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Preliminary formulation of a Nile tilapia (*Oreochromis niloticus*) feed with alternative ingredients and local by-products**Formulación preliminar de un alimento para tilapia del nilo (*Oreochromis niloticus*) con ingredientes alternativos y subproductos locales**¹Lenin Vicente Loayza Peñaranda  , ²María José Farías González  , ³Ledi Vergara Miranda  .¹Technical University of Machala, Faculty of Agricultural Sciences, Machala, Ecuador²Technical University of Machala, Faculty of Agricultural Sciences, Machala, Ecuador³Technical University of Machala, Faculty of Agricultural Sciences, Machala, Ecuador***Corresponding author:** Lenin Vicente Loayza Peñaranda 

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ABSTRACT

Aquafeed formulation with alternative raw materials requires distinguishing between theoretical estimates and experimentally determined composition. The objective was to preliminarily formulate and produce a feed prototype for Nile tilapia (*Oreochromis niloticus*) using alternative ingredients and local by-products, and to mathematically estimate its protein contribution. An applied, exploratory-descriptive study was conducted. The formulation included a fish-viscera-derived ingredient, soybean meal, duckweed (*Lemna minor*), rice, coffee residues, sunflower oil, and salt. The viscera were washed, ground, heat-treated, and acidified with acetic acid. The final mixture had a mass of 2462 g. Protein contribution was calculated using theoretical nutritional coefficients and reached 30.03%. This percentage was not determined through proximate analysis and depends on the coefficients adopted, some of which lack specific verification for the batch used. The prototype could be mixed, shaped, and dried using low-complexity operations. Digestibility, proximate composition, water stability, productive performance, cost per kilogram, and environmental impact were not evaluated. In conclusion, a technically producible preliminary formulation was obtained that was mathematically close to the protein target; its nutritional and productive adequacy requires experimental validation.

Keywords: aquaculture; feed formulation; alternative ingredients; duckweed; by-products; Nile tilapia**RESUMEN**

La formulación de piensos acuacuícos con materias primas alternativas requiere una clara distinción entre estimaciones teóricas y composición determinada experimentalmente. Este estudio tenía como objetivo formular preliminarmente y producir un prototipo de alimento para la tilapia del Nilo (*Oreochromis niloticus*) utilizando ingredientes alternativos y subproductos locales, y estimar matemáticamente su contribución proteica. Se llevó a cabo un estudio aplicado y exploratorio-descriptivo. La formulación incluía un ingrediente derivado de vísceras de pescado, harina de soja, lenteja de agua (*Lemna minor*), arroz, residuos de café, aceite de girasol y sal. Las vísceras de pescado fueron lavadas, molidas, tratadas térmicamente y acidificadas con ácido acético. La mezcla final tenía una masa total de 2462 g. La contribución proteica se calculó usando

coeficientes nutricionales teóricos y alcanzó el 30,03%. Este porcentaje no se determinó mediante análisis aproximado y depende de los coeficientes adoptados, algunos de los cuales carecen de verificación específica para lotes. El prototipo podía mezclarse, moldearse y secarse mediante operaciones de baja complejidad bajo las condiciones de laboratorio documentadas. No se evaluaron la digestibilidad, composición próxima, estabilidad del agua, rendimiento productivo, coste por kilogramo ni impacto ambiental. En conclusión, se obtuvo una formulación preliminar técnicamente producible con una contribución proteica matemáticamente estimada cercana al objetivo; su adecuación nutricional y rendimiento productivo requieren validación experimental.

Palabras clave: ingredientes alternativos; pienso para acuicultura; subproductos; lentilla de agua; tilapia del Nilo; proteína teórica

INTRODUCTION

Aquaculture is a strategic activity for the global supply of aquatic foods and for diversifying protein sources intended for human consumption. FAO emphasizes that sustained growth of the sector requires simultaneous improvements in productive efficiency, resource use, and the sustainability of farming systems (1). In this context, Nile tilapia (*Oreochromis niloticus*) holds a relevant position because of its adaptability, commercial acceptance, and widespread production. Its expansion increases the need for technically formulated feeds that respond to the nutritional requirements of the species and to specific production conditions.

Feed is one of the decisive components of tilapia farming because it affects growth, feed-conversion efficiency, and a substantial share of operating costs. Pressure on conventional marine-origin ingredients has encouraged the search for plant sources and by-products that can be incorporated into diets without compromising nutritional quality (2,3). Nevertheless, selecting alternative raw materials requires assessment of chemical composition, digestibility, amino-acid profile, energy, safety, and potential antinutritional factors. For this reason, a calculated protein percentage is only an initial criterion and cannot, by itself, be interpreted as evidence of the nutritional adequacy of the feed (4).

Raw materials with potential for aquafeed formulations include soybean meal, duckweed (*Lemna minor*), fishery by-products, and certain agro-industrial residues. These sources offer advantages related to availability or material valorization, but they also vary according to origin, moisture, processing, and storage conditions. Specialized literature warns that the actual usefulness of an ingredient depends on its characterization and on the biological response obtained within a complete diet (5). Consequently, using nutritional coefficients from bibliographic references makes it possible to construct preliminary estimates, although such values must be distinguished from results obtained through proximate analysis of the batch used.

In small-scale experimental contexts, preliminary formulation can be a useful stage for establishing proportions, documenting processing operations, and determining whether a mixture can mathematically approach a protein target. However, a methodological problem arises when the theoretical estimate is presented as though it represented the actual composition of the product. The requirements of *O. niloticus* vary according to size, physiological stage, culture system, and ingredient quality; therefore, an isolated percentage does not demonstrate nutritional sufficiency (6). This

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distinction is especially important when raw materials lack specific characterization and the process does not yet include digestibility assessment, bioassays, or physicochemical evaluation of the feed.

The problem formulation focuses on the limited evidence available to determine whether a local combination of alternative ingredients and by-products can be transformed, through low-complexity operations, into a reproducible prototype whose theoretical protein contribution approaches 30%, without confusing that estimate with experimentally measured crude protein. The difficulty involves both the physical feasibility of production and the consistency of the protein balance. Based on this delimitation, the research question is posed: is it feasible to preliminarily formulate and produce a Nile tilapia feed prototype with the selected ingredients, achieving a theoretical protein contribution close to 30% under the documented conditions?

As a provisional response to the problem, the following working hypothesis is established: the combination of a fish-viscera-derived ingredient, soybean meal, duckweed, rice, coffee residues, sunflower oil, and salt makes it possible to preliminarily formulate and produce a Nile tilapia prototype whose theoretical protein contribution approaches 30%. This hypothesis is restricted to the mathematical balance and the observed possibility of production; it does not imply that the product would reach that percentage in a proximate analysis or guarantee digestibility, growth, feed conversion, or sustainability. At this stage, hypothesis testing is limited to the available formulation, mass, and processing records.

In accordance with the question and hypothesis, the general objective was to preliminarily formulate and produce a feed prototype for Nile tilapia (*Oreochromis niloticus*) using alternative ingredients and local by-products, and to mathematically estimate its theoretical protein contribution. The study was limited to formulation design, documented treatment of raw materials, prototype production, mass balance, and qualitative observation of the process. No proximate analyses, digestibility trials, controlled feeding tests, economic estimates, or environmental assessments were performed. This delimitation makes it possible to interpret the results within their actual scope and avoids attributing nutritional or productive properties to the prototype that still require independent experimental validation.

LITERATURE REVIEW

- Nutritional requirements of tilapia and formulation criteria

Diet formulation for Nile tilapia must begin with the animal's requirements rather than with an isolated protein value. Glencross notes that the usefulness of an ingredient depends on its characterization, digestibility, and biological response, because two raw materials with similar protein percentages may differ substantially in nutrient availability (5). This distinction is central to preliminary studies: a mathematical balance can approximate a target composition, but by itself it does not demonstrate that nutrients are available to the fish or that the diet supports efficient growth.

The protein requirements of *O. niloticus* vary with developmental stage, size, culture conditions, and dietary energy density. Torres-Novoa and Hurtado-Nery synthesize this variability and show that there

is no single percentage applicable to all production phases (6). Therefore, using approximately 30% protein as a formulation target should be understood as an operational reference rather than proof of comprehensive nutritional adequacy. A complete evaluation requires consideration of essential amino acids, digestible energy, lipids, minerals, vitamins, and nutrient relationships.

- Soybean meal as a plant protein source

Soybean meal is one of the most widely used plant sources in aquafeeds because of its availability and protein concentration. In tilapia, Llanes and Toledo observed that high inclusion levels can be used, although performance depends on the overall balance of the diet and the degree of substitution (7). Its use requires attention to amino-acid profile, energy, and the presence of antinutritional factors. Consequently, assigning a protein coefficient to soybean is useful for a preliminary calculation, but the actual value of the batch and its effective nutritional contribution must be verified when the feed is to be validated.

- Duckweed (*Lemna minor*): composition and variability

Duckweed has received attention as a rapidly growing biomass with potential for animal feeding. A recent review describes its nutritional and environmental interest but also emphasizes that its composition changes according to cultivation, management, and processing conditions (8). This variability limits the direct transfer of a protein percentage from the literature to a specific batch. In a preliminary formulation, published information can serve as a reference for constructing the balance, provided that the result is explicitly presented as an estimate rather than as proximate composition determined in the laboratory.

Opiyo et al. found variations in the nutritional composition of *L. minor* grown under different fertilization schemes, confirming that the production environment influences the resulting biomass (9). Chakrabarti et al. also characterized amino-acid and fatty-acid profiles, showing that evaluation of this macrophyte should not be restricted to crude protein (10). From a historical perspective, Leng et al. had already proposed duckweed as a protein resource for animals and fish, although its use requires adaptation to production conditions and the target species (11).

Experimental evidence in tilapia provides an additional criterion. Opiyo et al. evaluated partial replacement of fishmeal with *L. minor* and observed growth and body-composition responses under controlled conditions (12). These results support the relevance of investigating the macrophyte, but they do not justify automatically extrapolating those findings to any formulation. Inclusion level, processing, moisture, composition of the rest of the diet, and biomass quality are variables that must be considered before attributing a specific nutritional effect to the ingredient.

- Alternative ingredients and replacement of conventional sources

Replacing fishmeal with plant proteins is part of a broad trend in aquaculture. Hussain et al. review several plant sources and emphasize that their potential depends on digestibility, amino-acid composition, antinutritional factors, and processing (13). Complementarily, Serra et al. indicate that selection of alternative proteins should integrate nutritional, technological, and availability criteria,

avoiding the assumption that one raw material is equivalent to another solely because of its protein concentration (14). These considerations support a cautious approach to prototypes produced from mixtures of heterogeneous ingredients.

- Fishery by-products, acidification, and silage

Fish-processing residues, including viscera, contain usable protein and lipid fractions, but their high moisture content promotes deterioration and requires stabilization measures. Suárez et al. studied tilapia-viscera silages and showed that processing modifies the characteristics of the final material (15). Therefore, a protein coefficient obtained from one processed material should not be transferred without verification to another product obtained under different conditions. Temperature, time, acid concentration, moisture, and possible defatting can modify composition and stability.

Recent reviews of fish-waste silage emphasize the importance of pH control, microbiological stability, and process standardization to obtain reproducible raw materials (16). In the type of formulation considered here, preservation acidification may contribute to by-product handling, but it should not automatically be equated with validated silage if critical parameters are not documented. This methodological distinction is relevant because the quality of the viscera-derived ingredient substantially affects any subsequent estimate of the protein content of the mixture.

- Coffee residues and valorization of agro-industrial by-products

Coffee residues are an agro-industrial by-product whose incorporation into feeds requires specific characterization. Phinrub et al. evaluated spent coffee grounds as a partial substitute for rice bran in tilapia diets and showed that their use can be studied at defined inclusion levels (17). However, that precedent does not establish a universal protein content for all coffee residues. Bean origin, extraction process, moisture, fiber, and other components may vary; therefore, the coefficient used in a formulation should be supported by analysis or documentation of the specific material.

- Recent evidence on unconventional ingredients in tilapia

A systematic review of unconventional ingredients for tilapia shows the diversity of raw materials evaluated and confirms that the response depends on the nature of the ingredient, its processing, and the inclusion level (18). In parallel, recent studies on tilapia protein requirements emphasize that needs change between production and reproductive phases, reinforcing the need to contextualize any percentage protein target (19). Taken together, this evidence supports using protein calculation as a design stage, but not as a substitute for chemical and biological validation.

Fishmeal-free formulation has also been investigated in fattening tilapia, showing that protein level must be evaluated within complete diets and under defined production conditions (20). Other studies have explored partial substitutions using black soldier fly larvae and freshwater shrimp in smallholder systems, expanding the range of available sources (21). These studies illustrate that ingredient innovation can respond to local contexts, although its relevance must be demonstrated through measurements of growth, feed conversion, survival, and nutritional quality.

The search for substitutes also includes leaf protein concentrates. Ayyat et al. evaluated concentrates derived from sugar beet and carrot as novel fishmeal replacements in juvenile tilapia (22). Amer et al. studied moringa protein hydrolysate as a substitute in *O. niloticus* diets, incorporating productive indicators to assess its response (23). These precedents show that the development of alternative ingredients is moving toward highly diverse materials, but useful evidence is obtained when ingredient characterization is linked to a defined formulation and a controlled biological evaluation.

Fermentation processes have also been investigated to improve plant raw materials. Van Doan et al. evaluated partial replacement of soybean meal with fermented corn husk in tilapia reared in a biofloc system, linking the ingredient to growth and physiological responses (24). Fermentation illustrates how processing can modify the functional value of a by-product and, therefore, why nutritional coefficients should not be considered invariant properties. For a preliminary prototype, traceability of the material and of the treatment applied is essential for interpreting any subsequent calculation.

- Synthesis of the basis for preliminary formulation

The reviewed evidence converges on three criteria. First, calculated protein is only one dimension of nutritional quality and must be distinguished from analytically determined protein. Second, alternative raw materials and by-products show variability associated with origin, moisture, processing, and batch. Third, the feasibility of mixing and shaping a prototype does not demonstrate digestibility, physical stability, productive efficiency, cost reduction, or environmental sustainability. Consequently, the value of the present approach lies in reproducibly documenting an initial formulation and production stage, while explicitly maintaining the limits of the theoretical estimate and defining the validations required before considering the product a nutritionally verified feed.

METHOD

Research approach, scope, and design

The study was conducted using a quantitative approach because the formulation was based on mass measurements, mixture proportions, material balances, and mathematical estimates of protein contribution. In terms of purpose, it was applied research with an exploratory-descriptive scope, oriented toward the preliminary development of a product.

An applied, exploratory-descriptive study of preliminary formulation and production of a prototype was conducted in the Chemistry Laboratory of the Faculty of Agricultural Sciences at the Technical University of Machala, within the Biochemistry course of the Aquaculture program. No treatments, control group, experimental replicates, or comparisons among formulations were established, and no bioassays with fish were performed.

Raw materials and materials

A fish-viscera-derived ingredient, soybean meal, duckweed (*Lemna minor*), rice, coffee residues, sunflower oil, and salt were used. Acetic acid at 95% was used to treat the viscera. Source records do not specify the species or origin of the viscera, the origin of the coffee residues, the condition of the duckweed, the commercial presentation of the soybean, the exact type of rice, or, for all ingredients,

whether the mass corresponded to wet, dry, or conditioned material. These variables were neither reconstructed nor assumed because they are not documented in the available material.

Selection, traceability, and theoretical characterization of ingredients

Nutritional coefficients were retained as operational coefficients of the formulation and compared with the literature when a relevant precedent existed. No proximate analyses of the batch were performed. The 8% value for coffee residues was used in the final calculation because it is the coefficient recorded in the definitive formulation; the preliminary value of 12% was discarded to avoid using two coefficients simultaneously. This 8% value has no batch-specific analytical verification. Likewise, the exact type of rice and its 8% coefficient could not be verified with the available documentation (Table 1).

Table 1. Nutritional values adopted for the formulation and level of data support

Ingredient	Protein used (%)	Data basis
Fish-viscera-derived ingredient	55	Operational coefficient from the original formulation; not analytically verified for the batch. Suárez et al. (15) studied different processed materials.
Soybean meal	44	Operational formulation coefficient; compatible with reference values but not measured in the batch (3,4).
Duckweed (<i>Lemna minor</i>)	30	Theoretical coefficient based on literature on dry <i>L. minor</i> biomass; not measured in the batch (8-10).
Coffee residues	8	Coefficient used in the definitive formulation. The preliminary value of 12% was discarded; the 8% value was not analytically verified for the batch (17).
Sunflower oil and salt	0	No protein contribution considered in the balance.
Rice	8	Inherited operational coefficient; the exact type of rice is not documented and the value was not verified for the batch.

The values in Table 1 show that the formulation was constructed using operational coefficients with different levels of supporting evidence. Soybean meal and duckweed have compatible references, whereas the viscera-derived ingredient, rice, and coffee residues present greater uncertainty for the batch used. Therefore, the resulting protein estimate should be interpreted as a mathematical

approximation requiring confirmation through batch-specific proximate characterization of the raw materials.

Source: Authors' own elaboration

Note: The percentages correspond to coefficients used to estimate protein contribution and not to results of proximate analysis of the experimental batch. The final estimate depends especially on the coefficients assigned to the viscera-derived ingredient and coffee residues.

Preliminary feed formulation

The initial formulation assigned 20% soybean meal, 26% viscera-derived ingredient, 3% coffee residues, 6% salt, 18% rice, 8% sunflower oil, and 19% duckweed. For each ingredient, the theoretical protein contribution was calculated by multiplying its inclusion proportion by the adopted protein coefficient and dividing by 100. The sum of the individual contributions provided the total estimated protein content of the mixture.

Ingredient protein contribution (%) = [Ingredient inclusion (%) × Adopted protein coefficient (%)] / 100

Using the coefficients employed in the original formulation, an initial protein estimate of 30.6% was obtained. This value was used only as an adjustment tool and not as a result of proximate composition.

Processing of fish viscera

The viscera were washed, cut into pieces, and ground. Heat was subsequently applied to reduce moisture before acidification. The source documentation does not record maximum temperature, heating duration, or an instrumental endpoint criterion; therefore, these parameters were not invented and constitute a limitation to the reproducibility of the procedure.

Acetic acid treatment

For 640 g of viscera, the addition of acetic acid was established at approximately 4% of the raw-material mass.

Acid mass = 640 g × 4/100 = 25.6 g

$V = m/\rho = 25.6 \text{ g} / 1.05 \text{ g}\cdot\text{mL}^{-1} = 24.38 \text{ mL}$

Approximately 24 mL of 95% acetic acid was used, added gradually with continuous mixing for 5–10 min. The treated material was kept in a closed plastic container protected from light until incorporation into the mixture. Available records do not state the exact time elapsed between acidification and incorporation or a final pH value. Consequently, the procedure is described as preservation acidification and not as standardized silage equivalent to published protocols (15,16).

Reformulation and definitive formulation

After processing, 640 g of the viscera-derived ingredient was available. Initial masses were obtained by applying the percentage proportions to the available reference mass. The first reformulation

retained 640 g of this ingredient and scaled the remaining components, reaching 2461.54 g and 30.66% theoretical protein. The final adjustment was iterative: soybean meal, with a higher protein coefficient, was reduced and rice, with a lower coefficient, was increased, while practically maintaining total mass until the calculation approached the nominal 30% target. No optimization algorithm or additional nutritional constraints were used.

The definitive formulation consisted of 640 g of fish-viscera-derived ingredient, 450 g of soybean meal, 443 g of duckweed, 510 g of rice, 197 g of coffee residues, 148 g of sunflower oil, and 74 g of salt, for a total mass of 2462 g.

Mixing, shaping, drying, and storage

The solid ingredients were mixed manually; salt and then sunflower oil were added gradually. The mixture was manually shaped into pellets or granules and subjected to solar drying for three days. Source records do not document temperature, relative humidity, irradiance, exact location, contamination-protection system, or an instrumental criterion for determining the end of drying. After drying, additional grinding was performed to reduce lumps, followed by manual homogenization and storage in a clean plastic bag in a dry, cool place protected from light.

Calculation of theoretical protein content

The protein content of the formulation was mathematically estimated from the mass of each ingredient and the adopted protein coefficient. The calculation does not correspond to a proximate determination of the final product.

Theoretical protein (%) = $[\Sigma(\text{mass of each ingredient} \times \text{adopted protein coefficient} / 100) / \text{total mass of the mixture}] \times 100$

Data recording and processing

Document review, material balance, percentage calculation, and direct observation were used. Information was recorded in formulation tables and through photographic evidence. No statistical technique was applied because no independent treatments or production replicates were developed.

Methodological limitations

The main methodological limitations were the absence of proximate analysis of the batch, lack of independent replicates, incomplete traceability of some raw materials and processing conditions, and the non-determination of moisture, lipids, ash, fiber, energy, digestibility, water stability, floatability, hardness, durability, cost per kilogram, and environmental impact. These limitations restrict interpretation to a preliminary formulation and a theoretical protein calculation.

RESULTS

Initial formulation and reformulation

The preliminary formulation produced a protein estimate of 30.6% based on the adopted coefficients. The final availability of 640 g of the viscera-derived ingredient required recalculation of the mixture. The first reformulation reached 2461.54 g and an estimated theoretical protein content of 30.66%.

The final adjustment reduced soybean meal from 492.31 to 450 g and increased rice from 467.69 to 510 g.

Composition of the definitive formulation

The definitive formulation reached a total mass of 2462 g. With the adopted operational coefficients, the sum of the estimated theoretical protein contribution was 739.46 g and the estimated theoretical protein content was 30.03% (Table 2). This result is mathematical and does not correspond to experimentally determined crude protein.

Table 2. Composition of the definitive formulation and estimated theoretical protein contribution

Ingredient	Amount (g)	Inclusion (%)	Protein coefficient (%)	Protein contribution (g)
Fish-viscera-derived ingredient	640	26.00	55	352.00
Soybean meal	450	18.28	44	198.00
Duckweed (Lemna minor)	443	17.99	30	132.90
Rice	510	20.71	8	40.80
Coffee residues	197	8.00	8	15.76
Sunflower oil	148	6.01	0	0.00
Salt	74	3.01	0	0.00
Total	2462	100.00	—	739.46

Table 2 shows that the viscera-derived ingredient provides the largest theoretical amount of protein, followed by soybean meal and duckweed. Together, these components account for most of the estimated 739.46 g in the mixture. The final value of 30.03% approaches the formulation target, but its accuracy depends directly on the adopted coefficients; therefore, it does not represent an experimental measurement of crude protein in the prototype.

Source: Authors' own elaboration

Note: The 30.03% estimate is conditional on the adopted coefficients and is not equivalent to analytically determined crude protein. The coefficients for viscera, rice, and coffee residues require batch-specific confirmation.

Theoretical protein (%) = $(739.46 \text{ g} / 2462 \text{ g}) \times 100 = 30.03\%$

Observed physical characteristics

During production, a moldable mass, the possibility of manual shaping, visible moisture loss during drying, and subsequent presence of lumps that required additional grinding were qualitatively recorded. These observations describe the process but do not validate physical properties of the feed because moisture, particle size, hardness, floatability, water stability, and durability were not measured (Table 3).

Table 3. Physical observations of the production process

Stage	Recorded observation	Action applied	Qualitative result
Mixing and kneading	Progressive integration of solids and lipid phase.	Manual mixing and kneading.	Integrated and moldable mass.
Shaping	Sufficient consistency for molding.	Manual formation of pellets or granules.	Shaped product.
Drying	Visible moisture loss after three days.	Direct solar drying.	Apparently drier product.
Post-drying	Lumps and visible size differences.	Additional crushing or grinding.	Reduction of lumps.
Final homogenization	Need to redistribute components.	Subsequent manual mixing.	More homogeneous appearance.

The observations in Table 3 confirm that it was possible to manually integrate, shape, dry, and homogenize the prototype under the recorded conditions. The appearance of lumps after drying made additional grinding necessary, evidencing visible variations in product structure. These results describe small-scale operational feasibility but do not allow inference of the physical quality of the feed because residual moisture, hardness, particle size, floatability, water stability, and durability were not measured.

Source: Authors' own elaboration

Note: The observations were qualitative. Residual moisture, particle size, hardness, floatability, water stability, and durability were not instrumentally determined.

Observed production evidence

The evidence obtained was limited to facts directly recorded during formulation and production. Economic viability, environmental sustainability, and productive efficiency are not presented as results because these dimensions were not quantified.

Table 4. Directly observed evidence during formulation and production of the prototype

Dimension	Directly observed evidence
Technical	2462 g of prototype were produced using basic equipment.
Nutritional	An estimated theoretical protein contribution of 30.03% was calculated using operational coefficients.
Physical	Manual shaping was achieved; after drying, lumps were observed and additional grinding was applied.
Raw materials	Fish viscera, duckweed, and coffee residues were incorporated together with other ingredients.
Process	Mixing, shaping, solar drying for three days, grinding, and homogenization were recorded.

Dimension	Directly observed evidence
Productive	No fish bioassay was conducted and productive performance was not measured.

Table 4 clearly defines the scope of the evidence obtained: the produced mass, theoretical protein calculation, manual shaping, and main processing stages were documented. The absence of bioassays prevents linking this evidence to fish growth, feed conversion, or survival. Likewise, the use of alternative raw materials only demonstrates their incorporation into the prototype; it does not support claims of economic or environmental advantages without specific comparative measurements.

Source: Authors’ own elaboration

Note: The table presents only recorded evidence; interpretations, validation needs, and perspectives are developed in the Discussion.

DISCUSSION

Interpretation of theoretical protein contribution

The main quantitative finding was a formulation whose protein estimate reached 30.03%. However, the traceability review shows that this result depends on coefficients that were not measured in the raw materials of the batch. This distinction is fundamental: a mathematically balanced formulation does not guarantee that the actual composition matches the estimate. The approach proposed by Glencross specifically emphasizes that ingredient assessment should be based on analytical characterization, digestibility, and biological response (5).

The 55% coefficient assigned to the viscera-derived ingredient is the main source of uncertainty. Suárez et al. reported very different compositions for fresh viscera, chemical silage, and defatted chemical silage obtained through procedures different from that used in this study (15). Therefore, neither those values nor the 55% coefficient can be automatically transferred to the current product. Recent literature on fish-waste silage confirms that final composition depends on treatment, moisture, and storage conditions (16).

Soybean meal and duckweed

Soybean meal represented 18.28% of the final mixture. Although the present study did not evaluate biological performance, the findings of Llanes and Toledo demonstrate that *O. niloticus* can use diets with high levels of soybean meal when the overall formulation meets its nutritional needs (7). This supports its inclusion as a protein source but does not by itself validate the protein coefficient of the batch used.

Duckweed represented 17.99% of the formulation. The 30% coefficient used is plausible compared with dry-matter protein values reported by Opiyo et al. and with the characterization by Chakrabarti et al. (9,10). Nevertheless, the compositional variability described by Jaimes Prada et al. and Opiyo et al. requires consideration of cultivation conditions, moisture, and biomass processing (8,9). In addition, tilapia feeding studies show that response depends on inclusion level and dietary balance; therefore, proximate composition must be complemented by bioassays (12).

Coffee residues and ingredient traceability

In the preliminary records for coffee residues, a coefficient of 12% appeared, whereas the definitive formulation used 8%. To maintain a single reproducible calculation, this manuscript adopts 8% exclusively as the operational coefficient of the final formulation. This value is not interpreted as the analytical composition of the residue used. Phinrub et al. support the relevance of investigating coffee residues in tilapia diets but do not validate a specific protein content for this batch (17).

Rice presents a comparable traceability limitation: the source record does not identify whether white rice, broken rice, rice flour, or rice bran was used. Therefore, the operational coefficient of 8% is retained only to reproduce the calculation performed and not as a confirmed compositional value. This lack of identification prevents attribution of a specific composition to the batch and must be considered when interpreting the protein estimate.

Relationship with tilapia requirements

The theoretical value close to 30% falls within ranges used in some tilapia diets, but protein requirements vary with stage, size, culture system, and ingredient quality (6,19). Consequently, mathematical proximity to 30% does not demonstrate nutritional adequacy.

The prototype also cannot be considered nutritionally balanced in a comprehensive sense: essential amino acids, energy, lipids, minerals, vitamins, digestibility, and proximate composition were not determined. Recent evidence on alternative ingredients confirms that evaluation must integrate composition, digestibility, inclusion level, and biological response (13,14).

Applied novelty, feasibility, and perspectives

The contribution of the study is applied and contextual: it documents a preliminary formulation integrating raw materials of different types that could be produced through low-complexity operations. The use of by-products represents a potential for valorization, but the study did not conduct life-cycle analysis, environmental-footprint assessment, quantitative comparison of impacts, or economic analysis; therefore, it does not demonstrate environmental sustainability, cost reduction, or productive superiority.

A subsequent stage should include proximate analysis of all raw materials and the final feed, moisture and water activity, water stability, floatability, hardness, particle size, microbiological evaluation, amino-acid profile, and calculation of cost per kilogram. A bioassay with *O. niloticus* should then be conducted using a reference commercial feed and replicates, measuring weight gain, specific growth rate, intake, feed conversion, protein efficiency, and survival. These determinations will establish whether the proposal can evolve from a formulated prototype into a technically validated feed.

CONCLUSIONS

In accordance with the general objective, a feed prototype for Nile tilapia (*Oreochromis niloticus*) was preliminarily formulated and produced using alternative ingredients and local by-products, reaching a final mass of 2462 g. The mathematical balance based on the adopted operational coefficients produced an estimated theoretical protein contribution of 30.03%, close to the nominal target of

30%. This result confirms the feasibility of the formulation procedure as an initial stage, but it is not equivalent to an experimental determination of crude protein and does not demonstrate comprehensive nutritional adequacy.

The production process made it possible to integrate the ingredients, manually shape the prototype, apply solar drying, and perform subsequent grinding to reduce the observed lumps. These operations show that the formulation could be materialized on a small scale with the described resources, fulfilling the production dimension established in the objective. Nevertheless, the observations were exclusively qualitative and do not validate moisture, hardness, particle size, floatability, water stability, or durability. Consequently, the observed technical feasibility should be understood as preliminary and limited to the documented process conditions.

Interpretation of the calculated protein contribution is conditioned by incomplete traceability and the absence of proximate analysis of the raw materials, especially the viscera-derived ingredient, rice, and coffee residues. Therefore, the product should continue to be described as a formulated feed prototype rather than as a validated nutritionally balanced feed. To advance from preliminary formulation toward performance evaluation, chemical characterization of the ingredients and final product, physical tests, digestibility assessment, and controlled bioassays with *O. niloticus* are required, together with independent economic and environmental evaluations if conclusions are to be drawn regarding those dimensions.

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ETHICAL CONSIDERATIONS

The study was limited to feed formulation and prototype production and did not involve human participants or experimental administration of the feed to live animals. Therefore, no interventions involving humans or animals were performed at this stage. Any future feeding trial involving *Oreochromis niloticus* must, before implementation, undergo the corresponding institutional review procedures for ethics and animal welfare.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE (AI)

The authors declare that ChatGPT (OpenAI) was used solely as a support tool for organizing the structure of the manuscript. Its use was limited to arranging and structuring the sections of the document, without involvement in the generation of data, results, calculations, references, scientific interpretations, conclusions, or substantive research content. Responsibility for the information presented, its accuracy, integrity, and scientific validity rests exclusively with the authors.

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