

FORMULATION AND PREPARATION OF SUPPLEMENTARY DIET FOR ORNAMENTAL FISH USING THE PEARSON'S SQUARE METHOD

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ABSTRACT

The present study was designed to formulate and prepare a nutritionally balanced supplementary diet using the Pearson's Square method with the specific objective of enhancing growth performance and reproductive output in ornamental fish. It is an established fact that feed represents both the largest recurring expense and the principal biological driver of productivity in aquaculture. Therefore, the development of a diet with a precisely defined protein concentration provides an essential foundation for controlled nutritional research. A basal diet containing about 40% crude protein was formulated using fish meal and groundnut oil cake as a protein source, rice bran with wheat flour to cope up the energy need and vegetable oil with a vitamin-mineral premix added for nutritional balance. The Pearson's Square method produced a calculated crude protein value of 39.99% in the formulated basal feed. The feed was prepared through grinding, mixing, steam cooking, pelleting and drying. Proximate analysis following AOAC procedures showed that the analyzed crude protein content (39.72%) closely matched the calculated value confirming the reliability of the formulation method. The diet also contained adequate crude lipid, crude fiber, ash, and moisture levels consistent with ornamental fish requirements. These findings indicate that the Pearson's Square method is a practical and reproducible approach for developing cost-effective and nutritionally complete supplementary feeds using locally available ingredients for ornamental fish culture.

Keywords: *Pearson's Square method, Ornamental Fish Nutrition, Feed Formulation, Supplementary Diet, Proximate Composition, Reproductive Performance*

INTRODUCTION

Feed constitutes one of the most critical determinants of aquaculture productivity, exerting a direct and measurable influence on growth performance, physiological health, and reproductive success in cultured fish species. The nutritional quality of a diet governs not only the rate at which fish gain weight but also the efficiency with which ingested nutrients are converted into body tissue, the robustness of the immune system, and the capacity of broodstock to produce viable offspring. For these reasons, the science of fish nutrition occupies a central position within the broader discipline of aquaculture research and development.

The aquafeed industry currently ranks among the fastest-growing sectors of global agriculture, and its sustained annual production growth rates reflect the rapid expansion and intensification of aquaculture worldwide. As capture fisheries approach or exceed their sustainable limits, aquaculture has assumed an increasingly prominent role in meeting the global demand for aquatic animal protein, and this transition has placed corresponding pressure on the feed sector to deliver diets that are simultaneously effective, affordable, and environmentally responsible. Because feed accounts for a substantial proportion of the total operational costs incurred by aquaculture enterprises, reported to range from 40–60% (Kumar *et al.*, 2017) to as high as 70% (Musyoka *et al.*, 2020), the development of nutritionally balanced, cost-effective, and environmentally sustainable diets represents a fundamental prerequisite for profitable and durable fish

farming (Tacon & M tiau, 2015). Advances in fish nutritional science and feed manufacturing technologies have enabled the formulation of complete, species-specific diets capable of supplementing or entirely replacing natural food resources. Such diets, in turn, improve feed utilization efficiency, growth performance, health status, and overall production sustainability across a wide range of aquaculture systems (Sathiaruban *et al.*, 2025).

Feed formulation and preparation entail the systematic combination of diverse ingredients to produce mixtures that are precisely aligned with specific production objectives. This process demands an accurate understanding of both the nutritional composition of individual feedstuffs and the physiological requirements of the target species, since even minor deviations in the balance of key nutrients can compromise growth or reproductive outcomes. Among the various techniques available for this purpose, the Pearson's Square method stands out as a straightforward and extensively validated approach for balancing dietary protein concentrations. It facilitates the rapid computation of the optimal proportions between proteinaceous and energy-rich ingredients, thereby allowing researchers to achieve a target protein level with a high degree of precision ("Fish Nutrition in Aquaculture," 1995). This method has been applied in numerous aquaculture feed formulation studies, including the preparation of isonitrogenous diets for African catfish (Emmanuel *et al.*, 2022), and it remains a favoured tool in research settings where transparency and reproducibility are paramount.

In the present study the fish diets were formulated using the Pearson's Square method and were designed to achieve a crude protein level of approximately 40%. The prepared diet was processed under standardized conditions and subsequently subjected to proximate composition analysis following established AOAC protocols (Horwitz & Latimer, 2006). By verifying that the analyzed nutrient profile corresponded closely to the calculated formulation, the study sought to confirm the reliability of the preparation procedure before its application in biological trials. The formulation of these supplementary diets therefore establishes the essential nutritional foundation upon which subsequent investigations into the growth and reproductive performance of ornamental fish can be constructed.

MATERIALS AND METHODS

Ingredient Selection and Rationale

The selection of appropriate dietary ingredients constitutes a fundamental prerequisite for formulating nutritionally balanced feeds that adequately satisfy both the macronutrient and micronutrient requirements of ornamental fishes. Because no single feedstuff can supply the full complement of nutrients required for optimal growth and reproduction, a judicious combination of animal- and plant-derived ingredients was

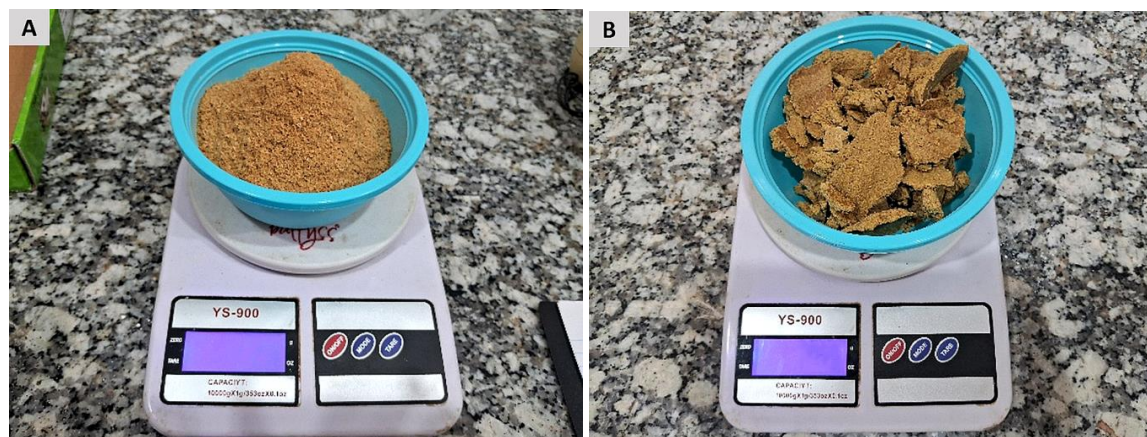


Figure 1. Protein rich ingredients used in diet formulation: (A) fish meal and (B) groundnut oil cake.

employed to meet these dietary specifications in a complementary manner. Fish meal and groundnut oil cake (GOC) were selected as the principal protein sources (Figure 1A-B), owing to their elevated protein content and favourable amino acid profiles, which together provide the essential amino acids necessary for tissue synthesis and physiological maintenance.

Rice bran and wheat flour served as the primary carbohydrate and energy substrates (Figure 2A-B) supplying readily available energy that spares dietary protein for its intended anabolic functions.

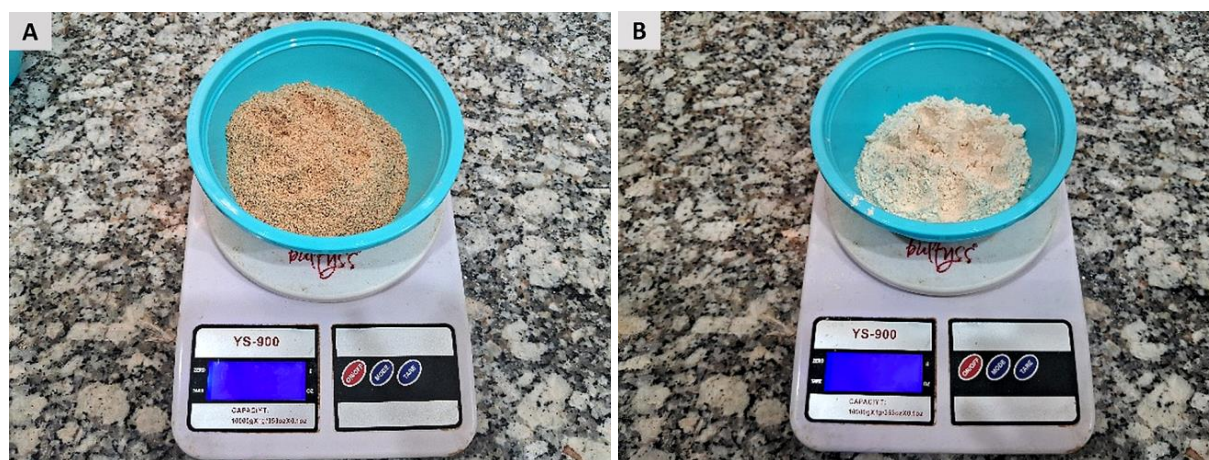


Figure 2. Energy rich ingredients used in diet formulation: (A) rice bran and (B) wheat flour.

Vegetable oil and a vitamin-mineral premix were incorporated to supply the essential fatty acids and micronutrients required for normal metabolic and physiological function (Figure 3).

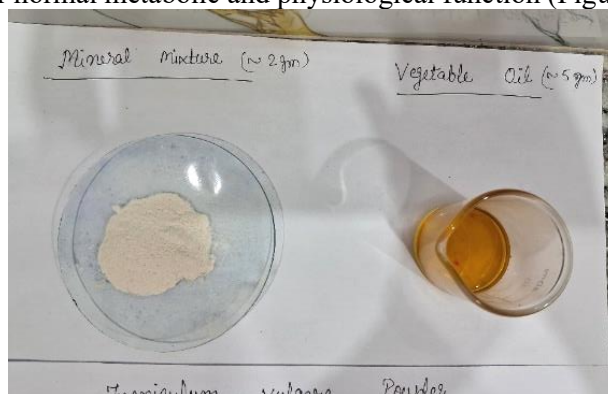


Figure 3. Supplementary ingredients used in diet formulation: vitamin-mineral premix and vegetable oil.

In addition to its nutritional contribution, wheat flour also functioned as a binder that enhanced pellet stability and water durability during feeding operations, thereby reducing nutrient leaching and feed wastage in the aquatic environment (Bhilave *et al.*, 2010; Tacon *et al.*, 2009).

Diet Formulation Using the Pearson Square Method

The supplementary diet was formulated to contain approximately 40% crude protein, a concentration deemed adequate for optimizing both growth and reproductive performance in the majority of ornamental fishes (Council, 2011; Ghosh *et al.*, 2007; Mandal *et al.*, 2011; Sathyaruban *et al.*, 2025). To achieve this target with precision, the Pearson's Square method was employed to formulate a nutritionally balanced

diet. This method offers a straightforward and reliable means of balancing dietary protein concentrations by calculating the optimal proportions of protein-rich and energy-rich ingredients relative to a defined target value (Emmanuel *et al.*, 2022; “Fish Nutrition in Aquaculture,” 1995).

For the purposes of this calculation, fish meal (60% crude protein) and groundnut oil cake (44% crude protein) were categorized as protein-rich ingredients, whereas rice bran (13% crude protein) and wheat flour (12% crude protein) constituted the energy sources. The standard crude protein values for the individual ingredients were adopted from FAO feed composition tables (Table 1) to ensure consistency with internationally recognized reference data (Hasan & New, 2014).

Preparation of Supplementary Diet

The diet was prepared from fish meal, groundnut oil cake, rice bran, wheat flour, vegetable oil, and a commercial vitamin-mineral premix. All dry ingredients were finely ground and accurately weighed according to the proportions derived from the Pearson's Square calculations and they were thoroughly homogenized in a mechanical mixer to obtain a uniform blend. Careful attention to particle size and uniformity at this stage was essential since consistent mixing directly influences both the nutritional homogeneity and the physical integrity of the final pellets.

Sufficient water was then added gradually until a homogeneous dough of appropriate consistency was achieved. All ingredients except vitamin -minerals premix were combined and kneaded into this dough to steam-cooked at 105 °C for 20 minutes in order to improve pellet binding and digestibility while simultaneously ensuring microbial safety (Figure 4).

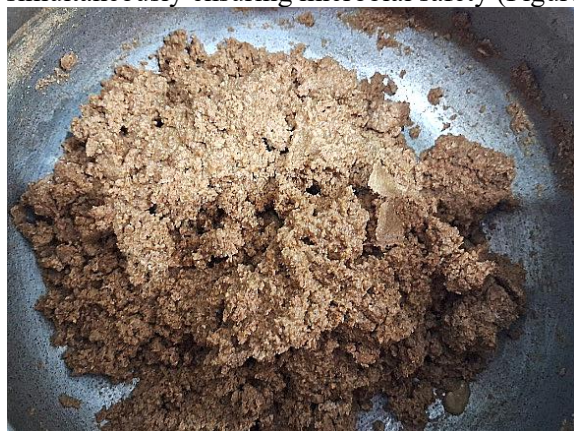


Figure 4. Steam-cooked basal diet following processing at 105 °C for 20 minutes



Figure 5. Shed drying of the prepared supplementary diet pellets

Following steaming, the vitamin-mineral premix was incorporated at approximately 1-2% of the total feed weight only after the dough had cooled, a precaution taken specifically to prevent the thermal degradation of heat-labile micronutrients that would otherwise be compromised by exposure to elevated temperatures. Pellets of 0.5 mm diameter were then produced using a hand pelletizer, air-dried for several hours (Figure 5), and finally oven-dried at 60°C until complete dehydration was achieved (Mandal *et al.*, 2011). This sequential drying regime was adopted to minimize surface cracking and to yield stable, storable pellets suitable for subsequent feeding trials.

Proximate Composition Analysis

The proximate composition of the basal formulated diet was analysed according to the standard procedures of the Association of Official Analytical Chemists (Horwitz & Latimer, 2006; Tacon *et al.*, 2009). The following nutritional parameters were determined:

- **Moisture content:** determined by oven drying at 105°C to a constant weight.

- **Crude protein:** estimated using the Kjeldahl nitrogen determination method, with the total nitrogen content converted to crude protein using the standard factor ($N \times 6.25$).
- **Crude lipid:** quantified by Soxhlet extraction using petroleum ether as the extraction solvent.
- **Crude fiber:** measured by sequential acid–alkali digestion.
- **Ash content:** determined by incineration in a muffle furnace at 550–600°C until a constant residue was obtained.

The resulting proximate analysis served to verify whether the formulated diet satisfied the desired nutritional specifications prior to the commencement of any feeding trial, thereby ensuring that the experimental feed met the intended standards before biological evaluation.

RESULTS

Pearson's Square Formulation of the Supplementary Diet

The supplementary diet was formulated to contain approximately 40% crude protein (CP) using the Pearson's Square method. Crude protein values for the individual feed ingredients were adopted from standard FAO feed composition tables (Hasan & New, 2014), and the ingredients together with their respective crude protein contents are presented in Table 1.

Table 1. Crude protein content of feed ingredients used for diet formulation

Ingredient	Crude Protein (%)
Fish meal	60
Groundnut oil cake	44
Rice bran	13
Wheat flour	12
Vegetable oil	0
Vitamin-mineral premix	0

Source: FAO feed composition tables (Hasan & New, 2014).

To simplify the formulation procedure, the ingredients were categorized into a protein-rich group and an energy-source group, and the average crude protein content of each group was calculated as follows.

Grouping of Ingredients:

- High-protein group: fish meal (60% CP) and groundnut oil cake (44% CP)
- Energy-source group: rice bran (13% CP) and wheat flour (12% CP)

Calculation of Average Crude Protein:

- High-protein group: total CP = 60 + 44 = 104%; average CP = $104 \div 2 = 52\%$
- Energy-source group: total CP = 13 + 12 = 25%; average CP = $25 \div 2 = 12.5\%$

Pearson's Square Application:

Based on a target dietary protein level of 40%, the differences between each group average and the target value were determined:

- Difference between the high-protein group and the target protein = $52 - 40 = 12$ parts
- Difference between the target protein and the energy group = $40 - 12.5 = 27.5$ parts

Proportion of Ingredients:

- Protein supplements: $27.5 \div (27.5 + 12) \times 100 = 69.62\%$
- Energy sources: $12 \div (27.5 + 12) \times 100 = 30.38\%$

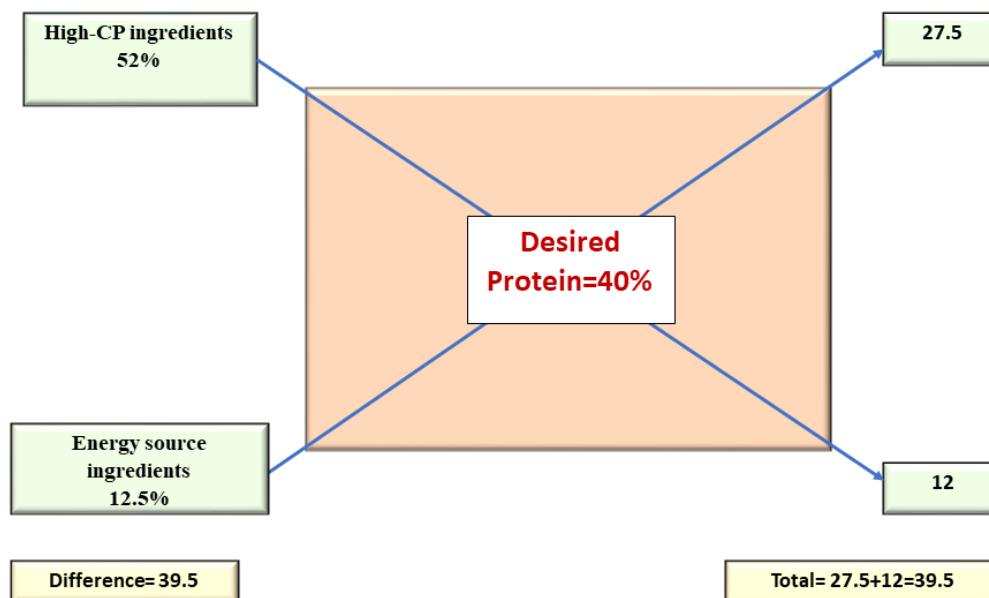
Conversion to a Quintal Basis (100 kg):

- Protein supplements = 69.62 kg (fish meal = 34.81 kg; groundnut oil cake = 34.81 kg)
- Energy sources = 30.38 kg (rice bran = 15.19 kg; wheat flour = 15.19 kg)

Calculated Crude Protein Content:

Total crude protein = $(69.62 \times 0.52) + (30.38 \times 0.125) = 39.99\%$

This value confirmed that the formulated diet closely met the targeted 40% crude protein requirement, thereby demonstrating the precision of the Pearson's Square approach in achieving a predetermined protein concentration.



Composition of the Supplementary Diet

The final ingredient composition of the supplementary diet, derived from the preceding is presented in Table 2.

Table 2. Ingredient composition of the supplementary diet (100 kg basis)

Ingredient	Composition (%)
Fish meal	34.81
Groundnut oil cake	34.81
Rice bran	15.19
Wheat flour	15.19
Total	100.00

Proximate Composition of the Basal Formulated Diet

The proximate composition of the basal formulated diet was analyzed by SSBP Bio green Analytical Research Pvt. Ltd., New Delhi, an NABL-accredited laboratory, following standard AOAC procedures (Horwitz & Latimer, 2006). The results of this analysis are summarized in Table 3.

Table 3. Proximate composition of the formulated diet

Parameter	Value (%)
Crude protein	39.72
Crude lipid	11.56
Crude fiber	10.22
Ash	10.57
Moisture	9.59

The analyzed crude protein content (39.72%) corresponded closely to the calculated protein level obtained through the Pearson's Square formulation (39.99%), thereby confirming the accuracy and reliability of the formulation procedure. The narrow margin between the calculated and analyzed values indicates minimal loss or variation during preparation and analysis. In addition, the diet contained adequate levels of lipid (11.56%), fiber (10.22%), ash (10.57%), and moisture (9.59%), all of which are consistent with the nutritional requirements reported for ornamental fishes (Sathyaruban *et al.*, 2025; Velasco-Santamaría & Corredor-Santamaría, 2011) and are therefore suitable for use in subsequent feeding experiments.

DISCUSSION

The present study successfully formulated a nutritionally balanced supplementary diet using the Pearson's Square method. The diet was deliberately designed to provide approximately 40% crude protein, a level widely considered adequate for supporting optimal growth and reproductive performance in ornamental fishes. Laboratory proximate analysis subsequently confirmed that the analyzed crude protein content (39.72%) closely corresponded to the calculated value (39.99%), indicating a high degree of accuracy and reliability in the formulation procedure. This close agreement demonstrates that the Pearson's Square method remains an effective and practical approach for preparing experimental aquafeeds with predetermined protein levels, a conclusion consistent with its long-established utility in aquaculture research (Emmanuel *et al.*, 2022; “Fish Nutrition in Aquaculture,” 1995).

Feed formulation represents one of the most critical aspects of aquaculture, since dietary nutrients directly influence fish metabolism, growth, feed conversion efficiency, immune competence, reproductive performance, and overall health. Among all dietary nutrients, protein is most important for tissue synthesis and the most expensive component of a formulated feed. Consequently, the accurate balancing of dietary protein is essential not only for achieving optimal biological performance but also for improving economic efficiency and for minimizing the quantity of nitrogenous waste released into the culture environment (Kumar *et al.*, 2017; Tacon & Météan, 2015). Previous studies have consistently demonstrated that inadequate dietary protein compromises growth, delays sexual maturation, reduces fecundity, and impairs larval quality (Council, 2011; Mandal *et al.*, 2011). For example, Ghosh *et al.* (2007) reported that nutritional interventions significantly influenced the gonadosomatic index, fecundity, and fry production in livebearing ornamental fishes, thereby underscoring the importance of adequate dietary protein for reproductive success. In a similar vein, Mandal *et al.* (2011) demonstrated that formulated feeds containing appropriate protein levels could partially replace live feeds without compromising reproductive performance or fry survival in *Betta splendens*, highlighting the practical viability of well-balanced prepared diets.

The present investigation employed the Pearson's Square method to formulate the supplementary diet on account of its simplicity, accuracy, and widespread application in experimental feed preparation. Although modern feed industries frequently utilize computer-assisted least cost formulation software capable of optimizing complex multi-ingredient blends (Mallick *et al.*, 2020), the Pearson's Square method continues to be extensively adopted in aquaculture research laboratories for balancing protein concentrations during experimental diet development, largely because of its transparency and ease of verification (“Fish Nutrition in Aquaculture,” 1995). Reinforcing this point, Akinloye Emmanuel *et al.* (2022) similarly employed this approach to formulate isonitrogenous diets containing 40% crude protein for African catfish, further validating its applicability across diverse aquaculture contexts and species.

Fish meal and groundnut oil cake were selected as the principal protein sources because of their complementary nutritional characteristics. Fish meal is widely regarded as the benchmark protein ingredient in aquafeeds, owing to its high digestibility, balanced amino acid profile, excellent palatability, and abundance of essential fatty acids and micronutrients (Gatlin *et al.*, 2007; Hardy, 2010). However, the

increasing cost and limited availability of fish meal have encouraged researchers to identify sustainable alternative protein sources capable of supplementing or partially replacing it (Francis *et al.*, 2001; Gatlin *et al.*, 2007; Hardy, 2010). Plant proteins constitute the most common fish meal replacements in aquafeeds because of their cost-effectiveness and broad availability, and the protein digestibility of properly processed plant-based feedstuffs typically ranges from 75 to 95% (Gatlin *et al.*, 2007). Within this framework, groundnut oil cake provides a relatively inexpensive plant protein source with good digestibility and an acceptable amino acid composition. The combination of an animal-derived protein with a complementary plant-derived protein therefore offered a balanced, palatable, and economically viable protein base for the formulated diet, uniting the nutritional strengths of both source categories.

Similarly, wheat flour and rice bran were incorporated as carbohydrate and energy sources in order to spare dietary protein for tissue synthesis rather than for energy production. This protein sparing strategy is particularly appropriate for the target species, since warm-water fish are able to utilize relatively high carbohydrate levels as an energy source (Ganguly *et al.*, 2014). Adequate carbohydrate supplementation additionally enhances feed pellet stability, improves feed manufacturing characteristics, and reduces the utilization of dietary protein as an energy source, thereby improving protein efficiency ratios and overall feed economy (Council, 2011). Beyond its carbohydrate contribution, vegetable oil supplied the essential fatty acids necessary for membrane integrity, hormone synthesis, and a range of normal physiological functions. The importance of essential fatty acids for reproductive performance has been well documented, as freshwater fish require the dietary supplementation of linoleic and linolenic acids that they are unable to synthesize *de novo* (Mandal *et al.*, 2011). Finally, the vitamin-mineral premix ensured sufficient micronutrient availability to support enzymatic reactions, metabolic regulation, and antioxidant defense, all of which are indispensable for sustained health and productivity.

The proximate composition of the formulated diet revealed crude lipid (11.56%), crude fiber (10.22%), ash (10.57%), and moisture (9.59%) levels that fall comfortably within the acceptable ranges reported for ornamental fish feeds (Sathyaruban *et al.*, 2025; Velasco-Santamaría & Corredor-Santamaría, 2011). Moreover, the nutritional profile obtained in the present study is comparable to that of formulated feeds employed in previous ornamental fish investigations. For instance, (Ganguly *et al.*, 2014) formulated iso-nitrogenous diets with protein fixed at 400 g/kg for *Poecilia sphenops*, while Kari *et al.* (2023) prepared formulated diets for *Betta splendens* and assessed their feed stability, growth performance, and physiological effects. The consistency of the present results with these established studies further supports the validity of the formulation approach adopted here and situates the current diet firmly within the range of nutritionally acceptable experimental feeds.

The practical significance of this work lies in its demonstration that nutritionally adequate feeds can be prepared from locally available, cost-effective ingredients without recourse to expensive live feeds or specialized additives. This finding is particularly relevant given that feed costs constitute the largest single proportion of operational expenditure in aquaculture (Kumar *et al.*, 2017; Musyoka *et al.*, 2020), and given that the ornamental fish industry additionally requires feeds capable of simultaneously supporting growth, reproduction, and aesthetic qualities such as pigmentation (Veeruraj *et al.*, 2023; Velasco-Santamaría & Corredor-Santamaría, 2011). By showing that a locally sourced, standardized diet can meet stringent nutritional specifications, the present study offers a replicable model that may reduce production costs while maintaining the biological performance required for successful ornamental fish culture.

CONCLUSION

In this study, we successfully created a nutritionally balanced supplementary diet using the Pearson's Square method. The formulation process effectively reached the desired dietary crude protein level of approximately 40%, which is considered adequate for meeting the nutritional needs of ornamental fish

(Council, 2011; Sathyaruban *et al.*, 2025; Velasco-Santamaría & Corredor-Santamaría, 2011). The close alignment between the calculated crude protein content (39.99%) and the laboratory-measured value (39.72%) confirmed the precision and reliability of the formulation method, highlighting the continued importance of this traditional approach in modern experimental aquaculture. By using fish meal and groundnut oil cake as the main protein sources, along with rice bran and wheat flour for energy, we established a balanced and cost-effective nutritional base for the supplementary diet. The proximate composition analysis showed that the formulated diet met the desired nutritional criteria across all measured parameters, indicating that the experimental feeds are suitable for assessing the biological impacts of dietary supplementation on the growth and reproductive performance of ornamental fish. Additionally, the formulation protocol developed in this study could serve as a practical guide for future research on feed additives and functional ingredients, potentially contributing to the ongoing development of cost-effective, sustainable, and functional diets for ornamental fish culture.

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