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STORAGE STABILITY OF AN ORNAMENTAL FISH DIET ENRICHED WITH PALMYRAH FRUIT (*Borassus flabellifer*) PULP

Sutharshiny SATHYARUBAN[1]*, Deepthi Inoka ULUWADUGE[2], Shivatharsiny YOHI[3], Sivashanthini KUGANATHAN[1]

[1] *Department of Fisheries, Faculty of Science, University of Jaffna, 40000, Northern Province, Sri Lanka.*
[2] *Department of Basic Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura, Nugegoda, 10250, Western Province, Sri Lanka.*
[3] *Department of Chemistry, Faculty of Science, University of Jaffna, 40000, Northern Province, Sri Lanka.*

Sutharshiny Sathyaruban: ssutharshi@univ.jfn.ac.lk, ORCID ID: https://orcid.org/0000-0002-5540-8578
Deepthi Inoka Uluwaduge: deepthiuluwaduge@yahoo.com, ORCID ID: https://orcid.org/0000-0002-0024-5054
Shivatharsiny Yohi: yshiva@univ.jfn.ac.lk, ORCID ID: https://orcid.org/0000-0003-3920-1478
Sivashanthini Kuganathan: sivashanthini@univ.jfn.ac.lk, ORCID ID: https://orcid.org/0000-0002-3005-2325

**Corresponding author: Sutharshiny SATHYARUBAN, ssutharshi@univ.jfn.ac.lk, +94774727648*

Abstract

The ornamental fish industry depends on high-quality diets for health and growth. Many available or formulated feeds degrade over time, reducing nutritional value, palatability, and cost-effectiveness. Over ten months, this study investigates the storage stability of an ornamental fish diet enriched with a novel feed additive, Palmyrah fruit (*Borassus flabellifer*) pulp. The quality of the experimental feed was assessed in terms of its nutritional composition, microbial properties, and cost-effectiveness, which were compared with the commercial diet. Users' perception of the experimental diet was assessed using a questionnaire. Statistical analysis was performed using Minitab Statistical Software 2021. The results showed that the experimental feed is not harmed by aflatoxin contamination (20 µg kg^{-1}) and total bacterial count (1.5 x 10^3 cfu g^{-1}). The protein content of experimental and commercial feeds remained constant for ten months (42.17 ± 0.06 and 40.53 ± 0.37, respectively), although the fat content of the experimental feeds did not differ significantly ($P < 0.05$) at the three storage periods. A downward trend in the proportion of all fatty acids in both feed types was observed after ten months of storage. Despite a rise in moisture, it remained under 10%, preventing microbial growth. The stakeholders have been receptive to the experimental feed for ornamental fish, which is 22 times more cost-effective than commercial feed on a small scale. Our findings indicate that the experimental feed remains of stable quality for ten months of storage under

room temperature (25 - 33 ºC), which offers a sustainable and cost-effective solution for the aquaculture industry.

Keywords: Aflatoxin, microbial properties, nutritional composition, physical properties

Introduction

The storage stability of fish feed is a crucial factor in aquaculture operations. The quality of fish feed can deteriorate over time due to various factors, including oxidation, moisture, and microbial activity (Pandey, 2016). Ornamental fish are an economic importance worldwide, and popular among hobbyists and researchers because of their attractive colouration (Ahilan & Kamalii, 2022). Feed colour additives are essential pigments for livebearers. They not only enhance the color of ornamental fish but also play a vital role in their overall health (Von Lintig et al., 2020).

The experimental feed in this study incorporates palmyrah fruit, which contains carotenoids (Priyadarshani & Jansz, 2014) and is widely found in the Northern, Eastern, and coastal North-West regions of Sri Lanka (Thillainathan & Inoka, 2019). Despite its potential benefits, palmyrah fruit has yet to be utilised globally for preparing ornamental fish feed (Sathyaruban et al., 2021). There is only one documented case of using palmyrah fruit for guppy fish in Sri Lanka (Sathyaruban et al., 2024). However, maintaining the shelf life of the experimental feed enriched with palmyrah fruit pulp presents significant challenges, especially given the climate conditions in tropical countries. The challenge is further complicated because palmyrah fruit is only available seasonally, while the feed may need to be stored for an entire year. To effectively utilise formulated fish feed in aquaculture systems, it is essential to study the feed's comprehensive nutrient composition, physical properties, microbial contamination, toxicity, and storage stability (Waghmare et al., 2022). A thorough analysis of the shelf life of this experimental feed is necessary before it can be marketed. Existing studies have determined the shelf life of formulated fish diets to be between two to ten months, depending on the nutrients, packaging materials, and storage conditions (Royes & Chapman, 2003; Hossen, 2013; Venugopal & Keshavanath, 2022). Thus, this study aims to investigate the storage stability of an experimental feed enriched with palmyrah fruit pulp, including an analysis of nutritional and microbial changes over a ten-month storage period.

Material and Method

Experimental feed formulation

A total of 11 fish feed ingredients were used in this study (Table 1). The soybean meal and maize were dust-free cleaned and ground into a fine powder in disk mill (FFC-23, China). All the powdered samples were stored in a sterilized container. Ripe palmyrah fruits were collected in the Northern region of Sri Lanka, and the pulp (PFP) was extracted manually without adding water from the ripe fruit.

The selected raw ground ingredients' nutrient composition [Moisture, ash, gross energy, digestible energy, crude fat, and crude protein] was determined using AOAC (2019) for formulating the experimental feed. Dry fish feed was formulated using a linear programming model at a 40% protein level (Velasco-Santamaría and Corredor-Santamaría, 2011). First, soybean meal and fish meal were mixed. Mazie, fish oil and wheat flour were gradually added. The feed ingredients were homogenized for 10 min in the mixer (AS – 40 Atlas). The required volume of the tap water was added, and the mixture was then slowly added into the mixed ingredients before adding mineral and vitamin premixes, DL- Methionine, L-Lysine, and Di-

Calcium phosphate. The wet mixtures were pelleted using a feed pellet machine (AKP 40 China). The dry feed pellets having similar diameters were processed in the machine. The pellets were sprayed with fish oil and dried in a forced-air oven (GEMMY-YCO-010) at 45 ºC for six hours.

The experimental feeds were kept in labelled sterilized containers at room temperature (25 to 33 ºC). Commercial feed (produced in China) was purchased from the local market and used as a test control.

Table 1. Nutrient compositions ((mean ± SD) %) of raw ingredients

Nutrient compositions	**FM**	**SB**	**Ma**	**WF**	**FO**	**PFP**
Moisture (%)	90.2 ± 0.1	87.7 ± 1.5	87.3 ± 0.9	88 ± 1	---	82 ± 0.5
Crude protein (% DW)	72.1 ± 0.7	41.4 ± 1.3	9.7 ± 0.3	10.8 ± 0.5	---	0.9 ± 0.04
Crude Fat (% DW)	10.8 ± 0.3	3.8 ± 0.07	3.9 ± 0.2	1.7 ± 0.07	100	0.3 ± 0.01
Gross Energy (kcal kg^{-1})	4833.3 ± 50.6	4220.3 ± 56.6	4021.7 ± 26.8	3864.3 ± 55.8	---	1026 ± 3.46
Digestible energy (kcal/kg^{-1})	3474.3 ± 22.3	2073.7 ± 109.5	2073.7 ± 109.5	2777.7 ± 48.5	--	ND
Fiber (% DW)	2.2 ± 0.1	6.5 ± 0.1	2.7 ± 0.1	0.8 ± 0.03	---	1.6 ± 0.03
Calcium content (g100g^{-1})	4.28 ± 0.03	0.27	0.017	0.318 ± 0.01	---	ND
Phosphate content (g100g^{-1})	3.2 ± 0.1	0.511	0.06	0.133 ± 0.01	---	ND
Ash (% DW)	13.16 ± 0.15	6.07 ± 0.06	1.2 ± 0.1	0.62 ± 0.03	---	0.83 ± 0.04

DW- Dry weight; FM – Fishmeal; FO-Fish oil; Ma- Mazie; PFP - Palmyrah fruit pulp; SB- Soybean; WF- Wheat flour, ND- Not determined

Evaluation of Feed Quality

Nutrient Quality

AOAC (2019) determined the nutrient composition of raw materials, experimental and commercial feeds. Total carotenoid content in experimental diets and commercial diets was performed according to the modified method described by Torrissen and Naevdal (1988). Homogenous samples of the diets (5 g) were taken and transferred to a motor and pestle. A small amount (2 g) of celite was added to it. The sample was ground and powdered well by adding 40 mL of cold acetone. It was transferred in a 100 mL conical flask (wrapped with aluminium foil) and closed tightly with a lid to minimize evaporation. The mixture was homogenized and mixed in a magnetic stirrer for 30 minutes. It was filtered with suction through a Buchner funnel with filter paper. The mortar and pestle, funnel, and residue of the sample were washed with 10 mL acetone and filtered through the funnel. The extraction was continued until the residue was devoid of colour. The mixture was centrifuged at 3500 rpm for

5 min and stored at 4 °C for 24 hours. The absorption of cooled supernatant was measured at 450 nm by UV-Vis Spectrophotometer. Petroleum ether was used as a blank.

The total carotenoid content (µg/g) was calculated using the following equation 3.11.

$$= [\text{Absorption} \times \text{dilution factor}] / [A_{1cm}^{1\%} \times \text{Sample weight (g)}]$$

Where, $A_{1cm}^{1\%}$ = Extinction coefficient (0.25)
A= Absorbance
volume = Total volume of extract.

To perform efficient extraction of carotenoids from the samples, all extractions were carried out at yellow light / dim light environment, temperatures at or below ambient, and all acetone solvents were cooled on ice prior to use.

Toxicity Level and Microbial Count
The aflatoxin level of the experimental feed was determined by ASTA (American Spice Trade Association) Method 24.2 (1986) with HPLC at Bureau Veritas Consumer Products Services Lanka (Pvt) Ltd., Sri Lanka.
The total bacterial count (cfu g^{-1}) was determined for the experimental and commercial feeds by following the dilution plate technique. Bacteria grown on nutrient agar medium were incubated for 48 h at 30 ºC (AOAC, 1990).

Storage Stability of Experimental Feed
The nutrient chemical composition [Moisture, crude fat (Soxhlet method), and crude protein (Kjeldahl method)], fatty acid profile (AOAC, 2012 -Trans esterification-FAME) of experimental feed fresh (0-month) and four-month storage feed was determined using AOAC (2019). After a ten-month storage, the experimental and commercial feeds were brought to the Norwegian College of Fishery Science, UiT, Norway and kept at 4ºC for further analysis. Moisture content (AOAC, 2019), Ash content (AOAC, 2019), crude fat (Folch et al., 1957), and fatty acid profile (AOAC, 2012 -Trans esterification-FAME) were determined.

Cost-Effectiveness for Diet Preparation
For this, the local retail sale market price of all the dietary ingredients at the time of the study was taken into account, and converted to US dollars (in 2021). The total expenditure incurred for formulating the experimental diets was then determined and compared with the cost of the commercial diet.

User's Perception
The user's perception was conducted by distributing questionnaires to various stakeholders, such as ornamental fish farmers, university students, staff, and administrative officers. Participants were given detailed information about the experimental diet, including its purpose, ingredients and potential health benefits. They were then asked to follow the diet for a specified period of time and give feedback through structured questionnaires and interviews.

Statistical Analysis
Data for all measured parameters were analysed using Minitab statistical software 2021. Variables are presented as mean ± standard deviation (SD). A two-sample t-test was used to compare differences between the two feed qualities. The storage stability data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance. Tukey's significant means

test was employed to identify groups that were significantly different. Statistical differences were considered when $P < 0.05$.

Results

Nutritional composition of commercial and experimental feeds

The nutrient requirements of ornamental fish probably vary based on the species, size, and environmental conditions (Jobling, 2012; Velasco-Santamaría & Corredor-Santamaría, 2011). Table 2 provides the nutrient composition of the both feeds.

Table 2. Nutrient composition of experimental and commercial feeds

Parameters	Experimental Feed*	Commercial Feed*
Moisture Content (%) (w/w)	3.42 ± 0.16	4.70 ± 0.04
Ash Content (%) (w/DW)	8.11 ± 0.24	8.32 ± 0.05
Gross Energy (MJ/kg)	17.95 ± 0.13	18.27 ± 0.40
Digestible Energy (MJ/kg)	12.49 ± 0.36	12.77 ± 0.29
Crude Protein (%) (w/DW)	42.08 ± 0.24	41.26 ± 0.18
Crude Fat (%) (w/DW)	5.22 ± 0.13	5.97 ± 0.18
Total Carotenoid Content (µg/g)	96.46 ± 7.53	100.24 ± 0.80

*All values are the mean of three replicates (n = 3).
w/w – Weight in total weight, w/DW – Weight in dry weight, ER- Expansion ratio, BD – Bulk density, WAI- Water absorption index, WSI- Water solubility index

Crude protein had the highest content of nutrient composition in experimental and commercial feeds. Feeds had a balanced crude protein level of 36 - 41%, with lipid content varying between 5.22% experimental and 5.9% commercial feed. The level of the TCC varies (5.95 to 100.28 µg/g) between the feeds. According to the current study, there is no significant difference ($P < 0.05$) in the crude protein, ash, moisture, digestible energy, and gross energy levels between the experimental and commercial feeds. In contrast, the crude fat content was higher in the commercial feed than in the experimental feed. However, both feeds meet the nutritional requirements of the selected ornamental fishes (Jobling, 2012). The present observations of the nutrient chemical composition of formulated feed are comparable to the previous studies on the feed of ornamental fish species: Guppy fingerlings (Mohanta & Subramanian, 2011), Guppy brood stock (Suting et al., 2013), Swordtail fingerling (Mohanta & Subramanian, 2011), Platy fish fry (Sapkale et al., 2017), Black molly (Mohanta & Subramanian, 2011). According to our study, the total carotenoid content of the experimental feed (96.46 ± 7.53 µg g^{-1}) showed a similar value to the total carotenoid content of the commercial feed.

Storage stability of the commercial and experimental feeds

Assessing the storage stability of a new product in the small-scale aquaculture industry is crucial as it could affect the nutrients' physical, chemical, or biological properties in the feed ingredients (Wagde et al., 2018). Our findings indicate that the experimental feed remains of stable quality after ten months of storage, with no significant changes in the moisture, protein, fat, aflatoxin, and total bacterial count. However, a few studies recorded that the experimental fish feed could be stored for four to twelve months. Venugopal and Keshavanath (2022) formulated dry fish feed incorporating fish meal, fish silage, or a mixture of taro leaves (Colocasia) for carp culture, which showed good keeping quality for up to three months under room temperature, coupled with satisfactory physical properties (Venugopal & Keshavanath, 2022). Based on the findings by Royes and Chapman (2003), the formulated fish feed can be stored in a double freezer bag but should be discarded after six months (Royes and Chapman,

2003). Similarly, Hossen (2013) recorded that the sealed feedstuff at two different temperatures (Room temperature -25-30°C and low temperature 5-8°C) can be utilized for the African catfish for up to two months (Hossen et al., 2013).

Moisture Content

Monitoring moisture content during storage is essential since it can impact the feed's physical and chemical properties and possibly lead to microbial contamination (Rezaei & VanderGheynst, 2010). Figure 1 illustrates the changes in moisture content (%) of both experimental and commercial feeds over different storage durations: initial, four, and ten months.

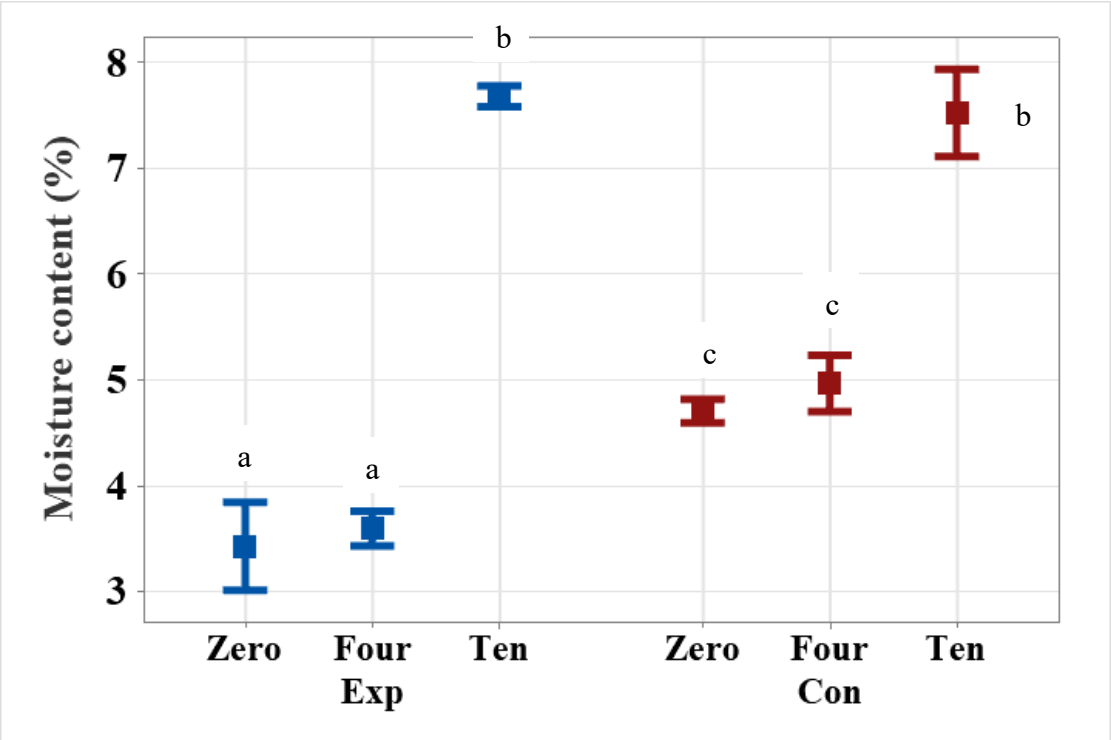

Figure 1. Effect of storage time (Zero-month, Four-month, and Ten-month) on the moisture content of experimental (Exp) and commercial (Com) feeds. Values are presented as mean ± SD. Values having different letters are significantly different ($P < 0.05$)

At zero months, the moisture content for the experimental feed was recorded at 3.43 ± 0.01%, while the commercial feed showed a higher moisture level of 4.73 ± 0.54%. As the storage period progressed, both feeds exhibited a significant increase in moisture levels, indicated by the statistical significance ($P < 0.05$) of the results. By the end of the ten-month storage period, the moisture content for the experimental feed rose to 7.68%, compared to 7.52% for the commercial feed. The rise in moisture content could be due to environmental factors. High humidity levels in the storage area result in moisture absorption (Snow et al., 1944). Inadequate temperature and humidity control in the storage space can allow moisture to penetrate, thereby elevating the water content of the feed. Additionally, certain ingredients in fish feed, particularly those that are hygroscopic (capable of absorbing moisture), can contribute to increased moisture content over time. Ingredients like grains and pelleted forms may have inherent moisture-absorbing properties (Buchanan & Moritz, 2009). Despite the increases in moisture content over time, it's noteworthy that both feeds maintained moisture levels below

10% throughout the storage duration, which is critical in discouraging microbial growth (Vera Zambrano et al., 2019). BIS (1999) ensures that feed moisture levels should not exceed 12% to prevent microbial growth and toxic production by potential pathogens. The graph effectively highlights the trend of rising moisture in both feed types while emphasizing the overall stability of moisture levels within acceptable limits. Several strategies can be implemented to prevent fluctuations in moisture content during storage, including proper Packaging, controlled storage conditions, use of antioxidants.

Crude Fat Content

In Figure 2, the impact of storage on the fat content of both feeds at three different points is displayed.

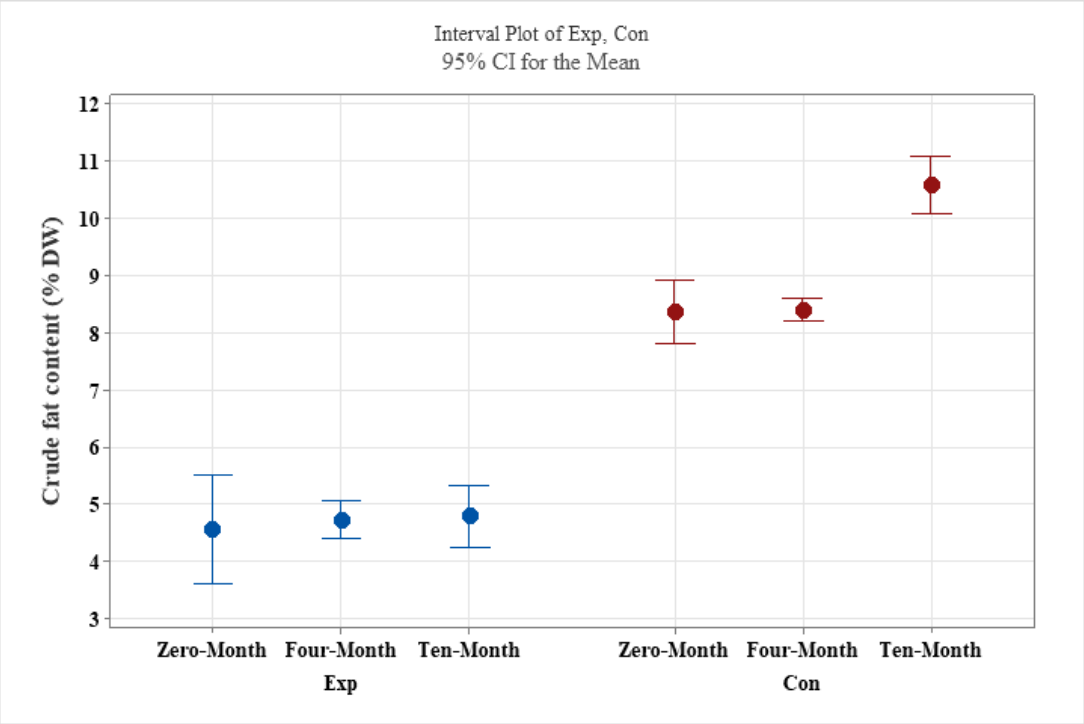

Figure 3. Effect of storage duration (Zero-month, Four-month, and Ten-month) on the fat content of experimental (Exp) and commercial (Com) feeds.

The initial fat content of the formulated and commercial feed was 4.5 ± 0.16% and 8.39 ± 0.20% respectively. The results show that after ten months, the total fat content of the formulated and commercial feed was found to be 4.80% ± 0.22 and 10.57% ± 0.81, respectively. The fat content of the experiment feeds did not differ significantly ($P < 0.05$) during the three storage periods. However, there was a significant difference in commercial diets at 10-month storage, compared to zero and four months. Our research on fish feed found that both feeds had the highest crude fat content after ten months of storage. This was surprising because our Soxhlet method had a lower extraction efficiency than the Folch method. Our findings support past research (Fonti-Furnols et al., 2015), which showed that the hexane solvent in the Soxhlet method is less effective at isolating the polar lipid fraction from the samples. In contrast, the Folch method uses a miscible mixture of polar and non-polar solvents. Further, one possibility is that the formulations have been adjusted to enhance energy density or improve palatability, leading to higher fat inclusion. Additionally, if the feed has been stored improperly, the breakdown of other components may result in an apparent increase in fat concentration as moisture content escalates. In fact, most of the previous studies reported that the fat content in the feed was

reduced at the end of the storage due to the breakdown of oil and vitamins and the lipid component's peroxidation (Siddhuraju & Becker, 2003). However, to prevent these nutrient breakdowns, feed manufacturers have attempted to utilise natural beneficial antioxidants (Siddhuraju & Becker, 2003). The present study utilized palmyrah fruit to achieve skin colouration. Apart from this, the fruit also functions as an antioxidant by providing a hydrogen molecule to the lipid peroxyl radical, which prevents the start of a new cycle in the auto-oxidation reaction chain. This ingredient is rich in carotenoids, and hence, it can be protected against oxidation (Pathberiya & Jansz, 2005). The study concludes that the carotenoid component of the palmyrah fruit can be effectively utilized for its antioxidant properties.

Fatty Acid Profile

Table 3 illustrates the fatty acid profile of the experimental and commercial feeds at zero, three, and ten months of storage.

Table 3. Fatty acid profile of experimental and commercial feeds at zero and ten-month storage

Fatty acids	Experimental feed			Commercial feed		
	Zero-month	Three-month	Ten-month	Zero-month	Three-month	Ten-month
Saturated						
C14:0 Myristic acid	4.71	4.21	0.134	3.04	3.00	0.192
C15:0 Pentadecanoic acid	0.97	0.96	0.45	0.90	0.84	0.61
C16:0 Palmitic acid	22.71	21.54	18.11	18.70	16.22	12.77
C17:0 Heptadecanoic acid	0.92	0.91	0.61	1.04	1.00	0.45
C18:0 Stearic acid	3.75	3.35	2.23	2.75	2.44	1.79
C20:0 Arachidic acid	0.20	ND	0.11	0.30	0.20	0.023
∑	**33.06^{a}**	**30.97^{a}**	**21.08^{b}**	**25.39^{a}**	**23.70^{a}**	**15.22^{b}**
Monounsaturated						
C16:1 Palmitoleic acid	3.4	3.3	1.13	2.75	2.55	1.84
C18:1 Oleic acid	16.49	15.68	14.52	23.2	23.1	21.52
∑	**19.89^{a}**	**18.98^{a}**	**15.65^{b}**	**25.95^{a}**	**25.65^{a}**	**23.36^{b}**
Polyunsaturated						
C18:2 Linoleic acid (ω-6)	18.39	17.25	10.9	27.5	22.5	15.96
C18:3 n-3 Linolenic acid (ω-3)	0.36	ND	0.16	0.12	ND	0.02
C20:5 Eicosapentenoic acid (EPA) (ω-3)	1.6	1.2	0.025	1.2	1.1	0.039
C22:1 (n=11)	ND	ND	0.025	ND	0.012	0.039
C22:5 -n-3 Docosapentenoic acid (ω-3)	0.5	0.2	0.4	0.4	0.2	0.3
C22:6 n-3 Docosahexenoic acid (DHA) (ω-3)	6.3	6.1	5.2	7.3	7.0	6.1
∑	**27.5^{a}**	**24.75^{a}**	**11.485^{b}**	**36.52^{a}**	**30.812^{b}**	**22.479^{c}**

ND- Not detected. Values in the same raw of that each feed having different superscript letters are not significantly different ($P < 0.05$).

Six types of saturated fatty acids are measured, including notable ones such as palmitic acid (C16:0), which is predominant in both feed types at zero months but shows a marked decrease by ten months. The total saturated fatty acids for the experimental feed decreased significantly ($P < 0.05$) from 33.06% to 21.08%, while for the commercial feed, it declined from 25.39% to 15.22%.

In the monounsaturated fatty acid category, the experimental feed contains higher levels of palmitoleic acid (C16:1) and oleic acid (C18:1) at zero months compared to the commercial feed. However, both feed types exhibited a decrease in total monounsaturated fatty acids over the ten-month storage period, dropping from 19.89% to 15.65% for the experimental feed and from 25.95% to 23.36% for the commercial feed.

The polyunsaturated fatty acids section reveals significant changes, particularly with linoleic acid (C18:2), showing a substantial decrease in both feeds after ten months of storage. The total polyunsaturated fatty acids decreased for the experimental feed from 27.5% to 11.485% and for the commercial feed from 36.52% to 22.479%, indicating that prolonged storage negatively affects these essential fatty acids, which are particularly susceptible to oxidation due to their high content of unsaturated fatty acids (Filipe et al., 2023).

According to Table 3, a considerable loss of fatty acids was observed in both feeds after ten-month storage. The possibility of microbial degradation, variations in feed composition, or altered metabolic pathways may play a role. Potential influences could include its susceptibility to enzymatic activity or the stability of other co-occurring fatty acids that may protect or enhance its retention. Our findings are in agreement with Secci and Parisi (2016); Wazir et al. (2021). However, the PUFA (C18:4, n=3; C22:1, n=11; C22:6, n=3) detected after the ten-month storage. In the present study, experimental feed contained Elaidic acid (C18:1 n-9) and palmitic acid (C16:0), which were most abundant and are reported to be important fatty acids for fish (Özogul & Özogul, 2007).

Overall, the profile illustrates a downward trend in the proportion of all fatty acids in both feed types after ten months of storage, highlighting the potential impact of storage duration on the nutritional quality of the feeds. Furthermore, the recommendations on strategies to protect fatty acids in feeds involve elements such as antioxidant supplementation, optimising storage conditions to minimise exposure to light and heat, and proper formulation techniques to enhance fatty acid stability during the storage and feeding phases.

Crude Protein

The protein content under storage conditions for ten months is presented in Figure 3. The results in our study showed that the crude protein content (%DW) was recorded with 42.03 ± 3.15 for experimental feed and 40.31 ± 0.96 for commercial feed at the zero-month. It was increased at 42.17 ± 0.06 and 40.53 ± 0.37, respectively, in ten-month storage before falling slightly in four-month storage. However, these changes were not statistically significant with respect to that of storage. The observation regarding the protein content is indeed intriguing. While the present study would typically expect protein levels to remain stable or increase due to potential degradation over time, fluctuations can occur due to various factors, including storage conditions, temperature, and exposure to moisture. This finding aligns with the research by Waghmare et al. (2022), which indicated that the crude protein concentration remained stable over 12 months of storage for feed enriched with microalgal biomass (*Picochlorum* sp.). In contrast, Camacho-Rodríguez et al. (2018) suggested that the total protein levels in fish feed incorporating NAN (*Nannochloropsis*) and ISO (*Isochrysis*) showed a gradual decrease from

the outset.

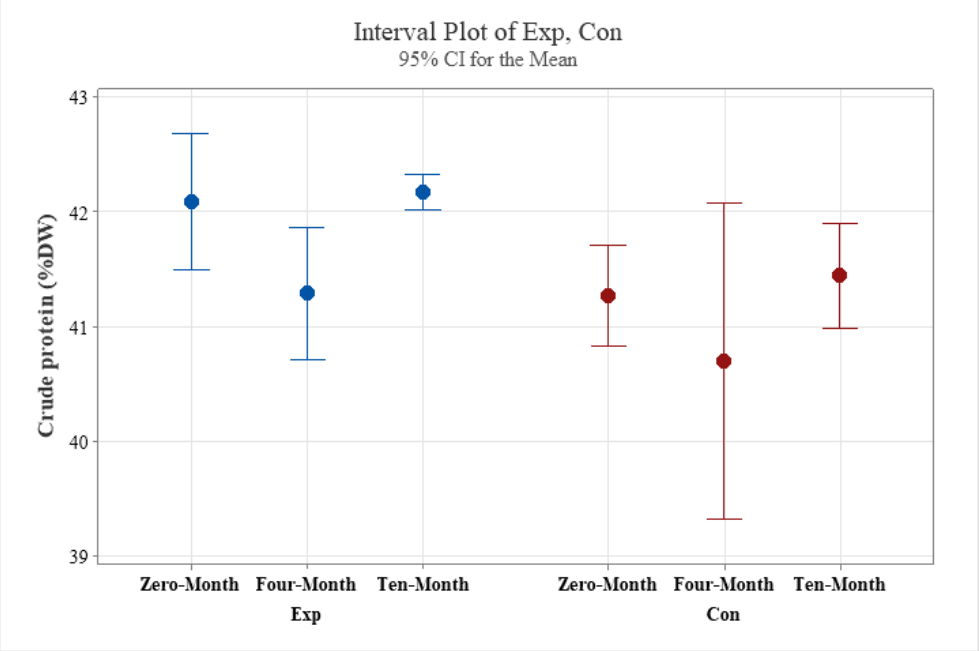

Figure 4: Effect of storage duration (Zero-month, Four-month, and Ten-month) on the protein content of experimental (Exp) and commercial (Com) feeds.

Aflatoxin and Total Bacterial Count

Feed contamination and toxicity are challenging factors in every newly formulated feed, particularly in tropical countries (Marijani et al., 2019). The findings indicate that the locally produced experimental feed is safe from aflatoxin contamination, as it was found to be below the quantification limit for B1, B2, G1, and G2 at (9.68, 1.33, Not detected, and 0.30 µg/kg) respectively, which is lower than the maximum permissible level (20 µg/kg) given by the European Commission standard for feed ingredients (EC, 2002). Additionally, the total bacterial count in the experimental feed was lower (1.0×10^3 to 2×10^3 cfu g^{-1}) than the Bureau of Indian Standards limit of 104 cfu g^{-1} (Bureau of Indian Standards, BIS, 1999).

However, previous studies from tropical countries reported that fish feeds were contaminated by aflatoxin ranging from 1.83–150 µg kg^{-1} (Marijani et al., 2019; Rokvic et al., 2020). Marijani et al. (2019) reviewed the occurrence of fungi and mycotoxins in formulated fish feeds and their impact on fish health. A study by Rokvic et al. (2020) aimed to determine the mycotoxin levels in carp aquaculture in Serbia. The carp were fed a diet consisting of plant-based products.

Summary of the Respondents

The respondents' (100) feedback on the questionnaire concerning the specially formulated feed enriched with palmyrah fruit pulp for the ornamental fish is summarized in Table 4. The survey aimed to gather opinions and insights from individuals who have experience in caring for ornamental fish. The respondents provided valuable information and perspectives on the formulated feed, which was designed to meet the specific colouration of the ornamental fish.

Many aquarium keepers have small glass and fibreglass tanks equipped with infrastructure, such as aerators, lights, and ornaments. Most people rear a limited number of fish, around 20 in total. However, they regularly clean the tanks five to seven times.

In terms of the economic section, most of the respondents agreed that the experimental feed enriched with PFP is cost-effective. After analysing the feed quality with the respondents, it

was found that the experimental feed has higher quality characteristics and is readily marketable in the local market. However, the texture of the feed needs to be assessed.
All of the respondents highly recommended this product and were asked to provide feedback on its shelf life.

Table 4. Summary of the 100 respondents' feedback on the questionnaires concerning the aquarium setup, economic, environmental, and product features.

Section 1: Aquarium setup		
Type of aquarium setup	Glass	50
	Fiberglass	50
	Plastic	--
Size	Small	50
	Medium	40
	Large	10
Number of fish	<5	--
	5--10	--
	10--15	--
	15--20	80
	20<	20
Species	Guppy	80
	Swordtail	60
	Barbs	50
	Tetra	70
	Gourami	30
	Catfish	10
	Angel	30
Accessories	Filter	100
	Aerator	100
	Ornamental plants	100
	Stones	100
	Ornaments	100
	Lights	80
Cleaning (days)	3--5	
	5--7	100
	7--10	
	>10	
Section 2: Economic		
Cost effective feed	Yes	80
	No	20
Section 3: Environment		
Causing turbidity	Yes	70
	No	30
Turbidity forming (days)	1--2	80

	3--5	20
Feed quality -Odour	Yes	
	No	100
Feed quality -Colour	Yes	80
	No	20
Environmentally friendly	Yes	100
	No	
Section 4: Product Features		
Which feature do you think is more valuable	Colour	10
	Size	100
	Odour	100
	Hardness	100
	Above all	90
	Other than the above	

The present survey examined the aquaculturists' perceptions and acceptance of an innovative fish-fed product enriched with PFP. Some clear evidence emerges from the results. First, the acceptability of an innovative feed product-experimental feed inclusion of the PFP seems more appropriate for most ornamental fish and is characterized by different aspects, from sustainability to food safety.

A few studies have stressed the importance of providing transparent information to increase aquaculturists' awareness of cost-effective feed in aquaculture (Menozzi et al., 2021; Sogari et al., 2022; Spartano & Grasso, 2021). Therefore, this study represents a step toward comprehending even more aquaculturists acceptance of a new product.

Cost-Effectiveness for Diet Preparation

Table 5 shows the production costs (0.52675 US $ per kilogram) of experimental feed.

Table 5. Production cost of experimental feed

Ingredients	**Price US $ per Kg (2021)**	**Required amount (g) of ingredients**	**Price US $**
Fish meal	0.6	290	0.174
Fish oil	EA	20	EA
Soybean meal	0.56	400	0.224
Maize	0.31	80	0.0248
Vitamin	1.79	20	0.0358
Mineral	1.79	20	0.0358
DL-Methionine	3.5	1	0.0035
L-Lysine	2.01	1	0.00201
Di-Calcium phosphate	0.72	5	0.0036
Wheat flour	0.28	83	0.02324
Palmyrah Fruit Pulp	EA	80	
Total		**1000**	**0.52675**

EA: Easily accessible

However, this cost would vary with varying ingredient costs in different seasons of the year and changes in ingredient production methods. The price (11.5 US $ per kg) of the imported feed (commercial feed) is higher than the experimental feed cost. The findings of this study suggest that producing an experimental feed on a small scale is 22 times more cost-effective than using commercial feed due to lower costs and the reliability of raw ingredients (Sutharshiny et al., 2022). If production is to expand to an industrial scale, costs for packing and storing the experimental feed would need to be considered.

Conclusion

In conclusion, the study highlights the significant impact of storage duration on the moisture content, crude fat levels, and fatty acid profiles of both experimental and commercial fish feeds. Monitoring these parameters is crucial to maintaining the stability and quality of feeds throughout their storage life, as increases in moisture content can lead to microbial contamination, while variations in fat content may affect the nutritional value and palatability of the feeds.

Despite the observed increases in moisture and fat content over ten months, both feed types remained within acceptable moisture levels, mitigating the potential for microbial growth. This underscores the importance of effective storage conditions, including proper packaging and temperature control, to sustain feed quality. The research also indicates that both feed formulations maintained a relatively high crude fat content, suggesting potential adjustments in formulation or storage practices to enhance energy density.

Moreover, the decline in saturated fatty acids over time, particularly for the experimental feed, points to the need for continuous evaluation of feed compositions to prevent nutrient degradation. The use of natural antioxidants, such as those derived from palmyrah fruit, reflects innovative approaches to preserving feed quality and highlights the potential benefits of incorporating functional ingredients in feed formulations. Further, it could be stored at room temperatures 25 to 33ºC for up to ten months without adding dehydrating agents.

Overall, these findings provide valuable insights that can guide feed manufacturers and researchers in optimizing storage practices, formulation strategies, and ingredient selection to enhance the nutritional and safety aspects of fish feeds, ultimately supporting sustainable aquaculture practices. Further research is warranted to explore the long-term effects of various storage conditions and formulations on feed quality and efficacy.

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Ethical approval

The research ethical committee of the Faculty Of Science, University Of Jaffna, Sri Lanka (No: AERC/2021/01) reviewed and approved all procedures involving the use of ornamental fish to ensure their optimised handling and minimize animal suffering.

The ethical clearance (ERC-HSS/FGS/2023/10/01) for providing the questionnaire was obtained from the Graduate Studies Ethics Review Committee, Humanities And Social Sciences, Faculty Of Graduate Studies, University Of Sri Jayewardenepura, Sri Lanka.

Informed consent
Not available

Data availability statement
The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing this study.

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Contribution of authors
Sutharshiny Sathyaruban: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Funding Acquisition, Project Administration and Writing - original draft
Shivatharsiny Yohi: Data Curation, Formal Analysis, Visualization, and Writing -review and editing
Deepthi Inoka Uluwaduge: Supervision, Writing -review and editing
Sivashanthini Kuganathan: Resources, Supervision, and Writing - review and editing.

References
Ahilan, B., & Kamalii, A. (2022). Ornamental Livebearers. CRC Press. https://doi.org/10.1201/9781003347323

AOAC. (2019). Official Methods of Analysis, 21st Edition (2019)—AOAC International. *Association of Official Analytical Chemists*, p. 2019. https://www.aoac.org/resources/official-methods-of-analysis-revisions-to-21st-edition/

ASTA Analytical Method 20.1. (1986). Official Analytical Method of the American Spice Trade Association. https://astaspice.org/resources/analytical-methods

Buchanan, N.P., & Moritz, J.S. (2009). Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. *Journal of Applied Poultry Research*, *18*(2), pp.274-283. DOI:10.3382/japr.2008-00089

Bureau of Indian Standards (BIS). (1998). *Food hygiene – general principles – code of practic*e. Manak Bhavan, 9 Bahadur Shah Zafar, Marg, New Delhi. https://law.resource.org/pub/in/bis/S06/is.2491.1998.pdf

Camacho-Rodríguez, J., Macías-Sánchez, M.D., Cerón-García, M.C., Alarcón, F.J. & Molina-Grima, E. (2018). Microalgae as a potential ingredient for partial fish meal replacement in aquafeeds: nutrient stability under different storage conditions. *Journal of Applied Phycology*, *30*, 1049-1059. https://doi.org/10.1007/s10811-017-1281-5

Dahlgren, B. T. (1980). The effects of three different dietary protein levels on the fecundity in the guppy, *Poecilia reticulata* (Peters). *Journal of Fish Biology,* 16(1), 83–97. https://doi.org/10.1111/j.1095-8649.1980.tb03688.x

Filipe, D., Gonçalves, M., Fernandes, H., Oliva-Teles, A., Peres, H., Belo, I., & Salgado, J.M. (2023). Shelf-life performance of fish feed supplemented with bioactive extracts from fermented olive mill and winery by-products. *Foods*, *12*(2),305. https://doi.org/10.3390/foods12020305

Hossen, M., Das, M., Sumi, K., & Hasan, M. (2013). Effect of Storage Time on Fish Feed Stored at Room Temperature and Low Temperature. *Progres Agriculture*, 22 (1 2), 115–122. file:///C:/Users/hp/Downloads/admin,+16473-59657-1-CE.pdf

Jobling, M. (2012). National Research Council (NRC): Nutrient requirements of fish and shrimp: The National Academies Press, Washington, DC, 2011, 376+ XVI pp,£ 128 (Hardback), ISBN: 978-0-309-16338-5.

Marijani, E., Kigadye, E., & Okoth, S. (2019). Occurrence of Fungi and Mycotoxins in Fish Feeds and Their Impact on Fish Health. *International Journal of Microbiology*, 1–17. https://doi.org/10.1155/2019/6743065

Menozzi, D., Sogari, G., Mora, C., Gariglio, M., Gasco, L., & Schiavone, A. (2021). Insects as feed for farmed poultry: are Italian consumers ready to embrace this innovation. *Insects*, *12*(5), p.435. https://doi.org/10.3390/insects12050435

Mohanta, K. N., & Subramanian, S. (2011). Nutrition of common freshwater ornamental fishes. *Technical Bulletin*, 27.

Özogul, Y., & Özogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, *100*(4), 1634–1638. https://doi.org/10.1016/j.foodchem.2005.11.047

Pandey, A., Kaur, V.I., Srivastava, A., Datta, S.N., & Singh, A. (2016). Effect of formulated feeds with different nutrient levels on growth and reproductive performance of molly, *Poecilia sphenops* (Valenciennes). *Animal Nutrition Feed Technology*, 16(1), 61-70. doi: 10.5958/0974-181X.2016.00006.8

Pathberiya, L. G., & Jansz, E. R. (2005). Studies on the Carotenoids and in vitro Antioxidant Capacity of Palmyrah Fruit Pulp from Mannar. *Journal of National Science Foundation Sri Lanka.*, 33(4), 269. https://doi.org/10.4038/jnsfsr.v33i4.2117

Rezaei, F., & VanderGheynst, J. S. (2010). Critical moisture content for microbial growth in dried food-processing residues: Critical moisture content for microbial growth. *Journal of the Science of Food and Agriculture*, 90(12), 2000–2005. https://doi.org/10.1002/jsfa.4044

Rokvic, N., Aksentijević, K., Kureljušić, J., Vasiljević, M., Todorović, N., Zdravković, N. & Stojanac, N. (2020). Occurrence and transfer of mycotoxins from ingredients to fish feed and fish meat of common carp (*Cyprinus carpio*) in Serbia. *World mycotoxin Journal*, 13(4), 545-552. https://brill.com/view/journals/wmj/13/4/article-p545_9.xml

Royes, J.A.B., & Chapman, F.A. (2003). Preparing Your Own Fish Feeds: Cir 97/FA097, 2/2003. EDIS, 2003(6). http://edis.ifas.ufl.edu

Spartano, S., & Grasso, S. (2021). UK consumers' willingness to try and pay for eggs from insect-fed hens. *Future Foods*, *3*,100026. DOI:10.1016/j.fufo.2021.100026

Sapkale, P. H., Patil, S. V., Yadav, S. R., & Gitte, M. J. (2017). Growth performance and feed conversion efficiency of Xiphophorus maculatus (Gunther, 1866) Juveniles at different daily feeding rates. *Ecology Environment and Conservation*, 23 (4): 2125-2128. https://www.researchgate.net/profile/Sv-Patil/publication/331895523

Sathyaruban, S., Uluwaduge, D. I., Yohi, S., & Kuganathan, S. (2021). Potential natural carotenoid sources for the colouration of ornamental fish: A review. *Aquaculture International*, 29(4), 1507–1528. https://doi.org/10.1007/s10499-021-00689-3

Sathyaruban, S., Perera, G.C., Amarasinghe, D.J.M., Uluwaduge, D.I., & Kuganathan, S. (2024). Efficiency of the experimental diet enriched with palmyrah (*Borassus flabellifer* L.) fruit pulp on growth, pigmentation, immune challenge, and breeding performance in

the guppy (*Poecilia reticulata*). *Aquaculture Reports*, *38*, 102285. https://doi.org/10.1016/j.aqrep.2024.102285

Secci, G., & Parisi, G. (2016). From farm to fork: Lipid oxidation in fish products. A review. *Italian Journal of Animal Science*, *15*(1), 124–136. https://doi.org/10.1080/1828051X.2015.1128687

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *Journal of Agricutural Food Chemistry*, 51(8), 2144–2155. https://doi.org/10.1021/jf020444+

Singh, S. K., & Muthukumarappan, K. (2016). Effect of feed moisture, extrusion temperature and screw speed on properties of soy white flakes based aquafeed: a response surface analysis. *Journal of Science Food Agriculture*, 96 (6), 2220 - 2229. https://doi.org/10.1002/jsfa.7339

Snow, D., Crichton, M.H.G., & Wright, N.C. (1944). Mould deterioration of feeding-stuffs in relation to humidity of storage: Part II. The water uptake of feeding-stuffs at different humidities. *Annals of Applied Biology*, *31*(2),111-116. https://doi.org/10.1111/j.1744-7348.1944.tb06220.x

Sogari, G., Menozzi, D., Mora, C., Gariglio, M., Gasco, L., & Schiavone, A. (2022). How information affects consumers' purchase intention and willingness to pay for poultry farmed with insect-based meal and live insects. *Journal of Insects as Food and Feed*, *8*(2),197-206. DOI:10.3920/JIFF2021.0034

Suting, P. S., Mandal, S. C., & Patel, A. B. (2013). Effect of Different Dietary Lipid Sources on Growth and Reproductive Performance of Guppy *(Poecilia reticulata)*. *Israel Journal of Aquaculture. ISR*, *65*: 1-6. http://www.siamb.org.il.

Sutharshiny, S., Deepthi Inoka, U., Sivashanthini, K., Harichandra, K., & Partheepan, T. (2022). Grouping the Potential Local Feed Ingredients for Ornamental Fish Feed based on their Nutrient Composition, Cost, and Availability. *Advances in Technology, 2*(2), 131-138. https://doi.org/10.31357/ait.v2i2.5445

Thillainathan, K., & Inoka, D.U. (2019). *Palmyrah research in Sri Lanka: A way forward*. https://doi.org/10.13140/RG.2.2.16244.60805

Velasco-Santamaría, Y., & Corredor-Santamaría, W. (2011). Nutritional requirements of freshwater ornamental fish: A review. *Revista MVZ Cordoba*, *16*(2), 2458–2469. https://doi.org/10.21897/rmvz.283

Venugopal, M.N., & Keshavanath, P. (2022). Formulation, stability and keeping quality of three pelleted feeds used in carp culture. *Fishery Technology*, 21(1), 11-15. http://hdl.handle.net/1834/33814

Vera Zambrano, M., Dutta, B., Mercer, D. G., MacLean, H. L., & Touchie, M. F. (2019). Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. *Trends in Food Science and Technology*, *88*: 484–496. https://doi.org/10.1016/j.tifs.2019.04.006

Vijayagopal, P., Gopakumar, G. N., & Vijayan, K. K. (2008). Empirical feed formulations for the marine ornamental fish, striped damsel, *Dascyllus aruanus* (Linné 1758) and their physical, chemical and nutritional evaluation. *Aquaculture Research*, *39*(15): 1658-1665. doi:10.1111/j.1365-2109.2008.02039.x

Von Lintig, J., Moon, J., Lee, J., & Ramkumar, S. (2020). Carotenoid metabolism at the intestinal barrier. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1865*(11):158580. https://doi.org/10.1016/j.bbalip.2019.158580

Wagde, M. S., Sharma, S. K., Sharma, B. K., Shivani, A. P., & Keer, N. R. (2018). Effect of natural β-carotene from carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) on

colouration of an ornamental fish—Swordtail. *Journal of Entomology Zooliogical Studies*, 6(5): 2112-2115. www.entomoljournal.com

Waghmare, A. G., Chugh, N., Sagaram, U. S., Arun, S., Menon, D., Subhash, G. V.,Nagle, V., Dattaroy, T., & Dasgupta, S. (2022). Characterization of storage stability of microalgal biomass for its applications as protein feed ingredients in animal and aquafeeds. *Animal Feed Science Technology*, 288: 115323.https://doi.org/10.1016/j.anifeedsci.2022.115323

Wazir, H., Chay, S. Y., Ibadullah, W. Z. W., Zarei, M., Mustapha, N. A., & Saari, N. (2021). Lipid oxidation and protein co-oxidation in ready-to-eat meat products as affected by temperature, antioxidant, and packaging material during 6 months of storage. *RSC Advances*, *11*(61), 38565–38577. https://doi.org/10.1039/D1RA06872E