



Utilizing Silage-Based Bioconversion of Myctophid Biomass to Enhance Protein Content and Nutritional Value of Aquafeed

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Abstract

Myctophids, commonly known as lanternfish, play a pivotal role in the oceanic food web and are one of the most unexploited fishery resources. In this regard, the present study has been carried out to utilize myctophids as an aquafeed ingredient by subjecting them to an ensilaging process. Two myctophid fishes, *N. microchir* and *D. watasei* mixed in a 1:1 ratio (w/w) were subjected to formic acid-mediated ensilaging. Biochemical changes were studied during the ensilaging process of 10 days, with sampling on every alternative day. The degree of hydrolysis (DH) increased throughout the ensilaging process, with the values ranging from 24.1% (0th day) to 59.45% (10th day). The increase in DH indicated the accelerated autolysis process by the formic acid addition. An increase in non-protein nitrogen content during the ensilaging process was found to be significant, and it indicates the hydrolysis of protein. Though the peroxide value has increased during the storage period, the values were within the acceptable range. Aquafeed prepared from the resultant silage by mixing with rice bran in a ratio of 2:1 (w/w) was found to have an increased protein content of 35.33%, suggesting that it can be a potential alternative for the protein source in fish feeds.

Keywords: Ensilaging, degree of hydrolysis, peroxide value

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Introduction

Fish and aquaculture sectors are often recognized for their pivotal role in addressing global food security and malnutrition-related challenges. Further, fish being an economical and affordable source of protein along with micronutrients and essential fatty acids is often promoted as a good nutrient source for a wider population globally. As per the recent global fish production statistics, aquaculture is reported to contribute 59% (130.9 million tonnes, valued at USD 312.8 billion) of the global production of aquatic animals (FAO, 2024). The production statistics indicate the significance of aquaculture as the fastest-growing food production system (Garlock et al., 2020). Over the years, aquaculture production has shown an increasing trend with Asia being the dominant player indicating their significance in the context of food and nutritional security. Further, in the present scenario of stagnant or declining capture fisheries, aquaculture is emerging as a promising solution to meet the demand gap for aquatic food.

The growth of fish is often influenced by the quality of feed, especially its nutritional profile. However, fish feeds account for nearly 60-75% of the total cost of fish production in many of the developing countries (Adéyèmi et al., 2020). The availability of good quality economical feeds is another constraint faced by the aquaculture sector. Hence, to maintain the sustainability of the aquaculture sector, affordable feed ingredients exhibiting potential for growth/immune performance need to be screened. Further, a judicious selection of ingredients has to be followed while formulating aquafeed by focusing on the nutritional and antinutritional factors available in the feed ingredients, their bioavailability, and economical aspects. Among the different feed components, protein is the costliest ingredient

influencing the production cost. Apart from cost, nutritionally protein is the much sought-after feed ingredient, since it can meet the amino acid requirement for the growth of fish. Several protein sources have been screened by researchers for substitution of fish meal in aquafeeds, which are either of plant, animal, and insect origin. The use of seafood processing waste, seaweeds, etc. has also been explored because of their nutritional characteristics (Tejpal et al., 2021). Rajamoorthy et al. (2013) reported the proximate composition of *D. watasei*, viz., moisture, fat, protein, and ash content as 63.19, 15.13, 21.40 and 1.33%, respectively, which indicates the nutritional potential of this species for human consumption and formulation of novel foods.

One such promising source that can be tapped for aquafeed production is myctophid fishes, as they are the most widely distributed and abundant group among the teleost fishes. Nutritionally, they are reported to be rich in protein, lipids, fatty acids, and minerals. Further, they are reported to have a protein content of 11% to 23% with fairly good amounts of essential amino acids (Zhang et al., 2023). Despite the well-proven nutrient profile, they are not employed for human consumption and are often discarded or kept as incidental catch and sold at low prices (Fernandez, Pradeep, Anandan, & Zynudheen, 2014). However, there lies a promising potential for the exploration of myctophids as a protein source in aquafeed formulation is immense. Fish silage is defined as a liquid product obtained from the whole fish or parts of it, to which acids, enzymes, or lactic-acid-producing bacteria are added. Liquefaction is then provoked by the action of enzymes from the fish (Dos Santos, da Silva, Zinani, Wander, & Gomes, 2015). Hence, this can also be employed for aquafeed formulation as a potential nutrient source for which myctophids can be a very good source. The present paper hence focuses on using myctophid as an aquafeed ingredient by subjecting it to protein concentration using an ensilaging process and investigating the nutrient profile and the biochemical changes of the product.

Materials and Methods

Two myctophid species (*Neoscopilus microchir* and *Diaphus watasei*) were collected from the Kollam harbor, India, and were brought to the Department of Biochemistry and Nutrition, Central Institute of Fisheries Technology (CIFT) Cochin in a Polypropylene container of 20L capacity. The commercial-

grade formic acid (85% strength) was purchased from Industrial suppliers, and rice bran used for co-drying was purchased from local feed suppliers. All the chemicals used in different analysis were of analytical grade.

Moisture, crude protein, lipid and ash content of the selected myctophid species were determined according to the method of AOAC (2009).

N. microchir and *D. watasei* were mixed in a ratio of 1:1 (w/w) in a blender and accordingly, a mince was prepared. For preparation of silage, 3% commercial grade formic acid (v/w) was added to the minced meat to lower the pH to 4 and mixed thoroughly to ensure proper mixing. Samples were stored at room temperature (26–28°C) throughout the experiment (10 days). During the storage period, silages were stirred daily to aid the hydrolysis. The proximate composition of the silage prepared from *N. microchir* and *D. watasei* were determined according to the method of AOAC, (2009).

The silage prepared was subjected to detailed biochemical analysis during the entire silaging process of 10 days with a sampling on every alternative day. The rate of protein hydrolysis was estimated as per the methodology described by Hoyle (1994). It was expressed as the degree of hydrolysis (Equation 1).

Degree of Hydrolysis =

$$\frac{\text{TCA soluble N in the sample}}{\text{Protein N in the sample}} \times 100 \quad (1)$$

Peroxide value (PV) was employed for measuring the primary lipid oxidation level and the iodometric method described by (Jacobs, 1951) was employed for measuring the peroxide value. The extent of protein breakdown during the ensilaging process was measured by analyzing the non-protein nitrogen (NPN) content as per the method described by (Lo, Liao, & Gao, 1993) and was expressed as mg nitrogen per 100g sample.

After the end of liquefaction, the silage sample was thoroughly mixed with rice bran (2:1 w/w) and sun-dried for 50h at an average temperature of 35°C. After sun drying, the samples were collected, pulverized, and stored at room temperature for further analysis. Nutrient profiling of the rice bran mixed silage was carried out by following AOAC methods (AOAC, 2009).

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software, version 29, and the results are expressed as mean \pm standard error. Analysis of variance (ANOVA) was performed to understand significant difference among the mean values. The level of significance was set at $p \leq 0.05$. Duncan's Multiple Range Test (DMRT) was used to determine the significant differences between the different treatments.

Result and Discussion

Two myctophid species, *N. microchir* and *D. watasei* were subjected to detailed nutrient composition analysis to investigate their potential as an aquafeed ingredient. The results are presented in Table 1.

Table 1. Proximate Composition (%) of two myctophids species, *N. microchir* and *D. watasei* on a dry weight basis

Constituents (%)	<i>N. microchir</i>	<i>D. watasei</i>
Crude protein	69.33 \pm 0.54	58.83 \pm 0.83
Crude fat	19.91 \pm 0.10	24.40 \pm 0.52
Ash	8.60 \pm 0.26	7.79 \pm 0.25

(Data expressed as mean \pm SE, n=3)

N. microchir had a significantly higher crude protein (69.33%) compared to *D. watasei* (58.83%). This translates to a 21.3% difference in protein content. On the other hand, *D. watasei* had a significantly higher crude fat content (24.4%) than *N. microchir* (19.91%). The ash content of *N. microchir* was considerably higher (8.6%) than *D. watasei*. The result obtained from the proximate analysis reveals that the *N. microchir* stood out with a significantly higher crude protein content, suggesting it could be a valuable source of essential amino acids and protein sources for the aquafeed formulation, compared to *D. watasei*. The higher fat content of *D. watasei* indicates its potential as a lipid-rich feed ingredient compared to *N. microchir* (Collins, Piatkowski, & Saunders, 2021)

(Fernandez et al., 2014) reported protein content in the range of 14.45-16.54% (wet weight basis) in *D. effulgens* and *D. hudson* suggesting the protein richness of myctophid fishes. (Chai et al., 2012) compared the nutrient composition of two myctophid species, *Benthosema pterotum* and *Diaphus splendidus*, and reported protein content of 14.7 & 12.56%, lipid content of 2.14 & 9.13% and ash content of 4.21 &

1.84%, respectively. In terms of lipid content, *D. splendidus* was reported to be superior suggesting as valuable lipid resource.

Studies have reported varying levels of protein, fat, and ash content in *D. watasei*, likely due to differences in methodology and sample origin. (Sebastine et al., 2011) reported 15.62% protein, 11.71% fat, and 0.47% ash (wet weight basis) in the edible portion of the benthopelagic fish, *D. watasei*. (Shaviklo, 2020) documented the nutrient profile of selected myctophid fishes and noticed wide variations in the nutrient composition with respect to different genera. The lipid content of myctophid fishes was found to vary from 2.4 to 28% with the highest lipid content reported for *Symbolophorus californiensis* followed by *Notoscopelus japonicus*. Similarly, the protein content was reported in the range of 11.5 to 22.3% whereas, ash content ranged from 0.5 to 11.5 to 22.3%. (Økland, Stoknes, Remme, Kjerstad, & Synnes, 2005) reported that other deep-sea fishes like *Macrourus berglax*, *Mora moro*, *Centroscymnus coelolepis*, *Etmopterus princeps*, etc. contained less than 1% fat and protein content between 16.7%-22.6%. In general, it was evident from the study and relevant literature that myctophids can be used as excellent protein sources owing to their promising content.

The fatty acid profiles of the myctophids *N. microchir* and *D. watasei* were examined to determine the presence of essential fatty acids like omega-3 and omega-6, which are required for human health and fish physiology. The fatty acid profile for *N. microchir* and *D. watasei* is given in Table 2.

Fatty acid profiling indicated that *N. microchir* have a higher content of polyunsaturated fatty acids. Among PUFA, Docosahexaenoic acid (DHA, C22:6) (23.12%), was found to be the most abundant fatty acid followed by Eicosapentaenoic acid (EPA, 20:5). Oleic acid (C18:1) (23.1%) and palmitic acid (C16) (17.99%) were found to be the most abundant monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA), respectively. The presence of DHA and EPA adds to a significant overall PUFA content in *N. microchir* suggesting that *N. microchir* could be a valuable source of omega-3 polyunsaturated fatty acids, which are essential for human health and brain development.

In the case of *D. watasei*, three dominant fatty acids were found; among which C16 (palmitic acid, 19.54%), C18:1 (oleic acid, 35.26%) C22:6

(Docosahexaenoic acid, 16.09%) were found to be the most dominant SFA, MUFA and PUFA, respectively. These three fatty acids together make up over 70% of the total fatty acid content in the sample. PUFA such as eicosapentaenoic acid (EPA, C20:5) and arachidonic acid (C20:4) are also present, contributing 4.88% and 2%, respectively. Other fatty acids were also present in lower quantities, including linoleic acid (C18:2), and other smaller chain and unsaturated fatty acids.

(Økland et al., 2005) reported that the content of PUFA varied from 48% to 63% out of total fat content among the different species of myctophids examined with C16:0 (palmitic acid), C18:1 (oleic acid) and C22:6 (DHA) being the dominant SFA, MUFA, and PUFA, respectively. (Navaneethan et al., 2014) reported that the fatty acid profile of *D. watasei* oil comprised of 37.49, 42.49 & 14.07% SFA, MUFA, and PUFA, respectively. Similar to our results, DHA was reported as the most dominant PUFA followed by EPA. The study also highlighted that when a comparison was made between *Sardinella longiceps* and *D. watasei*, the latter had significant amounts of

oleic acid and DHA suggesting their nutritional richness.

Chai et al. (2012) compared the fatty acid profile of two myctophid species, *Benthosema pterotum*, and *Diaphus splendidus*, and reported that palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n – 9), and docosahexaenoic acid (C22:6n – 3; DHA) as the most dominant fatty acids present in both the species. Further, *B. pterotum* was reported to have high ratio of MUFA and PUFA with a n3/n6 ratio of 7.8. In general, it can be reported that the selected myctophid species are par with the teleost fishes in terms of their nutrient potential qualifying them to be used as aquafeed ingredients.

The peroxide value (PV) of silage has been studied during the entire period of silaging (10 days). Peroxide value (Table 3) has increased over the storage period with an initial value of 0.14 to 0.37 meq oxygen/kg of fat. Though PV has increased during the storage period, the values are within the acceptable range. Sajib, Langeland, & Undeland (2022) reported that the peroxide value of herring

Table 2. Fatty acid profile of the myctophid species, *N. microchir* and *D. watasei*

Carbon Number	Fatty Acids	% of FA in terms of Total FA	
		<i>N. microchir</i>	<i>D. watasei</i>
Saturated fatty acids (SFA)			
C14	Myristic acid	3.86	3.62
C15	Pentadecyclic acid	0.69	0.33
C16	Palmitic acid	17.99	19.54
C17	Margaric acid	0.78	0.57
C18	Stearic acid	3.93	6.68
Monounsaturated fatty acids (MUFA)			
C16:1	Palmitoleic acid	6.20	2.84
C17:1	Cis-10 Heptadecenoic acid	1.20	0.71
C18:1	Oleic acid	23.1	35.26
C20:1	Gadoleic acid	1.33	2.30
Polyunsaturated fatty acids (PUFA)			
C18:2	Linoleic acid	1.63	0.79
C18:3	á-Linolenic acid	0.83	0.45
C20:2	Cis-11,14 – Eicosadienoic acid	0.49	0.34
C20:4	Arachidonic acid	3.00	2.00
C20:5	Eicosapentaenoic acid	7.06	4.88
C22:6	Docosahexaenoic acid	23.12	16.09

silage decreased significantly for a period of 7 days and afterward showed an increasing trend. The reason for the decreased peroxide value was reported as the effect of the addition of antioxidants, which might have imparted protection from oxidation. In the present study, no antioxidants were added, and any antioxidant peptides produced during the silaging process may not be effective per se in taking care of lipid oxidation. In line with our assumption, Sajib et al. (2021) reported that the inspite of the formation of short chain antioxidative peptides, the peroxide value and TBARS value has increased significantly during the ensilaging process of herring by-products. (Sajib & Undeland, 2020) reported that the PV and TBARS has shown an increasing trend for the first seven days of herring ensilaging process. (Ozyurt et al., 2020) reported that by the addition of synthetic antioxidants, the lipid oxidation of silage can be reduced.

When the degree of hydrolysis (DH) was analyzed, it was clear that DH values increased throughout the ensilaging process. The DH value measured on the first day of silaging was (Table 3) 24.25% and on the 10th day, DH was 59.15%. (Hoyos-Concha, Villada-Castillo, Fernandez-Quintero, & Ortega-Toro, 2022) reported a high DH value of 62.95% for high protein hydrolyzed flour of *Onchorhynchus mykiss* by products. In general, the high rate of hydrolysis can be related to the autolysis process, which might have accelerated with the formic acid addition. Further the ensilaging process was carried out at the

ambient conditions where temperature was around 32-35°C, which is favorable for the growth and proliferation of endogenous proteolytic bacteria. Sajib, Trigo, Abdollahi, & Undeland (2022) reported an increase in DH in case of silage stored at 22°C when compared to that stored at 4°C. This indicates that temperature also plays a significant role in determining the DH as it directly influences the activity of enzymes involved in the autolysis process. (Mohanty, Majumdar, Mohanty, Mehta, & Parhi, 2021) reported that the DH was influenced by several factors such as ensilaging time, temperature, enzyme to substrate ratio etc. Sajib et al. (2022) has reported a DH value of 38% after two days of ensilaging at ambient temperature conditions.

Non-protein nitrogen (NPN) content was observed to increase during the ensilaging process considerably. The initial NPN content (Table 3) was recorded as 421.66mg nitrogen/100g and after the 10th day the value has increased to 810.57mg nitrogen/100g. Tazim et al. (2021) reported similar results stating that the NPN value has increased during the ensilaging process. The increase in NPN value can be correlated to the breakdown of peptide bonds and the subsequent increase in free amino acid content. The increase in NPN values can be correlated to the accelerated rate of hydrolysis. Similar effects of increase in NPN was already reported by Gallardo et al. (2012). Ozyurt et al. (2020) also reported that in case of silage prepared from discarded fish with lactic acid bacteria, the

Table 3. Biochemical changes during the ensilaging process

Number of days	Non-protein nitrogen content (mg Nitrogen/100g)	Degree of Hydrolysis (%)	Peroxide value (PV) (meq oxygen/kg of fat)
1	421.66±0.29 ^a	24.25±0.28 ^a	0.14±0.006 ^a
2	490.78±0.28 ^b	26.91±0.33 ^b	0.19±0.006 ^b
3	521.86±0.44 ^c	32.23±0.52 ^c	0.21±0.006 ^c
4	591.99±0.43 ^d	38.80±0.54 ^d	0.24±0.006 ^d
5	622.00±0.47 ^e	42.75±0.36 ^e	0.27±0.006 ^e
6	673.34±0.39 ^f	49.67±0.30 ^f	0.29±0.006 ^f
7	759.45±0.44 ^g	53.58±0.38 ^g	0.32±0.003 ^g
8	789.03±1.0 ^h	54.79±0.30 ^h	0.33±0.003 ^h
9	798.64±0.80 ⁱ	56.77±0.40 ⁱ	0.33±0.003 ^h
10	810.57±0.73 ^j	59.15±0.53 ^j	0.37±0.003 ⁱ

Data expressed as mean±SE, n=3; Means in the row with different superscripts are significantly different (p≤0.05)

Table 4. Proximate composition of silage produced from *N. microchir* and *D. watasei*

Constituents (%)	Proximate composition (%)		
	Minced Myctophid	Myctophid Silage (Prepared with 3% formic acid)	Feed Mixture of Myctophid Silage and Rice bran
Moisture	74.23±0.63 ^b	79.05±0.53 ^c	13.29±0.35 ^a
Crude protein	15.3±0.57 ^a	15.28±0.52 ^a	34.43±0.62 ^b
Crude fat	6.83±0.34 ^b	5.33±0.09 ^a	4.8±0.06 ^a
Ash content	2.83±0.41 ^b	0.89±0.02 ^a	13.11±0.2 ^c

(Data expressed as mean±SE, n=3; Means in the row with different superscripts are significantly different (p≤0.05))

NPN value has increased considerably and stated that the use of acid may aid in more liquefaction due to high protein solubilization.

After the ensilaging process, the silage obtained was used for aquafeed preparation. For this, the prepared silage was thoroughly mixed with rice bran (2:1 w/w) and sun dried for 50h at average temperature of 35°C. This was then subjected to a basic nutrient profiling to compare the changes in the basic biochemical constituents with that of the minced myctophid and silage prepared out of it. The proximate composition of the minced myctophid, silage and the feed mixture is shown below (Table 4).

The proximate analysis of minced myctophid, myctophid silage, and a combination of myctophid silage and rice bran were analyzed. The moisture content was highest in the minced myctophid (74.23±0.63%) due to its fresh state. Similar to our results, Palkar et al. (2017) also reported a moisture content value of 77.09%. However, after the ensiling process, the moisture content has increased (79.05±0.53%). The addition of rice bran decreased the moisture content to considerably lower levels of 13.29±0.35% making it a concentrated feedstock and improving storage stability.

The myctophid silage showed a decrease in protein content (15.3±0.57%) compared to minced myctophid (15.28±0.52%). Palkar et al. (2017) reported a similar result stating that the protein content has decreased from 15.2% to 13.03% and 12.82% in silages prepared from 3.5 and 4.5% formic acid, respectively. Protein might have broken down during the ensiling process resulting in a lower value. However, it was observed that adding rice bran to the myctophid silage increased the protein content

(34.43±0.62%) and it can be due to the concentration of ingredients that occurred due to drying of feed mixture.

The crude fat content showed a similar trend to crude protein, where the myctophid silage (5.33±0.09%) showed a slight decrease when compared to minced myctophid (6.83±0.34%). The addition of rice bran (4.8±0.06%) resulted in a further decrease in the fat content of the combined silage.

On the other hand, the ash content differed across the samples. The highest value was found in minced myctophid (2.83±0.41%). The ensiling process reduced the ash content in the myctophid silage (0.89±0.02%), whereas the incorporation of rice bran increased the ash content in the feed mixture (13.11±0.2%).

The present study was carried out to bio valorize myctophid fishes, *N. microchir* and *D. watasei* as an aqua feed ingredient by subjecting to formic acid mediated ensilaging process. Ensiling of 10 days resulted in a degree of hydrolysis 59.45% suggesting the enhanced autolysis process by virtue of the acid silaging process. Though the peroxide value has increased over the storage period, the values were within the acceptable range suggesting the oxidative stability of the product. Further, the aquafeed mixture prepared from the silage by mixing the rice bran showed an enhanced protein content of 35.33% suggesting that it can serve as an affordable and sustainable alternative for protein source in aquafeed preparation. It can be hence concluded that myctophids can be effectively valorized for aquafeed preparation by focusing on the protein concentration as protein is one of the most sought out constituent in aquafeeds. The study shows potential

to use myctophids as a bioresource for aquafeed preparation. However, to establish this, comparative feed trials analysis has to be carried out using commercial aquafeed as reference and need to be carried out with special emphasis on the quality, palatability and the economic aspects. Nutritional impact assessment of the developed feed using fish models will further establish their safety and efficacy as an aquafeed ingredient by studying its growth and physio-metabolic changes.

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