjahan, M. (2022). Evaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. *Biological Trace Element Research*, 1-11..

Sabbir, W., Rahman, M. Z., Halder, T., Khan, M. N., & Ray, S. (2018). Assessment of heavy metal contamination in fish feed available in three districts of South Western region of Bangladesh. *International Journal of Fisheries and Aquatic Studies*, **6(2)**, 100-104.

Suchana, S. A., Ahmed, M. S., Islam, S. M., Rahman, M. L., Rohani, M. F., Ferdusi, T., ... & Shahjahan, M. (2021). Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish Pangasianodon hypophthalmus. *Biological Trace Element Research*, **199**, 3869-3885.

Saha, B., Mottalib, M. A., & Al Razee, A. N. M. (2018). Assessment of selected heavy metals concentration in different brands of fish feed available in Bangladesh. *Journal of Bangladesh Academy of Sciences*, **42(2)**, 207-210.https://doi.org/10.3329/jbas.v42i2.40054

Shamshad, B. Q., Shahidur, R. K., &

Tasrena, R. C. (2009). Studies on toxic elements accumulation in shrimp from fish feed used in Bangladesh. *Asian Journal of Food and Agro-Industry*, **2(4)**, 440-444.

Sharifuzzaman, S. M., Rahman, H., Ashekuzzaman, S. M., Islam, M. M., Chowdhury, S. R., & Hossain, M. S. (2016). Heavy metals accumulation in coastal sediments. Environmental remediation technologies for metal-contaminated soils, 21-42.

Sukumaran, K., Pal, A. K., Sahu, N. P., Dalvi, R. S., & Debnath, D. (2008). Haemato-biochemical responses and induction of HSP70 to dietary phosphorus in Catla catla (Hamilton) fingerlings. *Fish physiology and biochemistry*, **34**, 299-306..

Uddin, M. N., Shahjahan, M., & Haque, M. M. (2012). Manipulation of species composition in small scale carp polyculture to enhance fish production. *Bangladesh Journal of Progressive Sciences and Technology*, **10**, 9-12.

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., ... & Phillips, M. I. C. H. A. E. L. (2014). Improving productivity and environmental performance of aquaculture. WorldFish.

Ween, O., Stangeland, J. K., Fylling, T. S., & Aas, G. H. (2017). Nutritional and functional properties of fishmeal produced from fresh by-products of cod (*Gadus morhua* L.) and saithe (*Pollachius virens*). *Heliyon*, **3(7)**.

Zaman, M. F. U., Samad, M. A., Islam, M. A., Jaman, M. H. U., Khondoker, S., & Asif, A. A. (2017). Assessment of the sustainability of Pangasius (*Pangasius hypophthalmus*) farming at Jhikargachha upazila in Jessore district, Bangladesh. *International Journal of Fauna and Biological Studies*, **4(5)**, 109-119.