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Research article

Valorization of White Shrimp Shell Waste: Development of Chitosan-Based Pellet Feed for Enhanced Nile Tilapia (*Oreochromis niloticus*) Nutrition

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Abstract: This study demonstrates the development and application of an eco-friendly Nile tilapia (*Oreochromis niloticus*) feed formulation incorporating chitosan extracted from white shrimp shells. The extraction process yielded purified chitosan through ultrasonic deproteinization, acid demineralization, and alkaline deacetylation, confirmed by FTIR spectrophotometry. Three experimental feed formulations containing varying chitosan levels (10%, 15%, and 20%) were prepared and evaluated through proximate analysis. Formula 2 (15% chitosan) was identified as optimal, meeting standard nutritional requirements for aquafeeds while maximizing protein content and minimizing ash levels. A 14-week growth trial using the optimal feed showed that tilapia exhibited healthy growth performance, with an average weight of 14.90 g, length of 10.05 cm, and a survival rate of 77.50%. Water quality parameters remained within acceptable ranges, confirming the environmental compatibility of the feed. These results align with previous findings that chitosan supplementation enhances feed conversion and growth in aquaculture species. This work underscores the value of converting shrimp shell waste into a functional feed additive, offering a sustainable solution for improving aquafeed quality and promoting circular economy practices in aquaculture. By integrating waste valorization and local feed production, this research contributes to safer, more sustainable fish farming while reducing reliance on chemical additives. The approach supports both environmental stewardship and community livelihood development in regions where aquaculture is an essential economic sector.

Keywords: Tilapia; chitosan; white shrimp; pellet feed

1. Introduction

Animal feed plays a vital role in supporting livestock health, growth, reproduction, and milk production. Typically sourced from plant and animal materials, these feeds provide varying nutritional profiles. In recent years, chemical additives have been widely incorporated into feed formulations to boost productivity. However, prolonged use of such additives can lead to harmful residues in animal products, posing significant risks to consumers. This has led to increased interest in natural, environmentally friendly alternatives that promote both animal health and consumer safety. One promising approach

involves utilizing naturally derived compounds to enhance feed efficiency and overall productivity. For example, the outer shells and heads of crustaceans, particularly shrimp, are commonly ground and added to animal feed. Beyond their nutritional value, shrimp shells offer greater potential due to their rich content of chitin and chitosan, two biopolymers with diverse functional applications. Chitin is a fibrous, structural carbohydrate structurally similar to cellulose, but distinguished by the presence of a nitrogen-containing acetylamino group (–$NHCOCH_3$) at the C-2 position of the glucose monomer, which gives it distinctive reactivity. The chemical formula of its monomer is $C_8H_{13}NO_5$. Chitosan, derived from chitin via alkaline deacetylation, is a copolymer consisting of anhydro-*N*-acetyl-D-glucosamine and anhydro-D-glucosamine (commonly known as glucosamine, $C_6H_{11}NO_4$). This process modifies the structure of chitin, especially the nitrogen-based functional groups. While chitosan is insoluble in water, it dissolves readily in organic acids. Its solutions are viscous, transparent, and exhibit non-Newtonian behavior. Chitosan can naturally form flexible, plastic-like films and be processed into various forms, including membranes, gels, beads, fibers, colloids, and coatings.

Thanks to its unique physicochemical and biological properties, biocompatibility, non-toxicity, and biodegradability, chitin and chitosan are regarded as safe and versatile materials. They are widely used in agriculture, food technology, water treatment, separation processes, medicine, pharmaceuticals, and cosmetics. Chitin is naturally found in shrimp shells, crab carapaces, squid pens, mollusk shells, and the cell walls of certain fungi [1]. Chitosan is a white, odorless biopolymer that is biodegradable and exhibits low toxicity, with an LD_{50} in rats of approximately 16 g/kg body weight, comparable to common table salt or sugar [2-4]. In aquaculture, chitosan has been applied in various roles, including water purification, flocculation of suspended solids, waste adsorption, and bacterial control [5, 6]. It has also been used to manage phytoplankton in aquaculture ponds [7], stimulate immune responses in aquatic species [8, 9], control drug and vaccine release [10], and enhance stress resistance and survival in post-larval white shrimp [11]. Given these beneficial properties, the present study aims to develop an eco-friendly aquafeed by chemically extracting chitosan from shrimp shells and incorporating it into a high-quality, sustainable feed formulation. This initiative also seeks to transfer knowledge to local aquaculture communities, supporting the development of environmentally responsible practices and sustainable livelihoods.

2. Materials and Methods

2.1 Extraction of chitin and chitosan from Pacific white shrimp shells

First, selected white shrimp shells are thoroughly washed with clean water and sun-dried for two days. Once completely dry, the shells, approximately 12 kilograms, are weighed and subjected to deproteinization to remove residual proteins. This is carried out by soaking the shells in water using an ultrasonic cleaner operating at 30 kHz and 80 °C for 12 hours. This step is repeated twice more to ensure complete deproteinization. After ultrasonic treatment, the shrimp shells are washed again, then sun-dried for an additional two days. The resulting shells should appear cleaner and lighter in color. The dried shells are then coarsely ground to increase their surface area for the subsequent chemical treatments that remove impurities and minerals. Next, the ground shrimp shells are soaked in a 4% hydrochloric acid (HCl) solution for four days to eliminate residual minerals and contaminants. After soaking, the shells are thoroughly washed with clean water and filtered through a fine cloth until the rinse water is neutral. The cleaned shells are then sun-dried for another two days, yielding approximately 7 kilograms of dried material. The dried shells (7 kg) are then finely ground using an electric blender to produce a light brownish powder known as purified chitin. The purified chitin is weighed to calculate the yield percentage using the following formula:

$$\text{Yield (\%)} = (\text{Final weight of ground shrimp shells} \times 100) / \text{Initial weight of shrimp shells}$$

To convert the purified chitin into chitosan, the chitin powder is stirred in a 40% sodium hydroxide (NaOH) solution using a heated magnetic stirrer at 100 °C for four hours. This step removes the *N*-acetyl groups through deacetylation. After heating, the mixture is allowed to cool to room temperature (approximately 30-40 °C) for 30-40 minutes, and the deacetylation step is repeated once more to ensure completeness. The resulting mixture is filtered to separate the solid product from the NaOH solution. The

solid chitosan is then soaked and rinsed repeatedly with clean water until a neutral pH is reached, followed by filtration through a sintered crucible to remove residual solution. Finally, the chitosan powder is air-dried in a well-ventilated area for two days, producing a light brown purified chitosan powder ready for use in Nile tilapia feed formulations.

2.2 Formulation of Nile tilapia feed using chitosan extracted from Pacific white shrimp shells

The study focused on developing and testing feed formulations for Nile tilapia using chitosan extracted from white shrimp shells. The prepared ingredients were thoroughly mixed in a designated container, and clean water was added to achieve the desired consistency, maintaining a water-to-feed ratio of 14 liters per 10 kilograms of feed mixture. The moistened mixture was then processed through an animal feed pelletizer. The resulting pellets were sun-dried outdoors for one day and subsequently packed in suitable storage bags for later use. Three experimental feed formulations were initially developed to identify the optimal chitosan-based feed for Nile tilapia. All three formulations contained the same base ingredients: fish meal, duck eggs, ground broken rice, soybean meal, brewer's grains, distillery by-products, rice bran, ground corn, lead tree leaves (*Leucaena leucocephala*), water hyacinth, vegetable oil, and chitosan derived from shrimp shells. The only difference among the three formulations was the proportion of chitosan relative to the total feed:

Formula 1: 10% chitosan, 90% other ingredients

Formula 2: 15% chitosan, 85% other ingredients
Formula 3: 20% chitosan, 80% other ingredients

All three formulations will undergo proximate analysis to determine their protein, fat, fiber, ash, and moisture contents. These results will then be compared to select the single most suitable formulation, which will be used in further studies to assess its impact on the growth performance of Nile tilapia.

2.3 Growth performance testing of Nile tilapia fed with experimental diets

Before the feeding trial began, Nile tilapia fingerlings were obtained from a private farm. The fish had an average total length of approximately 6 centimeters (measured from head to tail). To allow the fingerlings to acclimate to the experimental conditions, they were kept in a holding pond and fed a commercial pellet feed for seven days. After the acclimation period, the fish were randomly sampled to measure their initial length and weight. They were then stocked into experimental cement tanks measuring 100 × 100 centimeters, each filled with water to a depth of 30 centimeters, at a stocking density of 50 fish per tank. The fish were fed the experimental diets twice daily (morning and evening) at a feeding rate of 3% of their body weight per feeding. Tank maintenance involved weekly partial water changes by draining half of the old water and refilling with clean water to maintain the original water level. Aeration was provided throughout the trial to ensure adequate dissolved oxygen levels. Growth performance data, including fish length, body weight, and survival rate (%), were recorded regularly to evaluate the effects of the feed formulations on the growth of Nile tilapia.

2.4 Water quality monitoring during the growth performance study of Nile tilapia

Water quality parameters were monitored weekly using standard methods. The parameters measured included temperature (electrometric method) [12], pH (electrometric method) [12], dissolved oxygen (membrane-electrode method) [12], nitrate as nitrogen (colorimetric brucine method) [13], and phosphate as phosphorus (ascorbic acid method) [14].

2.5 Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA), and mean differences were compared using Duncan's Multiple Range Test (DMRT) with the aid of statistical software.

3. Results and Discussion

3.1 Extraction of chitosan from white shrimp shells using a chemical method

The extraction of chitosan from white shrimp shells begins with 12 kilograms of raw, unpurified shrimp shells as the starting material. In the initial step, chitin is extracted from the shells and obtained as a light brown powder (Figure 1). This chitin is then converted into chitosan through a deacetylation reaction,

which removes the *N*-acetyl groups from the chitin molecules, resulting in purified chitosan in the form of a light brown powder in 58.33 % yield (Figure 1). The chemical equation for the deacetylation of chitin is presented in Figure 2.

Figure 1. Chitin (left) and chitosan (right) purified from white shrimp shells after chemical extraction

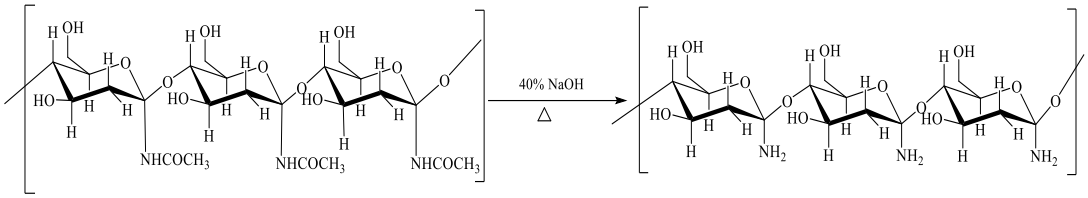

Figure 2. Deacetylation of chitin to chitosan using NaOH

3.2 Characterization of chitin and chitosan from white shrimp shells

The conversion of functional groups during the deacetylation of chitin was verified by confirming the transformation of *N*-acetyl groups into amino groups in chitosan. Functional group analysis was conducted using FTIR spectroscopy to characterize the chitin and chitosan extracted from white shrimp shells. The resulting spectra were compared with a standard chitosan reference. The FTIR spectra of chitin and chitosan from the shrimp shell samples are presented in Figures 3–5. As shown in Figure 3, the FTIR spectrum of chitin extracted from white shrimp shells exhibits a carbonyl (C=O) functional group characteristic of the amide molecule at a wavenumber of 1664 cm^{-1}. Additionally, an N–H bending vibration of the amide group appears around 1554 cm^{-1}. The FTIR spectrum for chitosan from the same shrimp shell sample is presented in Figure 4. Figure 4 shows that the FTIR spectrum of chitosan derived from white shrimp shells displays a similar pattern to that of chitin, with an N–H bending vibration of the amine group observed at approximately 1589 cm^{-1}. However, the C=O stretching vibration decreases significantly, confirming the success of the deacetylation process. This peak closely matches the spectrum of the standard chitosan shown in Figure 5.

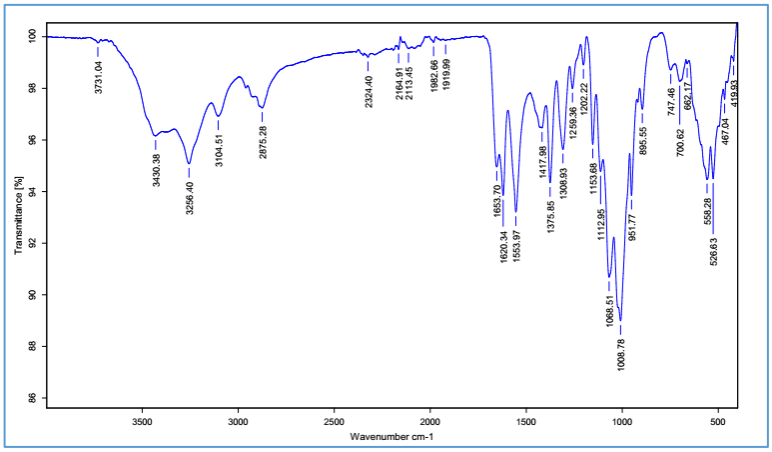

Figure 3. FTIR spectrum of chitin extracted from white shrimp shells

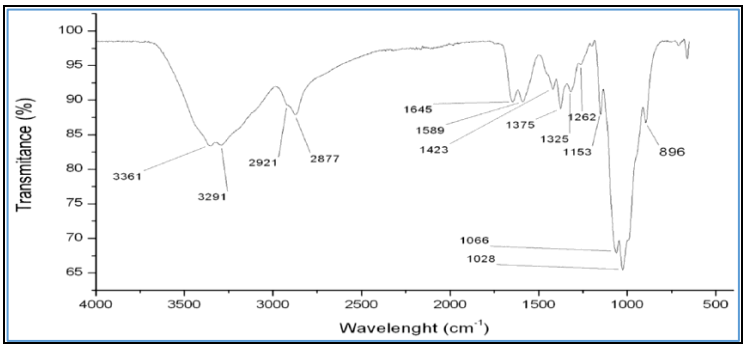

Figure 4. FTIR spectrum of chitosan extracted from white shrimp shells

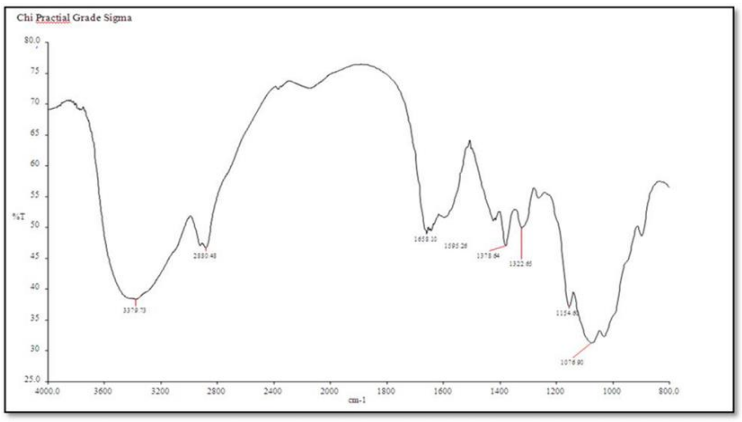

Figure 5. FTIR spectrum of standard chitosan

3.3 Identification of the optimal chitosan-based feed formulation from white shrimp shells

The optimal chitosan-based feed formulation for Nile tilapia includes the following ingredients: fish meal, duck eggs, ground broken rice, soybean meal, brewer's grains, distillery by-products, rice bran, ground corn, *Leucaena* leaves, water hyacinth, vegetable oil, and chitosan extracted from white shrimp shells. The experiment tested three variations of this base formula by adjusting the proportion of chitosan relative to the other feed components. The feed mixtures were as follows:

Formula 1: 10% chitosan and 90% other ingredients (fish meal, duck eggs, ground broken rice, soybean meal, brewer's grains, distillery by-products, rice bran, ground corn, *Leucaena* leaves, water hyacinth, and vegetable oil)

Formula 2: 15% chitosan and 85% other ingredients (same as above)

Formula 3: 20% chitosan and 80% other ingredients (same as above)

These three formulations were analyzed using a proximate analysis instrument to determine their protein, fat, fiber, moisture, and ash contents. The results are presented in Table 1.

Table 1. Standard requirements and proximate composition of the three chitosan-based feed formulations

Composition (%)	Standard Maximum	Standard Minimum	Formula 1	Formula 2	Formula 3
Protein	42	30	30.05	31.67	31.85
Fat	6	3	3.06	3.04	3.04
Fiber	6	5	4.60	5.07	5.04
Moisture	12	-	11.54	11.49	11.48
Ash	-	-	15.25	10.81	11.90

Note: The standard feed quality criteria are based on the Animal Feed Quality Control Act B.E. 2558 [15].

The results show that the protein content in Formulas 2 and 3 did not differ significantly and both fell within the standard range for aquafeeds [15]. Formula 1 had the lowest protein content at 30.05%. The fat content was similar across all three formulations. The fiber content was highest in Formula 2, followed by Formula 3 and then Formula 1. In contrast, the ash content was highest in Formula 1, followed by Formula 3, with Formula 2 having the lowest ash content. The moisture content was comparable across all three formulations. Based on the proximate analysis and in reference to the standard feed quality requirements, which specify minimum protein and fat levels and maximum fiber and moisture levels, Formula 2 was identified as the most suitable formulation. This feed will be used in further trials to evaluate its effects on the growth performance of Nile tilapia. The composition of the optimal chitosan-based feed formulation is as follows:

Fish meal — 10% by weight

Duck eggs — 10% by weight

Ground broken rice — 10% by weight

Soybean meal — 10% by weight

Brewer's grains — 5% by weight

Distillery by-products — 5% by weight

Rice bran — 10% by weight

Ground corn — 10% by weight

Leucaena leaves — 5% by weight

Water hyacinth — 5% by weight

Vegetable oil — 5% by weight

Chitosan from white shrimp shells — 15% by weight

3.4 Application of the optimal chitosan-based feed formulation from white shrimp shells to growth performance testing

3.4.1 Fish growth in terms of weight, length, and survival rate (%)

In this trial, Nile tilapia were fed the optimal chitosan-based feed formulation derived from white shrimp shells for a rearing period of 14 weeks. Throughout the experiment, all fish remained healthy, exhibited normal behavior, and showed no visible external deformities. Figure 6 presents the feed used and the general appearance of the fish at the end of the trial. By the conclusion of the experiment, the tilapia showed an average survival rate of 77.50% and demonstrated steady increases in both weight and length over time. The average final weight and length were 14.90 grams and 10.05 centimeters, respectively. Detailed results for fish weight and length are summarized in Table 2.

Figure 6. The experimental feed and the general appearance of the fish at the end of the trial

These findings are consistent with the reports by Wu (2020) and Rangkuti et al. (2025), who observed that tilapia fed chitosan-supplemented diets exhibited improved feed conversion ratios (FCR) and greater body weight compared to fish fed conventional diets. [16,17]. Similarly, Harikrishnana et al. (2012) reported that grouper (*Epinephelus bruneus*) fed chitosan-supplemented diets showed significantly better growth performance than those fed standard diets [18].

3.4.2 Water quality during the experiment

Water quality was monitored throughout the experiment, and the average values were as follows: pH 7.66, dissolved oxygen (DO) 4.11 mg/L, total phosphorus 1.18 mg/L, and nitrate-nitrogen 0.67 mg/L, as presented in Table 2.

Table 2. Growth performance and water quality parameters of Nile tilapia fed the optimal chitosan-based feed formulation

Week	Fish Weight (g)	Fish Length (cm)	pH	DO (mg/L)	Nitrate-Nitrogen (mg/L)	Total Phosphorus (mg/L)
1	6.66^{k}	6.05^{h}	8.62^{a}	3.63^{e}	0.00^{g}	0.00^{d}
2	7.62^{k}	6.69^{gh}	8.36^{b}	4.89^{a}	0.05^{fg}	0.51^{c}
3	9.08^{j}	7.69^{fgh}	7.81^{c}	3.66^{e}	0.44^{d}	0.80^{b}
4	10.17^{ij}	8.07^{fgh}	7.63^{cde}	3.70^{e}	0.45^{cd}	0.96^{b}
5	11.11^{i}	8.51^{fg}	7.50^{defg}	3.98^{cde}	0.26^{e}	1.33^{a}
6	12.62^{h}	9.11^{ef}	7.66^{cd}	4.88^{a}	0.15^{ef}	1.43^{a}
7	15.66^{g}	$9.96d^{ef}$	7.53^{defg}	3.70^{e}	0.44^{d}	1.51^{a}
8	16.94^{f}	11.02^{cde}	7.60^{def}	4.36^{b}	0.59^{c}	1.38^{a}
9	17.86^{ef}	11.87^{bcd}	7.41^{fg}	4.41^{b}	1.00^{b}	1.40^{a}
10	18.68^{de}	12.44^{abc}	7.46^{efg}	3.87^{de}	1.19^{a}	1.49^{a}
11	19.14^{cd}	13.30^{abc}	7.41^{fg}	4.29^{bc}	1.19^{a}	1.43^{a}
12	20.05^{bc}	13.70^{ab}	7.46^{efg}	3.89^{de}	1.21^{a}	1.46^{a}
13	20.86^{b}	14.07^{ab}	7.37^{g}	4.15^{bcd}	1.22^{a}	1.41^{a}
14	22.10^{a}	14.52^{a}	7.39^{g}	4.32^{bc}	1.24^{a}	1.48^{a}
Average	14.90	10.50	7.66	4.11	0.67	1.18
CV (%)	5.83	15.77	1.82	6.43	15.69	11.18

Note: Means in the same column with different superscript letters are significantly different at $P<0.01$.

The average pH values in the experimental tanks ranged from 7.37 to 8.62, which is within the optimal range for Nile tilapia growth. Generally, a pH range of 6.5-9.0 is considered suitable for aquaculture, allowing fish to maintain homeostasis without expending additional energy on osmoregulation [19]. If pH levels are too high or too low, they can stress or harm aquatic organisms. Specifically, pH values below 4.0 can be lethal; values between 4.0-6.0 may slow growth and disrupt reproduction; 6.5-9.0 is optimal; 9.0-11.0 is suboptimal for long-term survival; and levels above 11.0 are toxic [20,21]. The dissolved oxygen levels during the trial ranged from 3.63 to 4.89 mg/L, which is considered safe for tilapia. According to Abdel-Tawwab et al. (2014), the optimal DO range for Nile tilapia culture is 6.0-6.5 mg/L, but levels should not fall below 3.0 mg/L [22].

Insufficient DO can reduce respiration efficiency, growth, and metabolic function [23]. Nitrate-nitrogen levels ranged from 0.00 to 1.24 mg/L, and total phosphorus ranged from 0.00 to 1.48 mg/L, both of which are within safe limits for tilapia culture. Nitrate, as the final product of nitrification, should not accumulate beyond 50 mg/L; although not directly toxic at that threshold, high levels can stress fish and increase susceptibility to disease. Both nitrate and phosphorus are important nutrients for primary productivity in ponds and are not harmful at the levels observed.

4. Conclusions

This study successfully demonstrates the extraction, formulation, and application of an environmentally friendly tilapia feed enriched with chitosan derived from white shrimp shells. The optimized extraction process produced purified chitosan, confirmed by FTIR analysis, ensuring the functional integrity of the biopolymer for feed use. Among the three experimental formulations tested, the feed containing 15% chitosan (Formula 2) was identified as the most effective, meeting the required nutritional standards for protein, fat, fiber, and moisture content under the Animal Feed Quality Control Act B.E. 2558. When applied in a 14-week growth trial, the optimal chitosan-based feed supported healthy and consistent growth of Nile tilapia, with fish showing normal behavior, no visible deformities, and an acceptable survival rate of 77.50%. Importantly, the water quality parameters throughout the trial remained within safe and optimal ranges for aquaculture, indicating that the feed formulation does not adversely affect the culture environment. These findings are consistent with previous studies highlighting the benefits of chitosan in improving feed conversion efficiency and growth performance in various aquaculture species. Beyond demonstrating technical feasibility, this research emphasizes the significance of transforming crustacean shell waste, often discarded as an environmental pollutant, into a high-value, functional feed additive. This not only contributes to waste valorization and the circular bioeconomy but also offers practical benefits for small-scale farmers seeking cost-effective and safe alternatives to chemical feed additives. By integrating local waste resources into feed production, the approach strengthens the sustainability and self-reliance of rural aquaculture communities.

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