RESEARCH PAPER

Nutritional Evaluation of Fish Processing Waste and Chicken Intestine as Alternative Protein Sources in Formulated Aquafeed

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Abstract
The sustainability of aquaculture is increasingly constrained by the high cost and limited availability of fish meal, necessitating the exploration of alternative protein sources for aquafeed formulation. The present study evaluated the nutritional potential of fish processing waste (FW) and chicken intestine (CI) as alternative animal protein sources in formulated fish feeds. Six experimental diets (D1–D6) were prepared using a constant plant-based basal mixture, with graded replacement of conventional fish meal by FW and CI. All diets were formulated to be isonitrogenous, targeting a crude protein level of approximately 35%. Proximate composition, including moisture, ash, crude protein, crude fat, and carbohydrate content, was determined using standard analytical methods. Fish waste and chicken intestine exhibited high crude protein (40.42% and 71.04%, respectively) and lipid content (18.43% and 15.81%, respectively). Among the experimental diets, crude protein content ranged from 31.63% (D1) to 45.73% (D6), with diets containing FW and CI showing superior nutritional profiles compared to fish meal-based formulations. The findings indicate that fish processing waste and chicken intestine can serve as nutritionally rich, cost-effective, and environmentally sustainable alternatives to fish meal in aquafeed formulation.

Keywords: aquafeed formulation, fish processing waste, chicken intestine, alternative protein sources, proximate composition

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1. Introduction:

Aquaculture has emerged as one of the fastest-growing food production sectors worldwide, playing a crucial role in meeting the rising demand for animal protein. However, the sustainability and profitability of aquaculture are strongly influenced by feed costs, which account for approximately 50–70% of total production expenses (Tacon & Jackson, 1985). Fish meal remains the principal protein source in commercial aquafeeds due to its high protein content, balanced amino acid profile, and excellent digestibility. Nevertheless, increasing prices, fluctuating supply, and growing competition with human consumption have raised serious concerns regarding its long-term availability (Tacon, 1996; Hardy, 2010).

In response, extensive research has focused on identifying alternative protein sources capable of partially or completely replacing fish meal without compromising feed quality. Plant-based protein sources such as soybean meal have been widely studied; however, their utilization is often limited by anti-nutritional factors and amino acid imbalances (Boonyaratpalin et al., 1998; Daniel, 2018). Consequently, attention has shifted toward animal by-products and agro-industrial wastes that are nutritionally rich, locally available, and do not directly compete with human food resources.

Fish processing waste, which may constitute up to 50% of the harvested fish biomass, includes heads, scales, fins, and viscera generated during processing (Rustad, 2003). These wastes are rich in protein, lipids, and minerals, and their improper disposal poses significant environmental challenges (Arvanitoyannis & Kassaveti, 2008). Similarly, poultry processing by-products such as chicken intestines contain substantial amounts of high-quality protein and fat, making them promising candidates for aquafeed formulation (Giri et al., 2010; Bhaskar et al., 2015).

Utilizing fish processing waste and poultry by-products in aquafeeds aligns with the principles of waste valorisation and circular economy, reducing environmental pollution while lowering feed production costs (Ferraro et al., 2010). Despite their potential, comprehensive nutritional evaluation of aquafeeds formulated using fish waste and chicken intestine remains limited, particularly under Indian conditions. The present study was therefore undertaken to evaluate the proximate nutritional composition of experimental fish feeds formulated using fish processing waste and chicken intestine as alternative protein sources.

2. Materials and methods:

2.1 Collection and processing of feed ingredients:

Fish processing waste and chicken intestines were collected from Narengi fish market, Guwahati, Assam, during February 2025. Fish waste consisted of scales, gills, and intestines of commonly consumed freshwater fish species including *Labeo rohita*, *Catla catla*, and *Cyprinus carpio*. The samples were transported to the laboratory in sterile polyethylene bags under chilled conditions.

The collected materials were thoroughly washed with potable water to remove adhering impurities and oven-dried at 100°C for 18 hours to reduce moisture content and inhibit microbial activity. Dried samples were ground into fine powder using an electric grinder and stored in airtight containers until further use. Plant-based ingredients such as processed soybean, sesame seed, wheat flour, wheat bran, and corn powder were procured locally and processed using similar procedures.

2.2 Formulation of experimental diets:

Six experimental diets (D1–D6) were formulated using a constant plant-based basal mixture constituting 70% of the total feed. The remaining 30% comprised varying proportions of fish meal (FM), fish waste (FW), and chicken intestine (CI). All diets were formulated to be isonitrogenous, targeting an overall crude protein level of approximately 35%, based on the reported nutritional values of individual ingredients (AOAC, 2000). The formulated feeds were thoroughly mixed to ensure uniformity and stored in airtight containers for subsequent analysis.

2.3 Proximate composition analysis:

Proximate composition of fish waste, chicken intestine, and experimental diets was determined following standard procedures described by AOAC (2000). Moisture content was estimated by oven-drying samples at 105°C to constant weight. Ash content was determined by incineration in a muffle furnace at 550°C. Crude fat content was measured using Soxhlet extraction, while crude protein content was determined by the Bradford method. Carbohydrate content was estimated using the Anthrone method (Sawhney & Singh, 1996).

3. Results:

All proximate composition values are expressed on a dry weight basis. Data are presented as mean ± standard deviation (SD) of three replicates ($n = 3$). Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$.

3.1 Proximate composition of fish processing waste and chicken intestine:

The proximate composition of fish processing waste (FW) and chicken intestine (CI) is presented in **Table 1**. All measured parameters differed significantly between the two ingredients ($p < 0.05$).

Chicken intestine showed significantly higher crude protein content (71.04 ± 0.82%) compared to fish processing waste (40.42 ± 0.64%). Crude fat content was also high in both ingredients, with FW recording 18.43 ± 0.57% and CI recording 15.81 ± 0.49%. Ash content differed significantly between the two ingredients, with FW exhibiting higher ash content (27.91 ± 0.71%) than CI (9.62 ± 0.38%). Moisture content remained below 1% for both ingredients, while carbohydrate content was significantly higher in FW (12.42 ± 0.48%) than in CI (2.89 ± 0.21%).

Table 1: Proximate composition (% dry weight basis) of fish processing waste and chicken intestine

Parameter (%)	Fish processing waste (FW)	Chicken intestine (CI)
Moisture	0.82 ± 0.06	0.64 ± 0.05
Crude protein	40.42 ± 0.64	71.04 ± 0.82
Crude fat	18.43 ± 0.57	15.81 ± 0.49
Ash	27.91 ± 0.71	9.62 ± 0.38
Carbohydrate	12.42 ± 0.48	2.89 ± 0.21

(Values expressed as mean ± SD, n = 3)

3.2 Proximate composition of experimental diets:

The proximate composition of the six experimental diets (D1–D6) is summarized in **Table 2**. Moisture content across all diets ranged from 0.76 ± 0.02% to 0.91 ± 0.05%, with no significant variation among treatments ($p > 0.05$).

Crude protein content differed significantly among the experimental diets ($p < 0.05$), ranging from 31.63 ± 0.54% in D1 to 45.73 ± 0.76% in D6. Diets D3 to D6 recorded significantly higher crude protein levels compared to D1 and D2 (Table 2). The highest crude protein content was observed in D6, followed by D5 and D4.

Crude fat content also varied significantly among diets ($p < 0.05$). Values ranged from 10.42 ± 0.39% in D1 to 17.83 ± 0.61% in D6. Diets D4, D5, and D6 showed significantly higher crude fat content compared to D1 and D2.

Ash content differed significantly among diets ($p < 0.05$), with values ranging from 8.21 ± 0.33% in D1 to 14.62 ± 0.48% in D4. Diets containing fish processing waste (D3–D5) exhibited significantly higher ash content compared to fish meal-based diets (D1 and D2).

Carbohydrate content showed a significant inverse trend relative to crude protein and crude fat content ($p < 0.05$), decreasing from 48.83 ± 0.71% in D1 to 23.50 ± 0.49% in D6.

Table 2: Proximate composition (% dry weight basis) of experimental diets (D1–D6)

Parameter (%)	*D1*	*D2*	*D3*	*D4*	*D5*	*D6*
Moisture	0.91±0.05[a]	0.88±0.04[a]	0.85±0.03[a]	0.82±0.04[a]	0.79±0.03[a]	0.76±0.02[a]
Crude protein	31.63±0.54[a]	34.21±0.61[b]	38.46±0.68[c]	42.12±0.72[d]	44.05±0.74[e]	45.73±0.76[f]
Crude fat	10.42±0.39[a]	11.86±0.44[b]	13.92±0.48[c]	15.74±0.55[d]	16.98±0.58[e]	17.83 ± 0.61[f]
Ash	8.21 ± 0.33[a]	9.14 ± 0.35[b]	11.62 ± 0.41[c]	14.62 ± 0.48[d]	13.41±0.45[cd]	12.18 ± 0.42[c]
Carbohydrate	48.83 ± 0.71[f]	42.91 ± 0.68[e]	35.15 ± 0.62[d]	26.70 ± 0.56[c]	24.57 ± 0.52[b]	23.50 ± 0.49[a]

(Values expressed as mean ± SD, n = 3. Different superscript letters within the same row indicate significant difference at $p < 0.05$.)

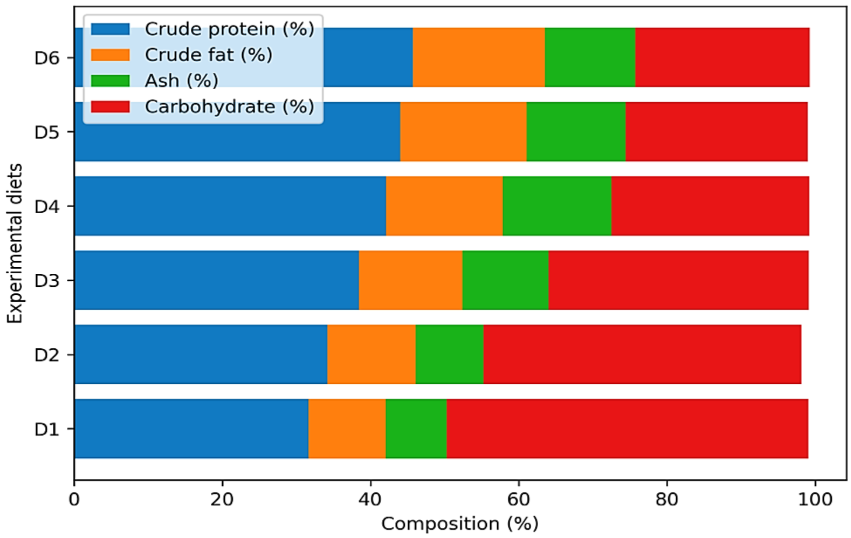

Fig.1: Proximate composition (% dry weight basis) of experimental diets (D1–D6), illustrating the relative proportions of crude protein, crude fat, ash, and carbohydrate.

3.3 Comparative analysis of crude protein and crude fat content:

Comparative variations in crude protein and crude fat content among experimental diets are illustrated in Figure 2. Crude protein content increased progressively from D1 to D6, with diets D4, D5, and D6 showing significantly higher values than D1–D3 ($p < 0.05$). Similarly, crude fat content increased significantly with increasing inclusion of animal by-products, with D5 and D6 recording the highest lipid levels among all experimental diets (Figure 2).

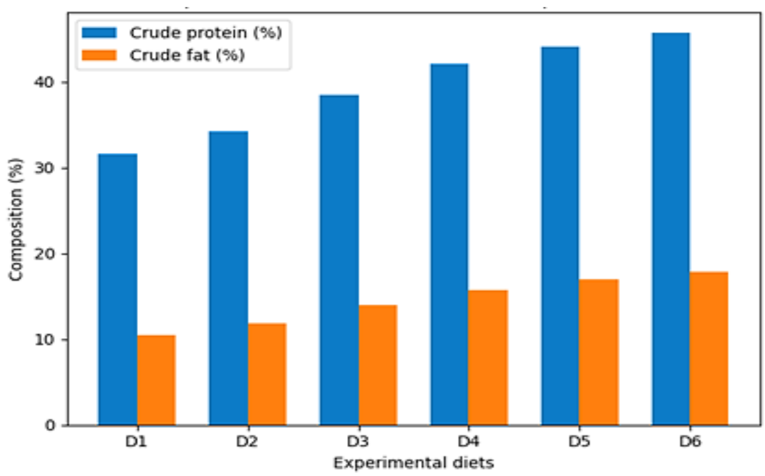

Fig. 2: Comparative crude protein and crude fat content (% dry weight basis) of experimental diets (D1–D6). Values are expressed as mean ± SD (n = 3).

Overall, inclusion of fish processing waste and chicken intestine significantly influenced the proximate composition of the experimental diets, particularly crude protein, crude fat, ash, and carbohydrate content ($p < 0.05$).

4. Discussion:

The present study evaluated the proximate composition of experimental diets formulated using fish processing waste (FW) and chicken intestine (CI) as alternative protein sources. The results demonstrated that incorporation of these animal-derived by-products significantly influenced the nutritional profile of the diets, particularly crude protein, lipid, ash, and carbohydrate contents, indicating their potential suitability in aquafeed formulation.

A progressive increase in crude protein content from D1 to D6 was observed, corresponding to increasing inclusion levels of FW and CI. Diets with higher inclusion levels, particularly D4 and D6, exhibited superior crude protein content compared to conventional formulations. These values fall within or exceed the recommended dietary protein requirements for many omnivorous freshwater fish species (Kaushik, 2000), suggesting their applicability in practical aquaculture systems. The high crude protein content observed in CI corroborates earlier findings that poultry viscera constitute nutritionally rich feed ingredients suitable for aquaculture species (Giri et al., 2010; Bhaskar et al., 2015). Similarly, previous studies have demonstrated that incorporation of animal by-products can significantly enhance dietary protein levels without compromising feed quality (Hamid et al., 2016).

The lipid content of the experimental diets also increased progressively with increasing levels of FW and CI. This trend can be attributed to the inherently high fat content of these animal-derived materials, particularly fish waste, which contains residual fish oils. The elevated fat content of FW observed in the present study is consistent with earlier reports (Güllü et al., 2014). Lipids serve as an important energy source in fish diets and play a vital role in improving feed utilization efficiency. However, excessive lipid levels may lead to metabolic imbalances, highlighting the importance of maintaining optimal inclusion levels during feed formulation.

Ash content was higher in diets containing greater proportions of FW, particularly in intermediate diets such as D3 and D4. This can be attributed to the presence of bone fragments and mineral residues in fish processing waste, which contribute to increased ash levels. Similar observations have been reported in previous studies (Tobinda and Butt, 2012; Güllü et al., 2014). While moderate ash content provides essential minerals required for fish growth and skeletal development, excessively high levels may negatively affect nutrient digestibility. In the present study, ash values remained within acceptable ranges reported for aquafeeds, suggesting that mineral content was within tolerable limits.

In contrast, carbohydrate content exhibited a decreasing trend from D1 to D6, reflecting the gradual replacement of plant-based ingredients such as rice bran and wheat flour with protein- and lipid-rich animal by-products. This inverse relationship between carbohydrate content and animal protein inclusion is consistent with earlier findings (Enyidi et al., 2014). Fish generally exhibit limited capacity to efficiently utilize high carbohydrate levels compared to proteins and lipids (Wasswa, 2007). Therefore, the reduction in carbohydrate content observed in higher inclusion diets may contribute to improved dietary energy utilization, although this requires validation through feeding trials.

Overall, the proximate composition results indicate a clear transition from carbohydrate-dominant diets to protein- and lipid-enriched formulations with increasing inclusion of FW and CI. This compositional shift aligns well with the nutritional requirements of many aquaculture species, particularly carnivorous and omnivorous fish, which depend primarily on protein and lipid as energy sources (NRC, 2011). Furthermore, experimental diets containing higher levels of FW and CI demonstrated comparable or improved nutritional profiles relative to conventional fish meal-based formulations, supporting their potential as alternative feed ingredients.

From a sustainability perspective, the utilization of fish processing waste and chicken intestine offers significant advantages in aquafeed production. These materials are often underutilized and may contribute to environmental pollution if improperly disposed of. Their incorporation into aquafeeds not only reduces dependency on conventional fish meal but also promotes waste recycling and resource efficiency. Previous studies have emphasized that animal by-products can effectively replace fish meal without compromising nutritional quality when properly processed and formulated (Abdel-Warith et al., 2001; Hardy, 2010; Tacon et al., 1994). The present findings further support the feasibility of utilizing these low-cost and locally available waste materials in aquafeed formulation, particularly under regional conditions.

Despite these promising findings, the present study is limited to proximate composition analysis and does not include feeding trials or digestibility assessments. Therefore, while the nutritional profiles of the formulated diets are encouraging, further research is necessary to evaluate their effects on growth performance, feed conversion ratio, nutrient digestibility, and overall fish health. Additionally, factors such as palatability, amino acid balance, and potential variability in raw material composition should be considered in future studies.

5. Conclusion:

The present study demonstrates that fish processing waste and chicken intestine are nutritionally viable alternatives to conventional fish meal in formulated aquafeeds. Diets incorporating these by-products exhibited enhanced crude protein and lipid content, with D4 and D6 emerging as nutritionally superior formulations. The utilization of fish waste and poultry by-products offers a sustainable and cost-effective strategy for aquafeed production while contributing to environmental waste reduction. Further studies involving feeding trials and digestibility assessments are recommended to validate the practical applicability of these formulations under aquaculture farming conditions.

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