



Nutrient composition of fish feeds formulated with poultry by-product meal, blood meal and meat-cum-bone meal

Bhattu Naveen Kumar¹, Irshad A^{2*}, Vasudevan V N³, Sathu T⁴, Sunilkumar N S⁵, Sanal Ebenezer⁶

¹ Department of Livestock Products Technology, CVAS, Mannuthy, Thrissur, Kerala, India

² Assistant Professor, Department of Livestock Products Technology, CVAS, Mannuthy, Thrissur, Kerala, India

³ Professor and Head, Department of Livestock Products Technology, CVAS, Mannuthy, Thrissur, Kerala, India

⁴ Professor, Department of Livestock Products Technology, CVAS, Mannuthy, Thrissur, Kerala, India

⁵ Assistant Professor, Dept of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

⁶ Scientist, Marine Biotechnology Fish Nutrition and Health Division, Central Marine Fisheries Research, Institute, Kochi, Kerala, India

Corresponding Author: Irshad A

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Abstract

The increasing cost and limited availability of fish meal have encouraged the search for sustainable alternative protein sources in aquafeed formulation. The present study was undertaken to evaluate the proximate composition and amino acid profile of poultry by-product meal (PBM), blood meal (BM) and meat-cum-bone meal (MCBM) and to assess their suitability as partial fish meal replacers in tilapia feed. Four isonitrogenous diets were formulated, including a control diet prepared as per FAO guidelines and three treatment diets in which 50 per cent of fish meal protein was replaced with MCBM, BM or PBM. The animal by-product meals and developed feeds were analysed for proximate composition and amino acid profile. Significant variation was observed among the animal by-product meals in moisture, crude protein, ether extract, total ash, crude fibre, acid insoluble ash and nitrogen free extract. Blood meal recorded the highest crude protein content, while PBM had the highest ether extract and MCBM showed the highest ash content. The developed feeds showed comparable crude protein levels, confirming successful formulation of isonitrogenous diets. Amino acid analysis revealed that replacement of fish meal protein with animal by-product meals maintained the essential amino acid profile within a nutritionally acceptable range. Among the treatment feeds, PBM-based feed showed favourable nutrient composition and amino acid balance. The study indicated that rendered animal by-products, particularly poultry by-product meal, can be effectively utilised as alternative protein sources in tilapia feed formulation, thereby reducing dependence on fish meal and promoting sustainable aquafeed production.

Keywords: Aquafeed, Tilapia, poultry by-product meal, blood meal, meat-cum-bone meal, fish meal replacement

Introduction

Aquaculture is one of the fastest-growing food production sectors and plays an important role in supplying quality animal protein for human consumption (Nathanailides, 2025) [17]. The expansion of aquaculture has increased the demand for nutritionally balanced and cost-effective aquafeeds (Verdegem *et al.*, 2023) [25]. Fish meal has traditionally been the major protein source in aquafeeds; however, its increasing cost, limited availability and sustainability concerns have encouraged the search for suitable alternative protein ingredients (Sarker *et al.*, 2020) [23].

India generates considerable quantities of slaughterhouse by-products such as blood, bones and poultry processing residues from the livestock and poultry sectors. Improper disposal of these by-products may cause environmental problems, whereas their utilisation in feed production offers scope for value addition and sustainable waste management (Kannatti *et al.*, 2024) [12]. Poultry by-product meal, meat-cum-bone meal and blood meal are promising alternative protein sources for aquafeeds due to their high protein content and favourable amino acid profile (Kari *et al.*, 2023; Macusi *et al.*, 2023) [13, 16].

Proximate composition and amino acid analysis are essential for evaluating the nutritional quality of feed ingredients and formulated feeds. Essential amino acids, particularly lysine

and methionine, are critical for optimum fish growth and feed efficiency (Small and Soares, 2000; Nguyen and Davis, 2009) [18, 24]. Therefore, the present study was undertaken to evaluate the proximate composition and amino acid profile of poultry by-product meal, meat-cum-bone meal and blood meal, along with the developed experimental feeds, to assess their suitability as alternative protein sources in aquafeed formulation.

Materials and Methods

Ingredients

Poultry by-product meal (PBM) was procured from Venkateshwara Hatcheries Pvt. Ltd., Bengaluru, blood meal (BM) from Fair Exports (India) Pvt. Ltd., Uttar Pradesh and meat-cum-bone meal (MCBM) from the Department of Livestock Products Technology, Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The ingredients were packed in LDPE bags and stored under ambient conditions until further use.

Other feed ingredients including fish meal, fish oil, clam meal, soybean meal, groundnut oil cake, wheat bran, wheat powder, sunflower oil and multivitamin supplement were purchased from local shops in Ernakulam. Food-grade additives such as vitamin C, mineral mixture, lysine, methionine, dicalcium phosphate, butylated hydroxytoluene and sodium metabisulphite were procured from NICE

Chemicals Pvt. Ltd., Kochi. Wheat gluten was purchased online. All ingredients were stored in sterile, dry plastic containers at room temperature until processing.

Proximate analysis

Animal by-product meals and developed feeds were analysed for proximate composition, including moisture, crude protein, ether extract and total ash, on the day of preparation as per AOAC (2016). The results were expressed as percentage.

Feed formulation

A basal control diet was formulated to meet the nutritional requirements of tilapia as per FAO (2004) guidelines, with fish meal as the major protein source. Three treatment diets were prepared by replacing 50 per cent of fish meal protein in the control diet with MCBM, BM or PBM. Four isonitrogenous diets were formulated as follows:

C: Control fish feed formulated as per FAO guidelines.

T1: Fish feed in which 50 per cent fish meal protein was replaced with MCBM.

T2: Fish feed in which 50 per cent fish meal protein was replaced with BM.

T3: Fish feed in which 50 per cent fish meal protein was replaced with PBM.

The composition of the control and treatment feed formulations is presented in Table 1.

Table 1: Formulation of control and treatment feeds

Ingredients	C	T1	T2	T3
Wheat gluten (%)	3	3	3	3
Soyabean meal (%)	20.6	20.6	20.6	20.6
GNOC (%)	15	15	15	15
Wheat bran (%)	21	21	21	20.5
Wheat powder (%)	20.75	20	21.5	21.75
Fish meal* (%)	8	4	4	4
MBM (%)	0	5	0	0
PBM (%)	0	0	0	4
BM (%)	0	0	3	0
Shrimp Meal (%)	4	4	4	4
Fish oil (%)	1.75	1.75	1.75	1.5
Sunflower oil (%)	1.25	1	1.5	1
Vitamin (%)	1.5	1.5	1.5	1.5
Mineral (%)	1.5	1.5	1.5	1.5
SMB+BHT (%)	0.2	0.2	0.2	0.2
Methionine (%)	0.5	0.5	0.5	0.5
Lysine (%)	0.2	0.2	0.2	0.2
Vitamin C (%)	0.25	0.25	0.25	0.25
DCP (%)	0.5	0.5	0.5	0.5

*Fish meal was replaced by PBM, BM and MBM in the treatment samples as suggested by the model design

Mixing of ingredients

The sieved ingredients were weighed according to the formulation and manually mixed in a plastic container. Liquid ingredients such as fish oil and sunflower oil were added and blended thoroughly, followed by the addition of 20 per cent water. The mixture was homogenised using a homogeniser at 1500 and 3000 rpm for one minute each, followed by mixing in a bowl mixer for 12 minutes. The prepared mix was sieved and transferred to plastic containers. The same procedure was followed for all control and treatment feeds.

Extrusion process

The blended feed mix was extruded using a twin-screw extruder fitted with a 1 mm die. The extrusion temperature was maintained at 110°C and each experimental diet was extruded separately. The extruded material was cut into pellets using a rotating blade, collected in trays and labelled accordingly.

Drying and packing

The extruded pellets were dried overnight in a hot air oven at 50°C. The dried pellets were sieved through a 1 mm sieve to remove unextruded particles, packed in HDPE bags and stored under refrigeration at 4°C until further use.

Estimation of serum biochemical indices

Serum biochemical parameters including total protein, albumin, globulin, albumin-globulin ratio, triglycerides and cholesterol were estimated in GIFT tilapia at CMFRI, Kochi (Abdel-Rahim *et al.*, 2024).

Sample collection and processing

After completion of the 60-day growth trial, two fish from each tank, making six fish per treatment, were anaesthetised using clove oil at 1 µL/L. Blood was collected from the caudal vein using a 24 G needle and a 2 mL sterile syringe. The blood samples were transferred to serum vials, allowed to clot at room temperature for 30 minutes and incubated at 4°C overnight. Serum was separated by centrifugation at 1000×g for 15 minutes, transferred to Eppendorf tubes and stored at -80°C until analysis. The frozen samples were transported to CMFRI, Kochi in an insulated box with ice packs.

Analysis

Serum total protein, albumin, triglycerides and cholesterol were estimated using commercial diagnostic kits. Absorbance was measured using a microplate spectrophotometer at the respective wavelengths recommended for each parameter. Globulin and albumin-globulin ratio were calculated using the following formulae: Globulin (g/dL) = Total protein (g/dL) - Albumin (g/dL)
A/G ratio = Albumin (g/dL) / Globulin (g/dL)

Statistical Analysis

All the experiments were replicated six times. The data obtained from the experiments were assessed statistically by repeated measures ANOVA, one-way ANOVA and independent sample T test using the SPSS software version 26.0.

Results and Discussion

Nutrient composition of animal by-product meals

The proximate composition of poultry by-product meal (PBM), blood meal (BM) and meat-cum-bone meal (MCBM) showed significant variation ($P < 0.01$) in all the parameters studied, including moisture, crude protein, ether extract, crude fibre, total ash, acid insoluble ash and nitrogen free extract (Table 2). This variation could be attributed to differences in the source of raw materials, rendering conditions, inclusion of bone and connective tissue and extent of drying during processing. Similar variability in animal by-product meals has been reported

earlier, particularly in poultry by-product meal, where the chemical composition is influenced by the nature and proportion of raw materials used for rendering (Johnson *et al.*, 1998)^[11].

The moisture content of all the meals was low, ranging from 5.46 to 6.48 per cent, indicating adequate drying and suitability for feed formulation. MCBM had significantly higher moisture content (6.48±0.30 per cent) than PBM (5.49±0.57 per cent) and BM (5.46±0.13 per cent). Low moisture content is desirable in feed ingredients as it improves storage stability and reduces the risk of microbial spoilage.

Blood meal recorded the highest crude protein content (86.79±0.72 per cent), followed by PBM (57.51±0.28 per cent) and MCBM (57.12±0.50 per cent). The higher protein content of BM is expected, as it is a concentrated animal protein source prepared from slaughterhouse blood. Previous studies have also reported blood meal as a protein-rich ingredient suitable for livestock and aquaculture feeds (Jędrejek *et al.*, 2016; Macusi *et al.*, 2023)^[16]. The crude protein content of PBM was comparable to the range reported for good-quality poultry by-product meal (Ravindran and Blair, 1993)^[19].

Ether extract was significantly higher in PBM (25.14±0.10 per cent), followed by MCBM (5.35±0.12 per cent) and BM (0.90±0.15 per cent). The higher fat content of PBM may be due to the presence of poultry skin, abdominal fat and other lipid-rich poultry processing residues. Although this can contribute to dietary energy, higher lipid content may increase susceptibility to oxidative changes during storage.

MCBM showed the highest total ash content (30.58±0.82 per cent), followed by PBM (6.38±0.12 per cent) and BM (4.70±0.35 per cent). This may be due to the presence of bone fragments and mineral-rich components in MCBM. Therefore, while MCBM can serve as a source of protein and minerals, its high ash content should be considered while deciding its inclusion level in fish diets.

Nitrogen free extract was higher in PBM (8.42±0.11 per cent) and MCBM (7.75±0.30 per cent) than BM (6.80±0.26 per cent). Crude fibre and acid insoluble ash were comparatively higher in PBM, which may be due to variation in raw material composition and the presence of indigestible fractions in the rendered product. Overall, the results indicated that BM, PBM and MCBM possess distinct nutritional characteristics and may be used as alternative animal protein sources in aquafeed formulation when included at appropriate levels.

Table 2: Proximate composition of PBM, BM and MCBM (per cent DM)*

Parameters	PBM	BM	MCBM	F value	P value
Moisture	5.49±0.57 ^b	5.46±0.13 ^b	6.48±0.30 ^a	8.552	<0.003**
Crude protein	57.51±0.28 ^b	86.79±0.72 ^a	57.12±0.50 ^b	1011.944	<0.001**
Ether extract	25.14±0.10 ^a	0.90±0.15 ^c	5.35±0.12 ^b	9720.348	<0.001**
Total ash	6.38±0.12 ^b	4.70±0.35 ^c	30.58±0.82 ^a	761.54	<0.001**
Nitrogen free extract	8.42±0.11 ^a	6.80±0.26 ^b	7.75±0.30 ^a	11.128	<0.001**
Crude fibre	2.49±0.11 ^a	0.29±0.20 ^c	0.55±0.54 ^a	239.344	<0.001**
Acid insoluble ash	2.52±0.25 ^a	0.33±0.60 ^c	1.60±0.18 ^a	209.614	<0.001**

PBM: Poultry by-product meal; **BM:** Blood meal; **MCBM:** Meat-cum-bone meal.

*Means bearing different superscripts in the same row differ significantly. P<0.01.

Proximate composition of the developed feeds

The proximate composition of control and treatment fish feeds showed significant differences in moisture, ether extract, total ash and nitrogen free extract, while crude protein content did not differ significantly among the feeds (Table 3). The crude protein content ranged from

36.03±0.22 to 36.56±0.26 per cent, indicating successful formulation of isonitrogenous diets. This confirms that replacement of 50 per cent fish meal protein with MCBM, BM or PBM did not alter the dietary protein level. A similar approach of isonitrogenous feed formulation was reported by Kannatti *et al.* (2024)^[12] in PBM-incorporated fish feed.

Table 3: Proximate composition of control and treatment fish feeds (per cent)

Parameters	C	T1	T2	T3	F value(P value)
Moisture	5.54±0.55 ^a	5.34±0.31 ^b	5.36±0.38 ^b	5.37±0.38 ^b	3.864 <0.025**
Crude protein	36.03±0.22 ^b	36.05±0.45 ^b	36.46±0.22 ^{ab}	36.56±0.26 ^a	2.864 0.62 ^{ns}
Ether extract	6.72±0.11 ^c	6.93±0.12 ^c	6.56±0.17 ^b	7.73±0.20 ^a	66.118 <0.001**
Total ash	7.64±0.11 ^b	7.81±0.12 ^a	7.47±0.14 ^c	7.72±0.13 ^{ab}	7.602 <0.001**
Nitrogen free extract	44.27±0.33 ^{ab}	43.90±0.26 ^b	44.49±0.29 ^a	42.94±0.43 ^c	24.321 <0.001**

** - significant at 1 per cent level, ns - non significant. Mean with same superscripts in a row does not differ significantly. The values are expressed as Mean ± Standard error.

C - Control feed (Fish feed formulation as per FAO guidelines), T1 - Fish feed by replacing 50 per cent FM from C with MCBM, T2 - Fish feed by replacing 50 per cent FM from C with BM, T3 - Fish feed by replacing 50 per cent FM from C with PBM.

The moisture content was significantly higher in the control feed (5.54±0.55 per cent) than in T1, T2 and T3. However, the moisture level of all feeds was low, suggesting effective drying after extrusion and better suitability for storage.

Goddard (2012)^[6] recommended a moisture content of 8 to 12 per cent for fish feeds to maintain storage stability.

Ether extract was highest in T3 (7.73±0.20 per cent), followed by T1, C and T2. The higher fat content of T3 may be attributed to the incorporation of PBM, which contains appreciable lipid depending on the proportion of skin, fat and other poultry processing residues. Ravindran and Blair (1993)^[19] reported that good-quality PBM may contain 12 to 20 per cent ether extract.

Total ash content was highest in T1 (7.81±0.12 per cent), followed by T3, C and T2. The higher ash content in T1 may be due to the inclusion of MCBM, which contains bone-derived minerals such as calcium and phosphorus. Nitrogen free extract was highest in T2 (44.49±0.29 per cent) and lowest in T3 (42.94±0.43 per cent), probably due to differences in the adjustment of carbohydrate-rich ingredients during feed formulation.

The results indicated that 50 per cent replacement of fish meal protein with MCBM, BM or PBM produced nutritionally comparable feeds without compromising crude protein content. Among the treatments, PBM incorporation increased ether extract, while MCBM inclusion contributed to higher ash content.

Amino acid analysis

The amino acid composition of the developed control and treatment feeds is presented in Table 4. Replacement of 50 per cent fish meal protein with animal by-product meals maintained the essential amino acid profile of the feeds within a nutritionally acceptable range. Among the essential amino acids, leucine was predominant in all feeds, followed by lysine, valine and phenylalanine. Leucine content ranged from 1.73 to 1.89 per cent, with the highest value in T1, followed by T3, C and T2. Lysine and methionine, which are important for fish growth and feed efficiency, were also higher in T1 and T3 than in the control and T2 feeds. The balance and availability of essential amino acids are critical for fish growth and nutrient utilisation (Li *et al.*, 2009).

T1 showed comparatively higher levels of most essential amino acids, including lysine, methionine, leucine, isoleucine, threonine and valine. This may be due to the contribution of meat-cum-bone meal and the balancing effect of other protein ingredients used in the formulation. However, the nutritional quality of MCBM may vary depending on raw material composition, processing conditions and bone content.

T3 also showed a favourable amino acid profile, with lysine and methionine levels higher than the control feed. Poultry by-product meal has been reported to contain protein of high biological value and amino acids that can complement plant protein ingredients (Wisman *et al.*, 1958; Galkanda-Arachchige *et al.*, 2019)^[5,26]. This supports the suitability of PBM as an alternative protein source in fish feed when used in a properly balanced formulation.

T2 recorded comparatively lower values for most essential amino acids, including lysine, methionine, isoleucine and valine. Although blood meal is rich in crude protein, it may be relatively deficient in certain essential amino acids such as isoleucine and valine, which can limit its utilisation at higher inclusion levels (Kari *et al.*, 2023; Santiago *et al.*, 2024)^[13,22].

Among the non-essential amino acids, glutamic acid was the most abundant in all feeds, followed by aspartic acid. Higher glycine and proline contents in T1 may be attributed to the presence of collagenous tissue and bone-associated protein fractions in MCBM. Overall, the results indicated that incorporation of MCBM, BM and PBM did not compromise the amino acid adequacy of the developed feeds. Among the treatment feeds, T3 showed a favourable

amino acid balance, supporting the potential use of PBM as a suitable fish meal replacer in tilapia feed.

The amino acid composition of the developed control and treatment feeds is presented in Table 8.

Table 8: Amino acid composition of developed feeds (per cent of amino acids)

Amino Acid (per cent)	C	T1	T2	T3
Essential amino acids				
Arginine	1.72	1.78	1.69	1.74
Histidine	0.62	0.66	0.61	0.64
Isoleucine	0.96	1.01	0.93	0.98
Leucine	1.78	1.89	1.73	1.84
Lysine	1.62	1.75	1.58	1.69
Methionine	0.82	0.86	0.80	0.84
Phenylalanine	1.02	1.08	0.99	1.05
Threonine	0.91	0.96	0.88	0.93
Valine	1.10	1.16	1.07	1.13
Non-essential amino acids				
Alanine	1.23	1.31	1.19	1.27
Aspartic acid	2.41	2.36	2.29	2.34
Glutamic acid	4.18	4.05	4.12	4.01
Glycine	1.12	1.42	1.08	1.25
Proline	1.36	1.48	1.31	1.40
Serine	0.97	1.01	0.94	0.99
Tyrosine	0.84	0.86	0.83	0.85

C- Control feed (Fish feed formulation as per FAO guidelines), T1 - Fish feed by replacing 50 per cent FM from C with MCBM, T2 - Fish feed by replacing 50 per cent FM from C with BM, T3 - Fish feed by replacing 50 per cent FM from C with PBM.

Conclusion

The present study indicated that poultry by-product meal, blood meal and meat-cum-bone meal can be effectively used as alternative animal protein sources in tilapia feed formulation by replacing 50 per cent of fish meal protein. The developed feeds were comparable in crude protein content, confirming successful formulation of isonitrogenous diets. The amino acid profile of the feeds remained within a nutritionally acceptable range after incorporation of animal by-product meals. Among the ingredients evaluated, poultry by-product meal showed better suitability as a fish meal replacer due to its favourable nutrient composition and amino acid balance. The utilisation of rendered animal by-products in aquafeed can reduce dependence on fish meal, lower feed cost and contribute to sustainable waste management.

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