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Improving the Nutritional Quality of Commercial Feed for Vannamei Shrimp Through Predigest Using Rumen Microbes

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ABSTRACT

Efforts to improve shrimp feed quality continue through the use of microbial pre-digestion. One promising approach involves the use of rumen microbes to enhance the nutritional value of feed. This study aimed to improve the nutritional quality of shrimp feed by applying rumen microbes as a pre-digestion agent. Fermentation was carried out using rumen microbes at a dose of 4.5mL per 100g of feed, with incubation times of 10, 15, and 20 minutes. The highest results were obtained with a 20-minute incubation, yielding dry matter digestibility of 77.98%, organic matter digestibility of 76.37%, soluble protein content of 26.76%, crude protein of 45.64%, crude fat of 5.55%, and nitrogen-free extract (NFE) of 36.83%. The lowest crude fiber content (3.13%) was also observed at the 20-minute incubation, while the highest ash content (10.98%) was recorded at the 10-minute incubation. Additionally, spectroscopic analysis revealed an increase in the intramolecular N-H bonds of carboxylic acid amines and a decrease in O-H functional groups in the shrimp feed fermented for 20 minutes, indicating molecular changes associated with improved digestibility. These results suggest that fermenting shrimp feed with rumen microbes for 20 minutes can significantly enhance its nutritional quality and digestibility, making it a promising strategy for sustainable aquaculture feed development.

INTRODUCTION

The continuous availability of quality feed is a key factor in the successful cultivation of Pacific white shrimp (*Litopenaeus vannamei*). This is particularly important given the high cost of commercial feed, which accounts for approximately 60–70% of total production costs **(Buccaro *et al*., 2023)**. In response, efforts have been made to improve feed quality through the addition of microbes, specifically probiotics. Predigestion

strategies aim to enhance nutritional quality by incorporating exogenous enzymes into the feed to increase digestibility, thereby improving growth performance and feed conversion ratio **(Indariyanti & Aprilia, 2022; Kusmiatun *et al*., 2022; Amiin *et al*., 2023)**.

Previous studies have reported that shrimp exhibit a relatively low ability to digest commercial feed due to limited enzymatic activity in the digestive tract. In response, many farmers have adopted the use of commercial probiotics to improve feed quality. However, these interventions have not yielded optimal results **(Tacon, 1987; Hidayat, 2017)**, likely due to the limited variety of enzymes present in commercial probiotic products. To address this, probiotics with a broader enzymatic profile—including protease, amylase, cellulase, and lipase—are needed **(Liang *et al*., 2021)**.

Rumen microbes offer a promising alternative, as they contain a variety of beneficial microorganisms, including cellulolytic, amylolytic, proteolytic, acid-utilizing, sugar-utilizing, and methanogenic bacteria. These microbes secrete a broad spectrum of enzymes capable of breaking down complex compounds such as fiber, starches, and lipids. These enzymes include α-amylase, galactosidase, hemicellulase, cellulase, xylanase, lipase (for fat hydrolysis), and phytase (for phytate hydrolysis) **(Won *et al*., 2020; Hinsu *et al*., 2021; Hua *et al*., 2022; Xie *et al*., 2022; Bugoni *et al*., 2023; Liang *et al*., 2023; Mei *et al*., 2023)**.

Research on the application of rumen microbes has demonstrated their potential to improve the nutritional quality of vegetable-based feed ingredients. Studies by **Murni and Darmawati (2016)** and **Murni *et al*. (2017, 2018, 2019, 2021)** have shown that the use of rumen microbes can enhance dry matter and organic matter digestibility, increase the degree of carbohydrate and fat hydrolysis, and reduce crude fiber content in feed materials. Additionally, rumen microbes used as fermentation agents in the bioconversion of vegetable waste have been shown to enhance digestive enzyme activity and growth performance in *L. vannamei*, as well as promoting the growth of tilapia.

Therefore, the use of rumen-derived microbes holds significant potential as a biological strategy to improve the nutritional quality of shrimp feed, making it a valuable component in sustainable and cost-effective aquaculture practices.

MATERIALS AND METHODS

The research was conducted through several structured phases. The initial phase involved media preparation and experimental layout, which took place at the Brackish Water Aquaculture Research Centre and Fisheries Extension Services in July 2024. In August 2024, pre-digestion testing of shrimp feed using rumen microbes was conducted at the Laboratory of the Aquaculture Study Programme. Subsequent analyses—including proximate composition, dry matter digestibility (DMD), organic matter digestibility (OMD), and soluble protein measurements—were carried out at the KAN-accredited

Integrated Laboratory of the Faculty of Animal Science, Hasanuddin University, Makassar. Additionally, Fourier Transform Infrared (FTIR) spectroscopy was performed at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University, to identify biochemical functional groups in the fermented feed samples.

Preparation of rumen microbes

The microbes used in this study were rumen microbes sourced from cow rumen fluid collected at local slaughterhouses. The rumen content was filtered using cotton cloth at a controlled temperature of 4°C. The microbial suspension was centrifuged at 2,000rpm for 15 minutes **(Sing *et al*., 2022)**. Total microbial populations were then estimated using turbidity and plate counting techniques.

Fermentation process

A total of 100 grams of shrimp feed was mixed with 4.5mL of rumen microbial suspension (4.5mL/ 100g feed) using a 10mL pipette. The mixture was thoroughly homogenized, sealed in airtight plastic containers, and incubated for the designated treatment durations of 10, 15, or 20 minutes. To terminate enzymatic activity after fermentation, the samples were steamed at 60°C for one minute. Proximate analysis followed the AOAC (2005) procedures, while FTIR analysis was conducted using a Shimadzu IRPrestige-21 spectrometer.

Experimental design

This study employed a Completely Randomized Design (CRD), consisting of three fermentation time treatments, each replicated three times:

- **Treatment A**: Fermentation time of 10 minutes
- **Treatment B**: Fermentation time of 15 minutes
- **Treatment C**: Fermentation time of 20 minutes

Parameters and data analysis

Proximate analysis

Feed samples were analyzed for protein, fat, ash, fiber, carbohydrate, and moisture content using standard proximate methods as defined by the **AOAC (2005)**.

- Protein was determined using the Kjeldahl method,
- Fat using the Soxhlet extraction method,
- Moisture and ash using the gravimetric method,
- Carbohydrate was calculated by difference from the proximate components.

Soluble protein testing

Soluble protein content was measured at the end of the fermentation period following the method of **Lowry *et al*. (1951)**. Briefly, 0.5g of fermented feed was treated to eliminate crude protease activity, then mixed with 1.5mL of 5% trichloroacetic acid and left at room temperature. Subsequently, 3mL of Tris-HCl buffer (pH 6.5) was added.

The mixture was centrifuged at 10,000 rpm for 20 minutes, and the supernatant was analyzed for soluble protein content using a Kjeltec 8400 Nitrogen Analyzer (FOSS, Hoganas, Sweden)

Analysis of organic matter digestibility and dry matter digestibility

The analysis of dry matter and organic matter digestibility refers to the method of **Minson and McLeod (1972)** and **Lunagariya *et al*. (2017)** and was calculated based on formulas (1) and (2) as follows:

$$\text{Dry Matter Digestibility} = \frac{\text{DM fresh sample (g)} - \text{DM residual sample (g)}}{DM\ fresh\ sample}) \text{ x100\%} \quad (1)$$

$$\text{Organic Matter Digestibility} = \frac{\text{OM fresh sample (g)} - \text{BO residual sample (g)}}{OM\ fresh\ sample}) \text{ x100\%} \quad (2)$$

Information: DM = Dry Matter; OM = Organic Matter; DM sample weight = sample weight × %DM; DM residue = weight after oven-drying with filter paper (crude protein determination); Blank = weight of filter paper after oven-drying without sample; OM sample weight = DM sample weight × %OM; %OM = 100% DM − (% ash content in DM); OM residue = weight after oven-drying − weight after ashing in furnace − filter paper weight.

Analysis fourier transform infrared (FTIR)

Sample analysis using Fourier Transform Infrared (FTIR) spectroscopy was conducted to identify the functional groups present in the test feed. For this purpose, 2mg of the feed sample (in pellet form) was homogenized with 200mg of potassium bromide (KBr) to form a transparent pellet. The infrared spectrum of the sample was then measured using an FT-IR Spectrometer (Spectrum S One, Model C69526, PerkinElmer) connected to a PC equipped with OPUS software. The measurement was carried out in the infrared (IR) region of 400– 4000cm^{-1}.

The absorption spectra obtained from the sample were displayed as a function of wavelength and subsequently converted into a tabulated format for data correction and interpretation. The absorption values were normalized, setting the highest absorption to 1 and the lowest to 0, to facilitate consistent comparison across treatments.

Data analysis

The results from the chemical analysis of the feed—comprising dry matter digestibility (DMD), organic matter digestibility (OMD), soluble protein, and proximate components (crude protein, crude fat, nitrogen-free extract [NFE], crude fiber, and ash)—as well as the FTIR data, were statistically analyzed using analysis of variance (ANOVA). Where significant differences were found, the analysis was followed by Duncan's multiple range test at a 95% confidence level, using SPSS version 26. To determine the optimal fermentation duration for each parameter, a quadratic polynomial regression analysis was performed.

RESULTS

Digestibility of dry matter and organic matter

Table (1) presents the results of research on dry matter digestibility, organic matter digestibility, and soluble protein of fish feed using rumen microbes with different incubation times.

Table 1. Digestibility of dry matter, digestibility of organic matter, and soluble protein of fish feed fermented using rumen microbes with different incubation times

Parameter	Treatments		
	A (10 minutes)	B (15 minutes)	C (20 minutes)
Digestibility of dry matter (%)	76.21±0.01[a]	77.56±0.03[b]	77.98±0.01[c]
Digestibility of organic matter (%)	75.64±0.02[a]	75.645±0.03[a]	76.37±0.52[b]
Soluble protein (%)	25.36±0.02[a]	25.58±0.01[b]	26.76±0.02[c]

Note: Different superscript letters (a, b, c) within the same row indicate statistically significant differences ($P < 0.05$).

Fermentation of shrimp feed using rumen microbes with varying incubation times had a significant effect ($P< 0.05$) on dry matter digestibility (DMD), organic matter digestibility (OMD), and soluble protein content, as shown in Table (1). Results from Duncan's multiple range test indicated significant differences among treatments for each parameter. The highest values were recorded at an incubation time of 20 minutes, with dry matter digestibility at 77.98%, organic matter digestibility at 76.37%, and soluble protein at 26.76%.

To further illustrate the relationship between fermentation time and these nutritional parameters, a quadratic polynomial graph depicting the interaction between incubation time (in minutes) and DMD, OMD, and soluble protein content is presented in Fig. (1).

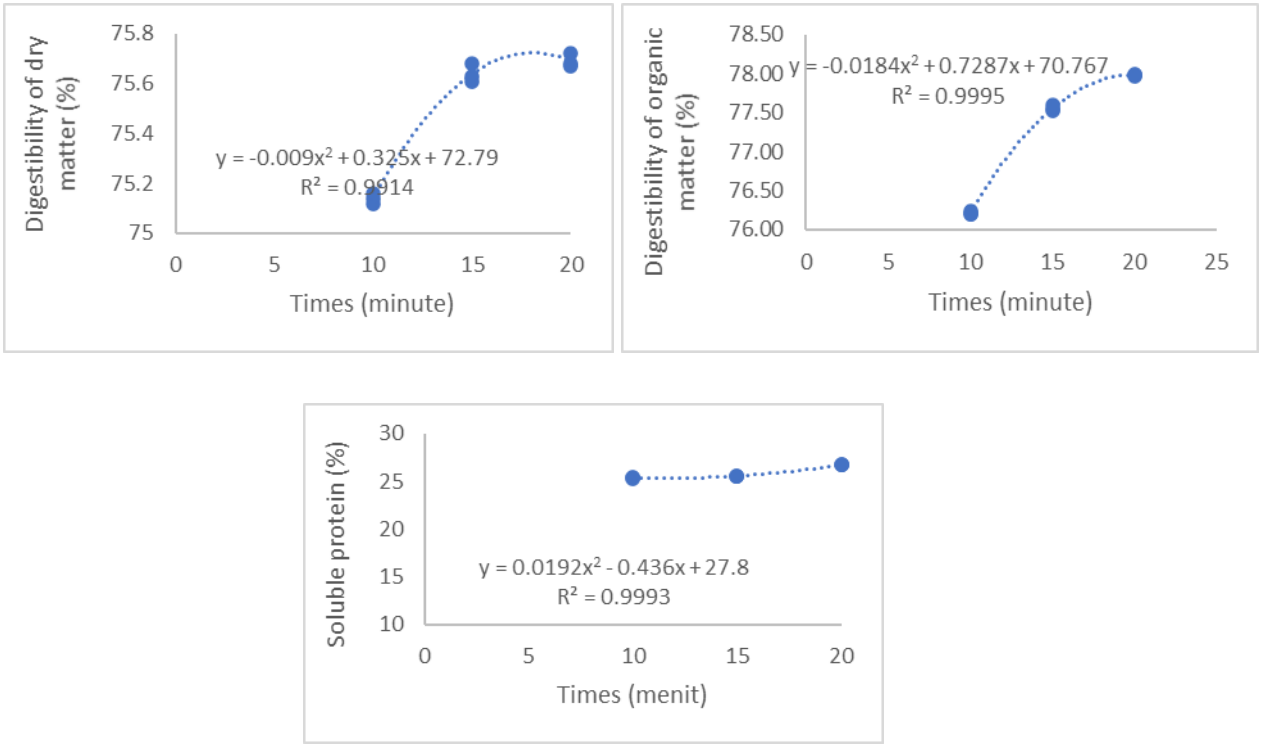


Fig. 1. Quadratic polynomial graph of the relationship between time (minutes) and dry matter digestibility (%), organic matter digestibility (%), and soluble protein (%) of feed fermented using rumen microbes at different incubation times

Based on Fig. (1), orthogonal polynomial analysis shows the relationship between time (minutes) and dry matter digestibility, organic matter digestibility (%), and soluble protein of feed fermented using rumen microbes at different times. The optimal point for dry matter digestibility is 84.41%, with a time of 19.70 minutes. The optimal point for organic matter digestibility is 72.80%, with a time of 0.06 minutes. The optimal point for dissolved protein is 27.64%, with a time of 0.09 minutes.

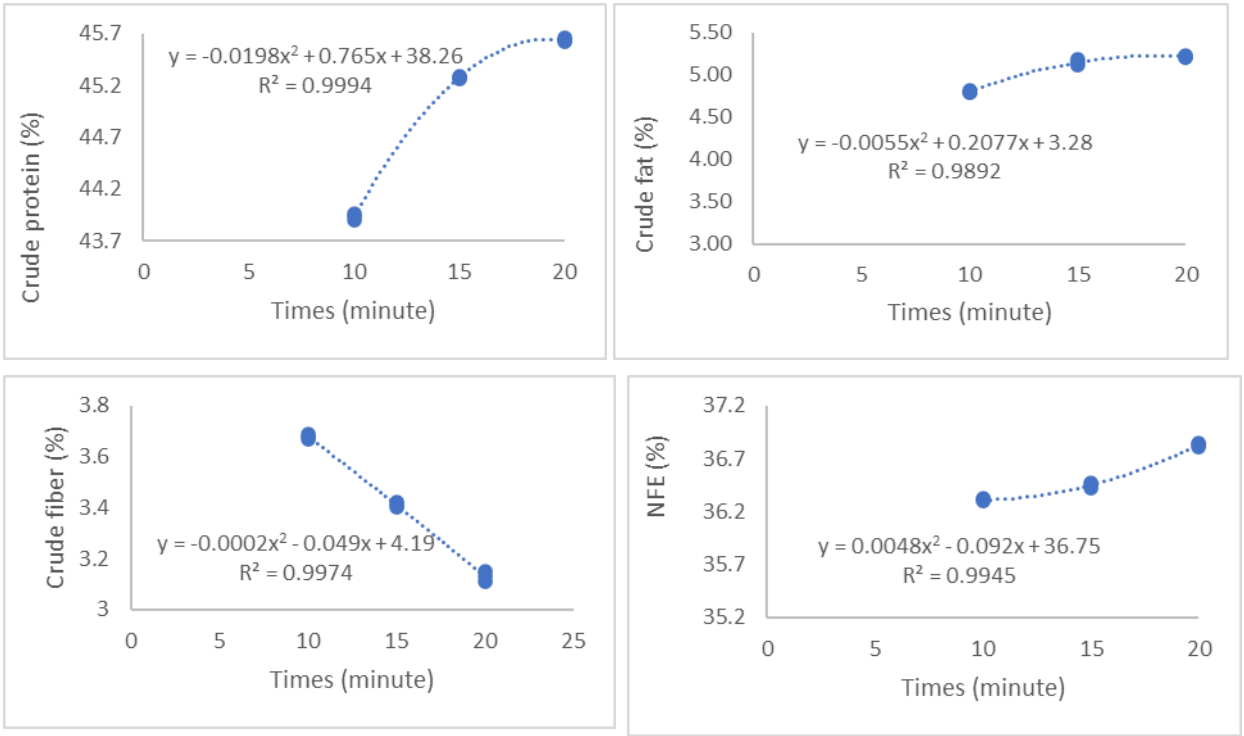
Proximate analysis feed

Table (2) presents the results of the proximate analysis of shrimp feed fermented by rumen microbes with different incubation times.

Table 2. The results of the proximate analysis of shrimp feed fermented by rumen microbes with different incubation times

Parameter	Treatments		
	A (10 minutes)	15 (minutes)	C (20 minutes)
Crude protein	43.93±0.03^{a}	45.28±0.01^{b}	45.64±0.02^{c}
Crude fat	4.80±0.01^{a}	5.15±0.03^{b}	5.55±0.01^{c}
Crude fiber	3.68±0.01^{c}	3.41±0.01^{b}	3.13±0.01^{a}
NFE	36.31±0.01^{a}	36.45±0.02^{b}	36.83±0.02^{c}
Ash	10.98±0.01^{c}	9.42±0.01^{b}	8.96±0.02^{a}

The results of variance analysis showed that the fermentation of shrimp feed using rumen microbes at different times had a significant effect ($P< 0.05$) on crude protein, crude fat, crude fiber, NFE, and ash. Further tests using Duncan showed differences in treatment for crude protein, fat, fiber, NFE, and ash (Table 2). The highest crude protein, fat, fiber, NFE, and ash were obtained at 20 minutes of incubation time. The lowest crude fiber was obtained at 20 minutes of incubation, while the lowest crude protein, crude fat, NFE, and ash were at 10 minutes. A quadratic polynomial graph of the relationship between time (minutes) and crude protein, crude fat, crude fiber, NFE, and ash (%) of shrimp feed fermented using rumen microbes at different times is presented in Fig. (2).



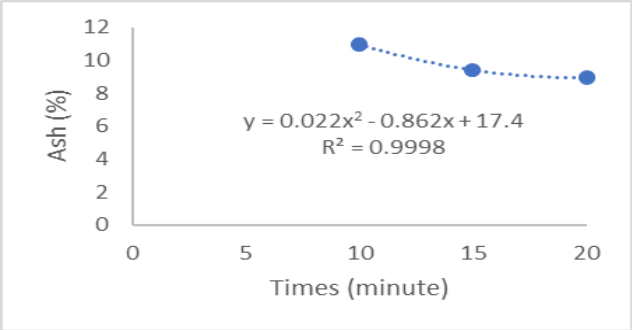


Fig. 2. Polynomial quadratic graph of the relationship between time (minutes) and crude protein, crude fat, crude fiber, NFE, and ash (%) of feed fermented using rumen microbes at different times

Based on Fig. (2), the results of the orthogonal quadratic polynomial analysis show a strong relationship between incubation time (minutes) and various chemical properties of shrimp feed fermented with rumen microbes.

- **Crude protein**
  The quadratic regression equation obtained was $Y = -0.0918X^2 + 0.765X + 38.26$ with $R^2 = 0.9994$, indicating a very strong correlation. The optimal crude protein content of 39.78% was achieved at an incubation time of 12 minutes. The R^2 value suggests that 99.94% of the variation in crude protein is explained by fermentation time, while only 0.06% is influenced by other factors.
- **Crude fat**
  The regression equation was $Y = -0.0055X^2 + 0.2077X + 3.26$ with $R^2 = 0.9892$. The optimal crude fat content of 3.39% occurred at an incubation time of 0.53 minutes. The R^2 indicates that 98.92% of the variation in crude fat is due to fermentation time.
- **Crude fiber**
  The regression equation for crude fiber was $Y = -0.0002X^2 - 0.049X + 4.19$ with $R^2 = 0.9974$. The predicted optimal point was 4.19% at 0.008 minutes, suggesting minimal fiber breakdown occurred with longer incubation. The model accounts for 99.74% of the variation in fiber content.
- **Nitrogen-free extract (NFE)**
  The equation was $Y = 0.0048X^2 - 0.092X + 36.75$, with $R^2 = 0.9945$. The optimal NFE value of 36.66% was achieved at an incubation time of 10 minutes, indicating effective carbohydrate availability at this duration.

- **Ash content**

  The regression for ash was $Y = 0.022X^2 + 0.862X + 17.4$, with $R^2 = 0.9998$. The model predicted an optimal ash content of 17.26% at an incubation time of 0.51 minutes. The R^2 value confirms that 99.98% of ash variability was due to incubation time.

Fourier transform infrared (FTIR) analysis

FTIR analysis was conducted to identify functional groups present in the fermented feed samples. The FTIR spectra of all samples (Treatments A–C) exhibited similar absorption patterns, indicating consistent functional group presence across treatments. Each band represents vibrational movements of molecular bonds, associated with specific chemical groups.

- A broad absorption band in the 3400 cm^{-1} region corresponds to O–H stretching, commonly associated with hydroxyl groups from alcohols or carboxylic acids. The relatively narrow shape of the band suggests that it is originated from primary alcohols rather than carboxylic acids.
- Additional confirmation of alcohol presence was noted with absorption in the 1020–1030 cm^{-1} region, representing C–O stretching vibrations typical of primary alcohols.
- A double absorption band around 2900 and 2800 cm^{-1} indicates C–H stretching of sp^3-hybridized carbon atoms (alkanes). This is supported by a band near 1450 cm^{-1}, associated with CH_3 group bending vibrations, confirming the presence of alkane chains.
- A strong absorption band at 1740 cm^{-1} was identified as C=O stretching, indicating the presence of ester compounds.
- Absorption bands in the 1600 and 1500 cm^{-1} regions signify C=C stretching vibrations of aromatic rings, while the absence of sharp bands near 3100 cm^{-1} suggests these are substituted aromatic compounds rather than free aromatics.
- The presence of an aromatic ether group (C–O–Ar) is confirmed by a band at 1240 cm^{-1}, while a non-aromatic ether group (C–O) is indicated by a band at 1150 cm^{-1}.
- Finally, absorption bands at 570 and 520 cm^{-1} suggest the presence of haloalkanes (C–X).

These FTIR results confirm the biochemical transformation of feed compounds during fermentation, including the formation of esters, alcohols, alkanes, ethers, and substituted aromatic compounds.

A summary of the functional groups and corresponding wavenumbers identified in Fig. (3) is presented in Table (3).

Table 3. Summary of wave number data along with the functional groups identified in the figure above as a whole

Number	Function group	Wave Number (cm^{-1})			
		A (control)	B (10 minutes)	C (15 minutes)	D (20 minutes)
1.	O-H	3444	3475	3448	3496
2.	Csp^3-H	2924 dan 2854	2924 dan 2854	2926 dan 2854	2924 dan 2854
3.	C=O	1741	1741	1743	1741
4.	C=C	1645 dan 1541	1643 dan 1541	1653 dan 1544	1645 dan 1543
5.	CH_3	1454	1454	1458	1456
6.	C-O	1242	1246	1246	1244
7.	C-O	1155 dan 1080	1153 dan 1076	1155 dan 1074	1155 dan 1082
8.	C-O	1028	1022	1022	1031
9.	C-X	572 dan 524	570 dan 524	572 dan 522	572 dan 526

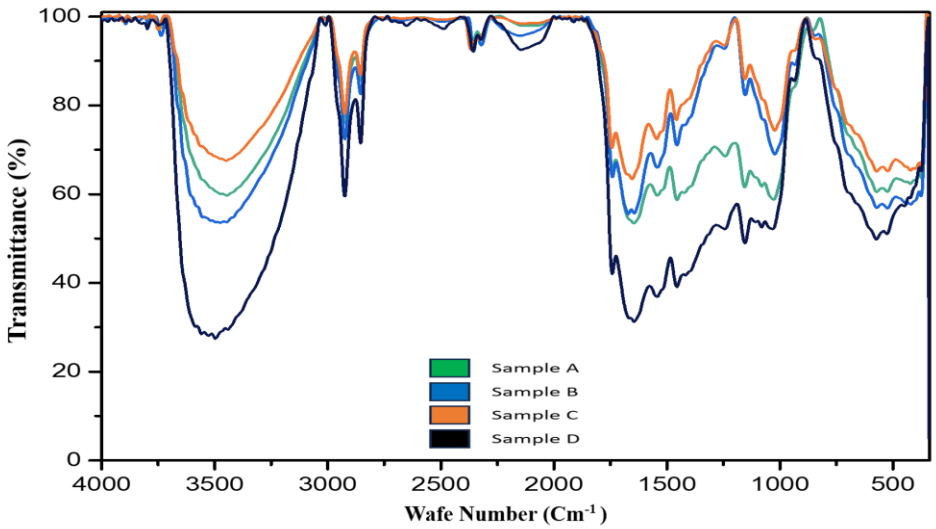


Fig. 3. Functional group analysis/FTIR of shrimp feed (A) Without Fermentation, (B) 10-minutes incubation, (C) 15-minutes incubation, (D) 20-minutes incubation using rumen microbes

DISCUSSION

Digestibility of dry matter, digestibility of organic, and soluble protein

Digestibility of both dry matter and organic matter increased with longer incubation times during fermentation of shrimp feed using rumen microbes. The highest values were recorded at an incubation time of 20 minutes, with dry matter digestibility reaching 77.98% and organic matter digestibility 76.37%. This result indicates that prolonged incubation allows rumen microbes to produce more hydrolytic enzymes, which, through

synergistic activity, effectively break down feed components **(Liang *et al*., 2023, 2024)**. These findings were further supported by the quadratic polynomial regression test, which predicted a peak dry matter digestibility of 84.41% at 19.70 minutes of incubation.

In contrast, the lowest dry matter digestibility (76.21%) occurred at 10 minutes, likely due to insufficient enzyme production at shorter durations. According to **Wang *et al*. (2022)**, the quantity of substrate and incubation duration are both critical in the fermentation process. If microbial activity is not sustained long enough or if the substrate availability is suboptimal, microbial efficiency is compromised **(Liang *et al*., 2021; Wang *et al*., 2022; Zhao *et al*., 2023)**.

Dry matter and organic digestibility are key indicators of feed quality. **Kearl (1982)** categorized feed based on digestibility levels: low (50–60%), moderate (60–70%), and high (above 70%). The values recorded in this study fall within the high-quality category. **Wang *et al*. (2021)** emphasized that higher digestibility implies better nutrient utilization by aquatic animals. The values reported here exceed those of previous studies using other microbial inoculants or substrates—for example, **Murni *et al*. (2019)** found dry matter and organic digestibility at 60.92% and 57.77%, respectively. On the other hand, **Sondakh *et al*. (2018)** reported 70.37% and 61.96%, and **Vargas-Ortiz *et al*. (2022)** obtained 69.5% and 66.9%. Differences across studies are attributed to variations in fermentation agents, substrates, and microbial diversity.

Soluble protein content

The highest soluble protein content (26.76%) was achieved at 20 minutes of incubation, significantly outperforming shorter incubation times of 10 and 15 minutes. This increase is attributed to higher microbial activity over time, enabling greater hydrolysis of polypeptides into peptides and essential amino acids **(Nugroho *et al*., 2022)**. Conversely, shorter incubation times resulted in reduced soluble protein due to limited enzyme production **(Anwar *et al*., 2023)**.

Proximate composition of fermented feed

Crude protein

The results of variance analysis showed a significant effect of incubation time on crude protein content. The highest crude protein content (45.64%) was recorded at 20 minutes, followed by 45.28% at 15 minutes and 43.93% at 10 minutes. Rumen microbes contribute to this increase by secreting protease enzymes, which hydrolyze proteins into amino acids, enhancing overall protein availability in the feed.

Crude fat

Fat plays a vital role in energy metabolism, survival, and growth in aquatic animals. The highest fat content (5.55%) was found in feed fermented for 20 minutes, followed by 5.15% (15 minutes) and 4.80% (10 minutes). The increased fat content is attributed to lipase enzyme activity from rumen microbes during extended incubation **(Liang *et al*., 2024)**.

Crude fiber

The study also showed a decreasing trend in crude fiber content with increasing incubation time. The lowest fiber content (3.13%) was observed at 20 minutes, which aligns with enzyme activity profiles of cellulase, hemicellulase, and esterase secreted by rumen microbes **(Liang *et al*., 2024)**. Shorter incubation durations (10 and 15 minutes) showed higher fiber content due to insufficient enzymatic breakdown, corresponding to early microbial growth phases **(Gra & Buchhaupt, 2022; Anwar *et al*., 2023)**.

Nitrogen-free extract (NFE)

NFE, which includes rapidly metabolizable carbohydrates like glucose, increased with longer incubation. The highest NFE content (36.83%) was recorded at 20 minutes, compared to 36.45% (15 minutes) and 36.31% (10 minutes). This increase is likely due to the enhanced activity of cellulase and amylase enzymes as the microbial population matured **(Graf & Buchhaupt, 2022; Senanayake *et al*., 2023)**. Prolonged fermentation supports greater hydrolysis of complex carbohydrates, boosting available energy substrates.

Ash content

Ash, which represents the total mineral content, was highest at 10 minutes (10.98%), followed by 9.42% (15 minutes) and 8.96% (20 minutes). The higher ash value in the earlier fermentation phase may result from less organic matter breakdown, leading to a relatively higher concentration of inorganic components.

FTIR analysis of functional groups

FTIR analysis confirmed chemical changes in the fermented feed. A consistent increase in the N–H stretching (amines and carboxylic acids) and a reduction in O–H stretching were observed with longer incubation times. Notably, the O–H stretching wavenumber was highest at 3496 cm^{-1} in feed incubated for 20 minutes, indicating the transformation of hydroxyl-containing compounds. Similar observations were reported by **Anwar *et al*. (2024)**, who found an increase in O–H bond absorption (3289 cm^{-1}) in fermented trembles seed flour using rumen microbes and *Bacillus* spp.

These molecular changes, supported by FTIR results (Fig. 3), reinforce the conclusion that 20-minute incubation enhances enzymatic hydrolysis, nutrient availability, and overall feed digestibility and quality.

CONCLUSION

The research results concluded that fermenting shrimp feed using rumen microbes with different incubation times increased nutritional quality. Means dry organic matter digestibility, soluble protein, crude protein, crude fat, VFE analysis, and FTIR were at their highest at 20 minutes of incubation. The highest crude fiber and ash were obtained at an incubation time of 10 minutes.

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