

Dual-Layer Gellan Gum–Chitosan Biopolymer Coatings Enhance Structural Integrity and Nutrient Retention of Fish Feed Pellets

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Fish feed pellets play a critical role in aquaculture; however, their performance is often compromised by structural degradation and nutrient leaching upon water exposure. To mitigate these issues, natural biopolymers were selected as coating materials. Gellan gum (GG), an anionic polysaccharide, exhibits strong gel-forming capacity and film integrity. Chitosan (CH), a cationic biopolymer, is recognized for its bioadhesive and antimicrobial characteristics. Pellets were sequentially coated with GG and CH in one or two cycles (GG:CH 1:1 and 2:2) and evaluated using Fourier-transform infrared spectroscopy (FTIR), elemental analysis, swelling behavior, optical microscopy, and scanning electron microscopy (SEM). FTIR analysis confirmed the incorporation of the coatings, indicated by broadening of the O–H band (3300–3400 cm⁻¹) and a shift in the carbonyl region. These spectral modifications suggest hydrogen bonding between GG and CH, supporting the existence of synergistic intermolecular interactions. Swelling ratio assessments demonstrated a marked reduction in water uptake for coated samples. The uncoated control exhibited the highest swelling ratio at 146.2%, while GG:CH (1:1) and GG:CH (2:2) samples showed ratios of 113.5% and 92.9%, respectively. These findings indicate that the crosslinked GG–CH network, despite containing polar functional groups, limits water penetration due to steric hindrance and intermolecular forces. Microscopy confirmed the formation of a uniform film, with the GG:CH (2:2) group displaying the thickest and most cohesive layer. Elemental analysis detected minor changes in carbon, hydrogen, and nitrogen composition, indicating altered surface chemistry that may enhance nutrient retention. SEM analysis further revealed improved adhesion and reduced porosity in coated pellets. Collectively, these results demonstrate that GG–CH biopolymer coatings enhance pellet stability and minimize nutrient loss, offering a sustainable approach to improving feed efficiency and promoting aquatic health in aquaculture.

Keywords: Gellan gum; chitosan; coating; fish feed pellets

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Aquaculture plays a crucial role in global food security, providing a sustainable source of protein to meet the growing demands of the population [1]. The efficiency of fish farming is heavily dependent on the quality of fish feed, which must be nutritionally balanced, environmentally sustainable, and cost-effective [2, 3]. One of the challenges in aquaculture nutrition is the loss of feed nutrients due to water solubility, leading to environmental pollution and decreased feed efficiency [4, 5]. It is estimated that 15–30% of nutrients leach from conventional feed within the first hour of immersion, which increases water pollution and decreases feed conversion efficiency [2]. To address this issue, various coating technologies have been explored to enhance feed stability and controlled nutrient release, with biopolymer-based coatings emerging as a promising solution [6, 7].

Gellan gum (GG), an anionic microbial exopolysaccharide produced by *Sphingomonas elodea*, has gained significant attention in the food and

pharmaceutical industries due to its excellent gelling, film-forming, and stabilizing properties [8–11]. It is widely used in encapsulation and coating applications due to its ability to form strong, flexible films with high water resistance [12, 13]. In the context of aquaculture, GG presents a viable option for coating fish feed pellets, potentially improving feed stability in water and modulating the release of nutrients [14, 15].

Similarly, chitosan (CH), a biodegradable and biocompatible polysaccharide derived from chitin, has been extensively studied for its antimicrobial, film-forming, and mucoadhesive properties [16–18]. Chitosan-based coatings have been explored in various food and biomedical applications, including drug delivery and food preservation [19–21]. In aquaculture, CH has been investigated as a feed additive due to its potential to enhance fish immunity and promote growth [22–24]. Additionally, its ability to form strong films makes it a suitable candidate for fish feed coating to improve water stability and reduce nutrient leaching [25].

Although biopolymers such as alginate, carrageenan, and cellulose derivatives have been explored for fish feed coating [26, 27], the sequential dual-layer application of GG and CH to aquaculture feed remains underexplored. Their synergistic interactions, driven by hydrogen bonding and electrostatic attraction, form polyelectrolyte complexes with improved film stability and controlled release properties [28, 29]. Leveraging this synergy can reduce nutrient leaching and improve pellet durability [30]. The synergistic interaction between GG and CH has been explored in various biomedical and food applications, including encapsulation and controlled release systems [31, 32]. Their electrostatic interactions lead to the formation of polyelectrolyte complexes, which enhance film integrity and functionality [33–35]. Applying this combination to fish feed coating could provide a dual advantage of improving pellet durability and modulating nutrient release, thereby enhancing feed efficiency and reducing environmental impact [36, 37].

This study evaluates the effect of coating thickness on water absorption, coating integrity, and nutrient retention in dual-layer gellan gum–chitosan (GG–CH) coated pellets. Fourier-transform infrared spectroscopy (FTIR) was used to confirm GG–CH interactions, elemental analysis to detect changes in surface chemistry, swelling tests to measure water uptake, optical microscopy to assess coating uniformity, and scanning electron microscopy (SEM) to evaluate morphology and thickness. Systematic characterization of these parameters addresses the identified research gap and supports the development of environmentally sustainable and nutritionally efficient aquaculture feed formulations. It is hypothesized that an optimized dual-layer GG–CH coating will significantly reduce water absorption and nutrient leaching compared to uncoated or single-layer coated feeds.

EXPERIMENTAL

Chemicals and Materials

Low-acyl gellan gum (GG; Gelzan™ CM, molecular weight $\approx 2\text{--}3 \times 10^5$ Da (provided by the supplier), product no. G1910, lot no. SLBB0374V), chitosan (CH; medium molecular weight, $\sim 75\%$ degree of deacetylation, viscosity ~ 453 cP, powder form), glycerine, and acetic acid were sourced from Sigma Aldrich (USA). Key parameters of Gelzan provided by the supplier's certificate of analysis (CoA) include moisture content ($\sim 16\%$), particle size distribution ($\geq 95\%$ through 42-mesh / ~ 355 μm), and trace inorganic ions such as Ca, Mg, K, Na, and phosphate. Purity in terms of molecular weight distribution and endotoxin level is not reported in the public CoA. Commercial fish feed pellets (product code 6243-1) were obtained from Cargill Malaysia. The fish feed pellets in this study were spherical and had an average diameter of 0.50 ± 0.02 cm. Measurement of bulk

density, based on the mass of a known pellet volume, resulted in a value of 0.62 ± 0.01 g/cm³. Proximate composition included crude protein at $32.5 \pm 0.4\%$, crude fat at $6.8 \pm 0.2\%$, crude fiber at $4.1 \pm 0.1\%$, ash at $9.7 \pm 0.3\%$, and moisture at $8.3 \pm 0.2\%$. All reagents and materials were used directly without any additional purification steps.

Preparation of Gellan Gum and Chitosan Solutions

To prepare the GG solution, 2% (w/v) GG was dissolved in deionized water (18.2 M Ω) and blended with 1.0% (w/v) glycerine. The mixture was heated to 90°C and stirred continuously for 2 hours to ensure homogeneity. For the CH coating, 2% (w/v) CH was dissolved in 0.5 M acetic acid, followed by 1.0% (w/v) glycerine under similar heating and stirring conditions. After heating, the solutions were cooled to room temperature ($\approx 25^\circ\text{C}$).

Fish feed pellets were sequentially immersed in each solution for 5 seconds per coating step. After dipping in the GG solution, pellets were dried at room temperature for 24 hours. The same procedure was repeated with the CH solution. For double-coating, this sequence was repeated once more. The samples were designated as follows: GG:CH (1:1) for single-layer coating and GG:CH (2:2) for double-layer coating. For every formulation—which includes the uncoated control, GG:CH 1:1, and GG:CH 2:2—three independent pellet batches were prepared and subsequently tested. To preclude any systematic bias, a random selection of replicate pellets from each batch was chosen for characterization. Accounting for potential differences between batches, samples for characterization tests (like FTIR, elemental analysis, swelling ratio, microscopy, and SEM) were drawn from various batches. Minimization of potential batch effects, alongside ensuring the reproducibility of the findings, was achieved through the randomization of pellet selection and cross-batch analysis. Coated samples were conditioned at room temperature for another 24 hours before characterization.

Characterization of Coated Fish Feed

Fourier Transform Infrared (FTIR) Spectroscopy

Coated fish feeds were analyzed using the potassium bromide (KBr) pellet technique. Samples were finely ground and then combined with KBr using 1:100 mass proportion (sample:KBr). A hydraulic press was then used to press the mixtures into see-through pellets. To remove leftover water that might otherwise affect the O–H stretching part of the spectra, an overnight vacuum-drying of both the polymer-coated feed samples and the KBr was conducted before measurement. The FTIR measurements of the samples were obtained by averaging over 16 scans with a resolution of 4 cm⁻¹ in the spectral range of 4000 cm⁻¹ – 600 cm⁻¹ using a spectrometer (Perkin Elmer Spectrum 100, USA) equipped with a DTGS detector. The FTIR

spectrometer used automatic background correction to scan empty beam paths before each sample measurement. The instrument's software performed atmospheric compensation of CO₂ and H₂O vapor to reduce spectral interference during measurements.

Elemental Analysis

Elemental analysis quantified the percentages of carbon (C), hydrogen (H), and nitrogen (N) in the coated fish feed pellets. A Euro EA 3000 analyzer (EuroVector, Italy) was used for all measurements. The instrument underwent daily calibration with acetanilide and benzoic acid standards. For each analysis, 2.0 ± 0.2 mg of dried, ground pellet sample was weighed into a tin capsule. Each formulation was measured in triplicate (n = 3) to address variability. Moisture and ash content corrections were applied using pre-determined proximate analysis values. Background blanks were analyzed between samples to prevent carryover. Elemental composition was assessed for both unimmersed pellets, representing baseline composition, and pellets immersed for 24 hours in phosphate buffer at pH 7.03 and 37°C. The change in elemental composition, with emphasis on nitrogen content, served to evaluate nutrient retention after exposure to an aqueous environment.

Swelling Properties

Swelling ratio was calculated using the weight ratio of absorbed water (W_{wet}) to dry weight (W_{dry}) of the coated fish feed. A coated fish feed was placed in a beaker filled with phosphate buffer solution (pH 7.03), which was heated to 37°C in a water bath. Each formulation was measured in triplicate (n = 3) to address variability. To achieve equilibrium swelling, the weight of the wet samples was measured after 24 hours. The swelling ratio for each sample was then calculated using the following formula:

$$\text{Swelling ratio} = [(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}}] \times 100$$

Optical Microscope

An optical microscope, which utilizes visible light to examine the morphology of a substance, was used to analyze the coated fish feed pellets. The pellets were carefully sliced using a razor blade and mounted on a microscope slide with a small amount of mounting medium. The samples were then observed under the microscope at varying magnifications to assess their structural characteristics. Optical images were captured using a Leica Z16 APO microscope, equipped with Leica Application Suite software (version 3.1.0 R1), ensuring precise visualization and analysis.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was employed to examine the surface morphology and structural characteristics of the coated fish feed pellets,

including coating thickness, uniformity, and distribution. Before imaging, the samples were sputter-coated with a thin layer of conductive material to prevent surface charging and enhance image resolution. The coated pellets were then placed in the SEM chamber, where a focused electron beam was scanned across the surface. The interaction between the electron beam and the sample generated secondary and backscattered electrons, producing signals that were detected and processed to generate high-resolution images. SEM analysis was conducted using a JEOL JSM-6300, providing a detailed visualization of the coating's microstructure, operated at 15 kV accelerating voltage while maintaining a 10 mm working distance for SEM imaging.

Data Analysis

All analysis data were performed in triplicate and reported as mean ± standard error. Statistical analysis for this study was performed by one-way analysis of variance (ANOVA) using Sigma Plot version 15.0 (Systat Software Inc., CA, USA), followed by the test of post hoc Dunnett or analysis of differences with p values (p < 0.05) considered statistically significant.

RESULTS AND DISCUSSION

FTIR Spectroscopy

Figure 1 displays the Fourier-transform infrared (FTIR) spectra of fish feed pellets coated with either a single layer of gellan gum (GG), a single layer of chitosan (CH), a 1:1 blend of GG and CH, or a 2:2 blend of GG and CH. Table 1 summarizes the characteristic absorption peaks and molecular interactions identified in these spectra. All samples containing GG or CH exhibit broad absorption bands in the 3200–3500 cm⁻¹ region, which correspond to O–H stretching vibrations from hydroxyl groups and associated hydrogen bonding networks [38]. The presence of OH stretching also confirms the inherent carbohydrate content in the fish feed pellets, as carbohydrates exhibit strong hydrogen bonding interactions in this region [39].

In the chitosan (CH) spectrum, the absorption band at 1649.98 cm⁻¹ is attributed to the Amide I vibration, corresponding to C=O stretching. A distinct peak at 1361.67 cm⁻¹, observed exclusively in the CH sample, corresponds to C–N stretching vibrations in chitosan's amine groups, highlighting its characteristic functional moieties [40]. These vibrations indicate the presence of functional groups that enable chitosan's film-forming properties and ionic cross-linking capacity. Additionally, all samples exhibit a broad peak within the 1000–1100 cm⁻¹ range, attributed to C–O stretching vibrations, which is consistent with previous studies on polysaccharide-based biopolymers [41].

In the gellan gum (GG) spectrum, the weak absorption band near 1748 cm⁻¹ is atypical for

polysaccharides and is likely due to minor acetyl C=O stretching or residual esterified components such as glycerine or processing residues, rather than a high acetyl content. This observation aligns with previous findings that GG typically exhibits only a weak carbonyl shoulder near 1730 cm^{-1} . The primary spectral feature of GG is the C–O stretching in the $1000\text{--}1100\text{ cm}^{-1}$ region, which is attributed to glycosidic linkages and carboxylate groups ($-\text{COO}^-$) [42]. A peak is observed at 1748 cm^{-1} , a feature that could be attributed to esterified adventitious components such as residual solvent or glycerine, rather than to a high degree of acetylation within the polymer backbone. Usually, it does not exhibit a pronounced acetyl carbonyl (C=O) band at approximately 1748 cm^{-1} .

In the blended coatings containing GG and CH at ratios of 1:1 and 2:2, the carbonyl absorption bands shifted from 1649.98 cm^{-1} for CH and 1748.38 cm^{-1} for GG to 1632.86 cm^{-1} and 1618.60 cm^{-1} , respectively. This downshift and broadening of the C=O and Amide I bands indicate the presence of hydrogen bonding and ionic interactions between the NH_3^+ groups of chitosan and the COO^- groups of gellan gum. These

spectral changes confirm a synergistic molecular interaction rather than a simple additive effect [43]. Another broad peak in the $2900\text{--}3000\text{ cm}^{-1}$ range, observed in all samples, corresponds to C–H stretching vibrations. These vibrations contribute to van der Waals interactions between polymer chains, which can improve the overall integrity and cohesion of the coating layer [44]. To account for variations in sample thickness and attenuated total reflectance (ATR) penetration depth, peak intensities were normalized to the constant C–H stretching band at 2927 cm^{-1} , and relative absorbance ratios were compared. The results indicate a greater contribution of gellan gum in the dual-layer coatings, especially in the GG:CH (2:2) formulation. Additional analyses, such as difference spectra or superposition analysis, further demonstrate the disappearance or shift of specific absorption bands, supporting the interpretation of polymer–polymer interactions. Overall, the FTIR spectra demonstrate that the GG–CH coatings are not simple physical mixtures. Instead, they involve specific hydrogen bonding and ionic cross-linking interactions, which contribute to the increased stability and reduced swelling observed in the coated fish feed pellets.

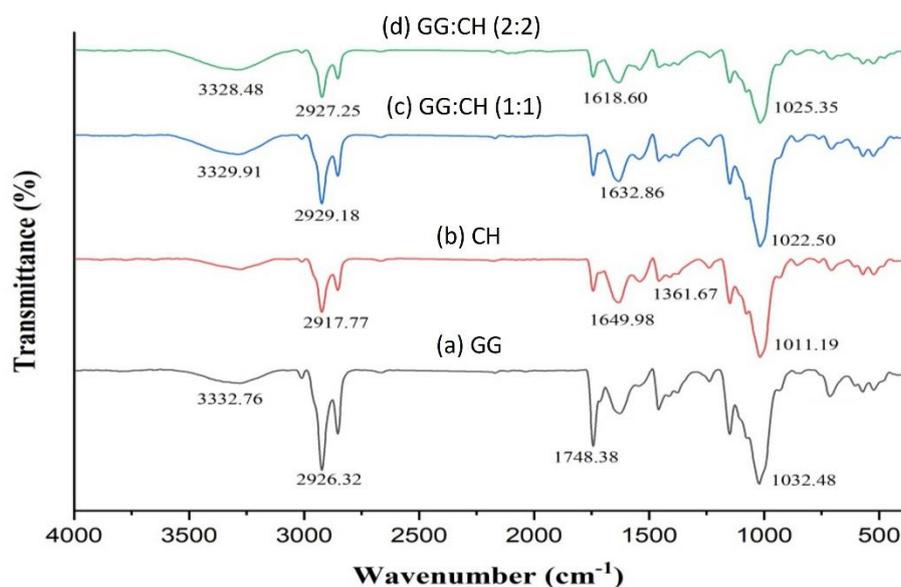


Figure 1. FTIR spectra of fish pellets coated with (a) gellan gum (GG), (b) chitosan (CH), (c) GG:CH (1:1), and GG:CH (2:2).

Table 1. Band assignment of FTIR spectra of gellan gum (GG), chitosan (CH), GG:CH (1:1), and GG:CH (2:2).

Samples	Band assignment (cm ⁻¹)				
	O-H stretching	C-H stretching	C=O stretching	C-N stretching	C-O stretching
GG	3332.76	2926.32	1748.38	-	1032.48
CH	-	2917.77	1649.98	1361.67	1011.19
GG:CH (1:1)	3329.91	2929.18	1632.86	-	1022.50
GG:CH (2:2)	3328.48	2927.25	1618.60	-	1025.35

Elemental Analysis

Elemental analysis was performed to evaluate the nutritional composition of fish feed pellets, focusing on carbon (C), hydrogen (H), and nitrogen (N) contents. These elements are essential for fish metabolism, with carbon serving as an energy source, hydrogen contributing to water balance, and nitrogen playing a critical role in protein synthesis [45]. This analysis aimed to determine whether the GG and CH coatings influenced the retention or leaching of these key elements, potentially affecting the overall nutritional availability of fish. The results of the elemental analysis are presented in Table 2. As shown in Table 2, all coated samples exhibited slightly higher carbon (C) content compared to the uncoated fish feed pellets. This increase can be attributed to the natural composition of GG and CH, both of which contain carbon as a structural component. A similar trend has been reported in previous studies, where biopolymer coatings contributed to minor variations in the elemental composition of coated feed materials [46]. Hydrogen (H) content remained relatively consistent across all samples, although a slight increase was observed in coated pellets, likely due to the hydrogen-rich functional groups present in the coating materials.

The most notable variation was observed in nitrogen (N) content. The GG:CH (1:1) and GG:CH (2:2) coated pellets displayed a slight increase in N compared to the uncoated and single-coated samples. This can be attributed to the nitrogen-rich structure of chitosan, which contains amine groups that contribute to its overall nitrogen content [47]. The presence of a thicker coating in the GG:CH (1:1) and GG:CH (2:2) coated pellets had a more pronounced effect on the elemental profile than the thinner, single-coated formulations. Although the observed increases in C, H, and N content were relatively minor, the improved water resistance of the coatings may enhance feed stability and reduce nutrient leaching, indirectly benefiting fish nutrition [48]. Studies have shown that biopolymer coatings can reduce nutrient loss and improve feed efficiency by prolonging the release of essential nutrients [49]. While the slight increase in nitrogen suggests a potential enhancement in protein retention, the magnitude of this change may be insufficient to significantly impact fish growth and development. Further studies assessing protein digestibility and bioavailability would be necessary to fully understand the implications of these findings.

Table 2. Summary of composition of uncoated and coated fish feed pellets determined using elemental analysis.

Samples	Elemental Percentages (%)		
	C	H	N
Uncoated	41.05 ± 0.5	6.44 ± 0.1	5.21 ± 0.1
GG	41.70 ± 0.1	6.64 ± 0.1	5.24 ± 0.1
CH	41.12 ± 0.1	6.49 ± 0.1	5.31 ± 0.1
GG:CH (1:1)	41.15 ± 0.3	6.59 ± 0.1	5.63 ± 0.1
GG:CH (2:2)	42.29 ± 0.5	6.77 ± 0.2	5.56 ± 0.1

Table 3. Swelling ratios of gellan gum (GG), chitosan (CH), GG:CH (1:1), and GG:CH (2:2).

Sample	Swelling ratio (%)
Uncoated	202 ± 1.13
GG	183 ± 0.98
CH	148 ± 1.92
GG:CH (1:1)	136 ± 1.41
GG:CH (2:2)	93 ± 1.65

Swelling Properties

A swelling properties test was used to evaluate the interaction between fish feed pellets in phosphate buffer solutions (pH 7.03). This measures the ability of fish feed pellets to absorb water and expand in size, as shown in Table 3. The results show that GG and CH can act as efficient water-resistant barriers, where a significant decrease in swelling ratio is seen in the coated samples. The pellet is surrounded by a semi-permeable barrier made of these natural polymers, which prevents water absorption and limits swelling [50].

The observed pattern within the coated samples shows that as the concentration of GG:CH increases, the swelling ratio gradually lowers. The uncoated fish feed pellets have the highest percentage of swelling at 202 ± 1.13%. This absorption leads to rapid disintegration, which potentially causes environmental pollution and nutrient loss [51]. Furthermore, excessive swelling can also cause pellets to break apart and lose form. This makes pellets look less attractive to fish and leads to waste.

As the GG:CH ratio increases, the swelling ratio progressively decreases and reaches the lowest value for GG:CH (2:2). The observed reduction results from both the formation of a thicker, denser coating layer and ionic cross-linking between NH_3^+ groups of chitosan (CH) and COO^- groups of gellan gum (GG). This cross-linking increases coating compactness and restricts water penetration. Consequently, the GG:CH (1:1) and GG:CH (2:2) samples exhibit a greater decrease in swelling compared to samples coated with only GG or CH. Pellets that are coated show less swelling ability, which helps them keep their integrity and form in water. This lessens feed waste and nutrient leaching, which may improve fish nutritional uptake. Slowdowns in swelling can delay the release of nutrients and facilitate a more gradual and effective consumption by fish. However, excessively low swelling might make it difficult for fish to eat pellets with limited water absorption, thus affecting their total feed intake and nutritional intake [52]. The best swelling ratio for fish feed pellets typically ranges between 50% to 100% and the fish feed coated with GG:CH (2:2) falls within this range. Although the GG–CH coatings substantially reduced pellet swelling and bulk nutrient loss, low-molecular-

weight, highly water-soluble nutrients (e.g., free amino acids, vitamin C, and B-group vitamins) may still diffuse across the coating matrix. This limitation highlights the need for future studies to quantify the retention of these critical nutrients in coated feed formulations. Thus, the key to optimizing fish feed performance is the coating composition that balances swelling properties with sufficient water intake and nutrient accessibility.

Optical Microscopy

Optical microscopy was employed to characterize the surface and inner layer morphology of fish feed pellets, providing insights into the effects of gellan gum-chitosan (GG:CH) coatings. The surface morphology of the uncoated and coated pellets is illustrated in Figure 2, highlighting distinct differences in texture and appearance. The uncoated fish feed pellet exhibited a granular texture with a matte brown color, indicative of its porous structure (Figure 2 (a)). In contrast, the pellet coated with GG:CH (1:1) (Figure 2(b)) displayed a thin, slightly translucent coating, imparting a glossy appearance to the surface. The pellet coated with a double-layer GG:CH (2:2) (Figure 2(c)) featured a thicker, more uniform coating, resulting in a pronounced shiny surface. These findings align with previous studies demonstrating the ability of biopolymer coatings to modify surface characteristics and enhance the physical integrity of fish feed pellets [36].

To understand the inner-layer morphology of the samples, the cross-section of the fish feed was investigated. The uncoated fish feed pellet (Figure 2(d)) exhibited a porous internal structure with numerous air-filled voids and a consistent brown coloration. In contrast, the pellet coated with GG:CH (1:1) showed partial penetration of the coating into the inner matrix, leading to a slight color change to pale brown (Figure 2(e)). The most significant coating penetration was observed in GG:CH (2:2) (Figure 2(f)), where the thicker coating layer was deeply embedded, resulting in a more pronounced yellowish hue and the presence of fewer air pockets. These observations suggest that increased coating thickness enhances the mechanical integrity of the pellets by reducing porosity and improving structural cohesion, consistent with previous findings on biopolymer-coated feed formulations [53].

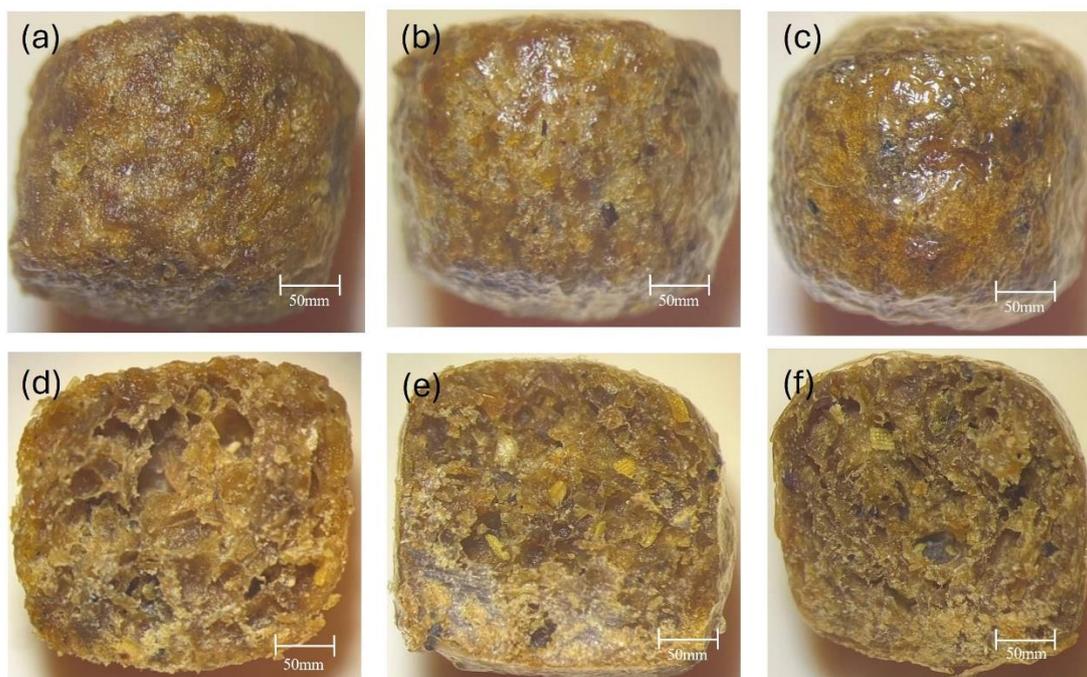


Figure 2. Surface morphology of (a) uncoated fish feed pellet, (b) fish feed pellet coated with GG:CH (1:1), and (c) fish feed pellet coated with GG:CH (2:2); cross-section of (d) uncoated fish feed pellet, (e) fish feed pellet coated with GG:CH (1:1), and (f) fish feed pellet coated with GG:CH (2:2).

The GG-CH coating demonstrated strong adhesion to fish feed pellets in all coated samples. Optical microscopy revealed no delamination or detachment, including the edges and corners, which indicates robust interfacial bonding between the coating and pellet surface. This improved adhesion likely results from electrostatic interactions and hydrogen bonding between GG and CH, as documented in previous studies on biopolymer-based encapsulation systems [54]. However, although optical microscopy confirmed intact adhesion, scanning electron microscopy (SEM) of the GG:CH (1:1) coating identified localized cracks and apparent delamination. These observations may represent surface irregularities or SEM charging artifacts rather than actual bulk adhesion failure.

The differences in coating thickness and penetration depth correlated positively with the swelling properties data. The GG:CH (2:2) samples, which exhibited the thickest and deepest coating penetration, demonstrated the highest water stability and the lowest swelling ratio among all samples. A denser and more uniform coating serves to delay pellet disintegration, allowing the feed to remain intact for an extended period in aquatic environments. This property is crucial for reducing nutrient leaching, minimizing environmental pollution, and optimizing feeding efficiency by extending the duration between feedings [55]. The findings of this study support the potential of GG and CH as effective coating materials for improving the stability and performance of fish

feed pellets, offering a promising strategy for sustainable aquaculture feed management.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was employed to investigate the morphology of the coated fish feed pellets, with a focus on coating distribution, surface defects, and coating thickness. This analysis provided critical insights into the structural integrity and performance of the gellan gum-chitosan (GG:CH) coatings. The SEM images in Figure 3 reveal distinct differences in surface morphology between uncoated and coated fish feed pellets.

The surface of the uncoated fish feed pellet (Figure 3(a)) exhibited a grainy texture, likely representing individual feed components, along with numerous pores. These structural features indicate high porosity, which can promote rapid water absorption and nutrient leaching when exposed to an aquatic environment. In contrast, the GG:CH (1:1) coated pellet (Figure 3(b)) displayed a continuous yet uneven coating layer with minimal cracks. The pellet coated with GG:CH (2:2) (Figure 3(c)) demonstrated a relatively uniform coating with no visible cracks. These findings highlight the role of biopolymer coatings in modifying the surface characteristics of fish feed pellets, as coatings have been shown to enhance feed stability and reduce water absorption [56]. By forming a smoother and less porous surface, coatings can slow nutrient leaching and improve the overall durability of fish feed in water.

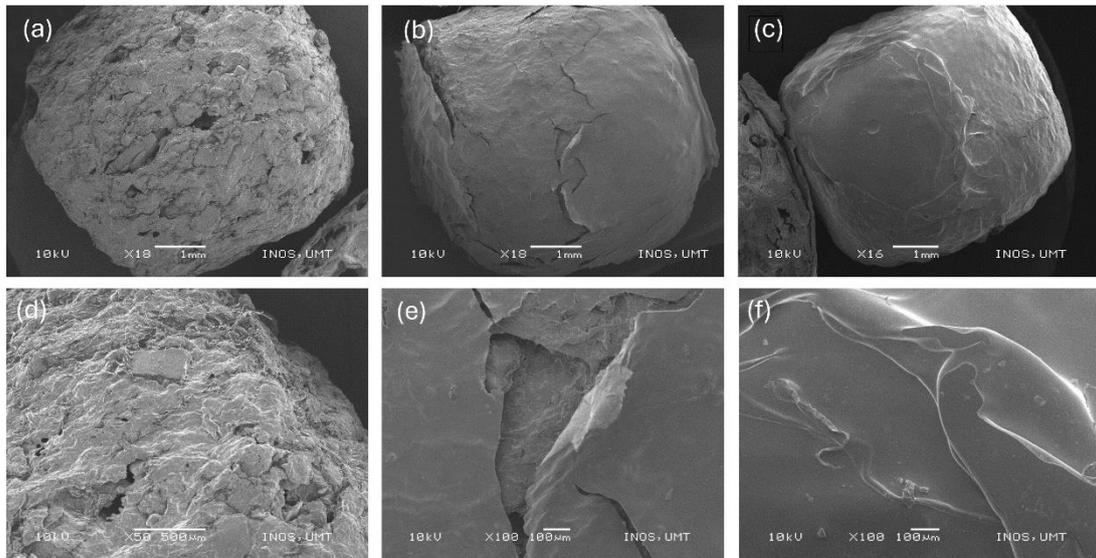


Figure 3. Surface morphology under SEM of (a, d) uncoated fish feed pellet, (b, e) fish feed pellet coated with GG:CH (1:1), and (c, f) fish feed pellet coated with GG:CH (2:2).

Detailed analysis of coating defects, as shown in Figure 3(d–f), identified structural inconsistencies with potentials to compromise coating performance. Multiple regions of the GG:CH (1:1) coating (Figure 3(e)) exhibited hairline cracks and delaminated areas, which exposed the underlying pellet substrate [57]. These features were not detected using optical microscopy, indicating that they are likely localized surface imperfections or artifacts from scanning electron microscopy (SEM) charging, rather than evidence of widespread detachment. In comparison, the GG:CH (2:2) coating (Figure 3(f)) demonstrated a more irregular surface texture but maintained superior adhesion, with no observable delamination. While some surface roughness was observed, this coating was structurally intact, suggesting an improved barrier function compared to the thinner GG:CH (1:1) coating. The cross-sectional SEM analysis of the fish feed pellets was observed and further underscores the impact of coating thickness, as shown in Figure 4.

The uncoated fish feed pellet exhibited a porous internal structure, with irregularly shaped voids of various sizes dispersed throughout the matrix (Figure 4(a)). In this sample, no distinct separation between an outer crust and an inner core was observed, indicating a uniform but highly porous composition. In contrast, the GG:CH (1:1) coated pellet (Figure 4(b)) featured a well-defined coating layer effectively separating the pellet's core from its external surface. This separation may contribute to improved water resistance and delayed nutrient release. Meanwhile, the GG:CH (2:2) coated pellet (Figure 4(c)) exhibited a significantly thicker coating layer. The increased thickness of this coating provides enhanced barrier properties, thereby minimizing water absorption and nutrient diffusion [58]. These results confirm that the GG:CH coating effectively alters the structural and functional properties of fish feed pellets, acting as a physical barrier against moisture ingress and nutrient loss (Figure 5). The average coating thickness of the GG:CH (1:1) is at $28.07 \pm 1.08 \mu\text{m}$, while that for GG:CH (2:2) is at $58.20 \pm 6.50 \mu\text{m}$.

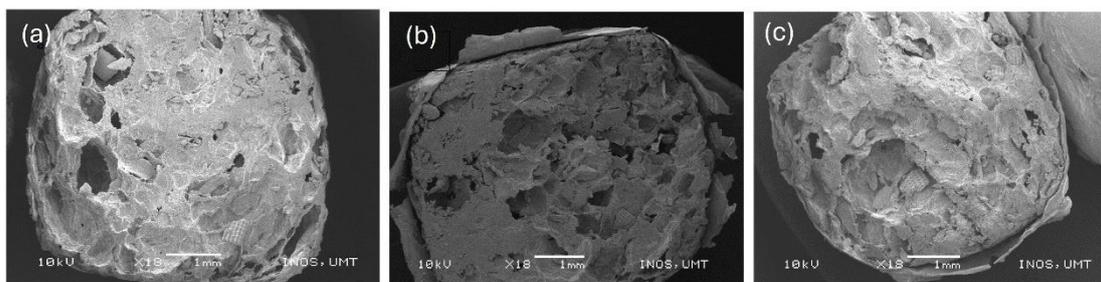


Figure 4. Cross-section of (a) uncoated fish feed pellet, (b) fish feed pellet coated with GG:CH (1:1), and (c) fish feed pellet coated with GG:CH (2:2).

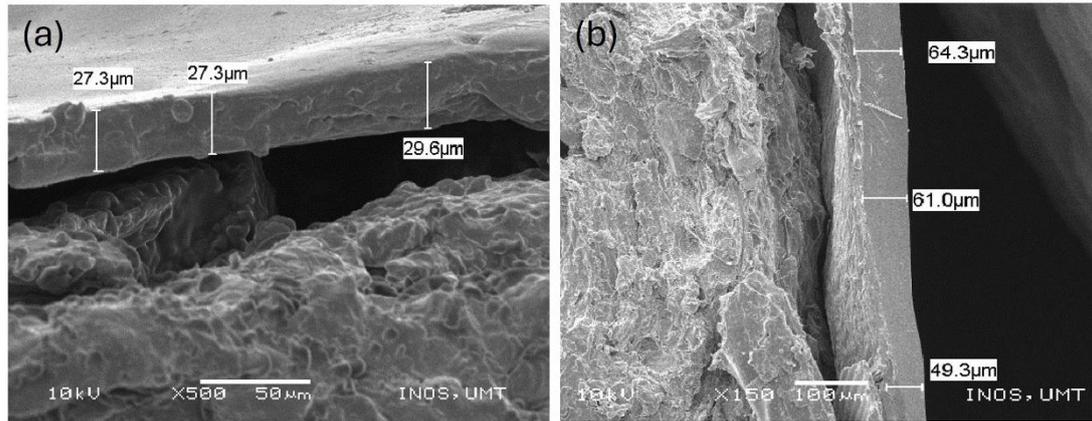


Figure 5. The coating thickness of (a) fish feed pellet coated with GG:CH (1:1) and (b) fish feed pellet coated with GG:CH (2:2).

The observed improvements in coating integrity, reduced porosity, and increased thickness support the use of biopolymer coatings as a promising strategy for enhancing feed stability in aquaculture applications. The findings align with prior studies demonstrating that biopolymer coatings can enhance feed retention and reduce nutrient loss in aquatic environments [59].

CONCLUSION

The effectiveness of gellan gum (GG) and chitosan (CH) biopolymers as fish feed pellet coating materials is demonstrated in this study. Both single-layer (GG-CH 1:1) and double-layer (GG-CH 2:2) coatings showed improvements in water stability and nutrient retention by reducing water absorption and swelling. Scanning electron micrographs revealed that thicker coatings (GG:CH 2:2) had better adhesion and integrity, along with reduced surface defects and improved structural cohesion compared to thinner coatings (GG:CH 1:1). FTIR, together with elemental analyses, confirmed the incorporation of additional functional groups and indicated minor improvements in retention of nutrients, especially nitrogen. From swelling tests, GG:CH (2:2) exhibited the lowest swelling ratio, which suggests this formulation is most effective in minimizing disintegration and nutrient loss in aquatic environments. Compared with uncoated pellets, GG:CH (2:2) reduced 24-h swelling by 54% and increased nitrogen content by 0.35% (w/w), with an average coating thickness of 58 μm. This study demonstrates that GG–CH biopolymer coatings enhance feed utilization and reduce waste, indicating significant potential for advancing sustainable aquaculture practices. The current conclusions are based primarily on laboratory-scale performance data. A comprehensive evaluation of sustainability will require life-cycle analysis, biodegradability testing under aquaculture-relevant conditions, and economic feasibility studies. These

areas represent important directions for future research. The present findings support further investigation and the potential commercialization of GG–GG-CH-coated fish feed products to address the requirements of the expanding aquaculture industry.

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