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"People, Planet, and Profit"

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THE WATERFRONT HOTEL KUCHING, SARAWAK, MALAYSIA

Editors:
Masnindah Malahubban, Juriah Kamaludeen, Hadi Hamli, Leong Sui Sien, Johan Ismail, Mohammad Nasir Hassan, Latifah Omar, Mohd Hamim Abdul Aziz and Dayang Shobiha Abang Abai

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Preface

The International Conference on Fisheries and Animal Sciences 2022 (FISAS2022) is one of the conferences under one umbrella of the Congress on Sustainable Agriculture and Food Security 2022 (COSAFS2022). The FISAS2022 was held to support efforts on the agricultural sustainability and food security agendas by gathering current knowledge and updates from diverse scientists, policymakers, and industry on fisheries and livestock. The information obtained is crucial to setting up new plans and hoping for a brighter, more sustainable future.

Sustainable fisheries and livestock have a crucial role in nutrition, food, and livelihood security. Collecting ideas and recommendations from FISAS2022 is essential to kickstarting new strategies that empower innovations for the industry.

Moreover, the innovative and transformative value chains will benefit farmers, breeders, fishermen, and many others. Empowering farmer communities, strengthening participatory approaches, building capacity among all stakeholders, promoting decent work and socio-economic measures, and addressing climate impacts. These efforts will help them stay relevant in ever-changing environments.

FISAS2022 garnered four main tracks: diversity and conservation, animal health and production, feed nutrition, and sustainable production. All topics of various organisms, from crustaceans, fishes, and poultry to ruminants, were highly anticipated and essential for understanding the industry's current state.

To all presenters, writers, participants, and editors: Your efforts to make it a success are greatly appreciated, and we can meet and work together again one day. Our efforts have been successful with the appearance of this electronic proceeding, which can serve as a research reference for all those involved in the fisheries and livestock sectors in their efforts to empower sustainable fish and livestock production.

To the Ministry of Food Industry, Commodity and Regional Development Sarawak (M-FICORD), thanks and appreciation for all the invaluable support given to ensure the success of FISAS2022.

Thank you.

Dr. Masnindah Malahubban
Head of Scientific Committee
The International Conference on Fisheries and Animal Sciences 2022 (FISAS2022)

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EFFECTS OF DIETARY OIL PALM CAROTENE ON COLOUR INTENSITY OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii)*

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Introduction

Giant freshwater prawn, *Macrobrachium rosenbergii* is among the commercial aquaculture species being given high priority by the Department of Fisheries, Malaysia (Anon, 2010) as food and food products for consumption and export (Rubi and Annie, 2016). This species has become the most important cultured freshwater prawn species due to its superior cultivable attributes such as fast growth rate, large size and tolerance to salinity fluctuations (New and Valenti, 2000). Demand for this species is increasing especially throughout Asia for its good taste and ability to be integrated with other agricultural farms such as rice or fish production (New, 2005). In many aquaculture operations, feed contributes to more than 60% of production costs. Hence, it is essential to look for feed ingredients that can improve the growth rate and survival of the cultured species to reduce the production cost. Carotenoids are a family of over 600 natural lipid soluble pigments that are produced by algae (including phytoplankton), higher plants and photosynthetic bacteria (Meyers and Latscha, 1997). Carotenoid pigments are used as key nutritional additives in several fish and crustacean diets due to their beneficial properties. Prawns have the metabolic ability to convert dietary carotenoids, such as beta-carotene, into astaxanthin, which increases the colour intensity and boosts immunity, thereby improving prawn growth performance. Currently, prawn feed manufacturers are using expensive synthetic astaxanthin as a feed supplement which contributes to higher feed costs. Oil palm carotene can be used in prawn feed production to replace the commercial synthetic astaxanthin in the market. A study was conducted to evaluate the effects of dietary oil palm carotene on the colour intensity of giant freshwater prawns (*Macrobrachium rosenbergii*).

Materials and Methods

The feeding trial was conducted at the Aquaculture Complex, Feed Research Group (FRG), MPOB Keratong Research Station, Pahang. The experimental system consists of 15 rectangular glass aquaria (48 (L) x 13 (W) x 20 (H) inches) with a capacity of 250 L water each, complete with continuous aeration and supplied with freshwater from reservoirs. Five treatment groups of prawns with equal in length 25 mm and weight 3.00 g, were stocked in triplicates in the aquaria at a density of 10 prawns per aquarium. The treatment groups that are isonitrogenic and isocaloric were used in this study, including: T1 (commercial feed); T2 (control, without oil palm carotene); T3 (3% crude palm oil (CP

O) inclusion); T4 (3% palm pressed fibre oil (PPFO) inclusion); and T5 (3% commercial oil palm-mixed carotene inclusion). Prawns were fed to satiation five times daily for a 10-weeks feeding trial.

At the end of the feeding trial, prawn samples were collected for colour intensity analysis. The analysis was carried out using three random samples from each aquarium. Prawn colour from each treatment was determined before and after cooking (1 min, 200 ml water at 100^{o}C) and evaluated right after collection using a Konica Minolta Chroma Meter CR-400 (Osaka, Japan, 2002) colorimeter, calibrated with white reference. Measurements were taken from three parts along the prawn body (close to head; middle; close to tail) and performed in the colorimetric space L* (lightness), a* (redness), b* (yellowness), at 25 ± 1^{o}C.

Results and Discussion

Colour differences were observed manually (Figure 1) and have been confirmed by instrumental colour measurement. The colour of raw prawn was darker, much greener and bluer than cooked samples. After cooking, the prawns from each treatment presented more intense orange colour. According to Parisenti et al.(2011), the blue/green colour observed is due to the accumulation of stacyanin, a protein-astaxanthin complex that becomes orange with complex dissociation. This dissociation may occur because of heat (cooking) or the addition of substances such as acetone (Muriana et al., 1993; Velu et al., 2003).

Figure 1. Color analysis of raw and cooked giant freshwater prawn samples fed with different oil palm carotenes

Results on instrumental colour analysis on raw and cooked prawn samples in Table 1 showed that following treatment, no significant variations in L* values were seen across all diet groups with T2 had the highest L* value ($P<0.05$). Meanwhile, T4 had the highest value of a* and b* ($P<0.05$) compared to prawn samples from other treatment groups but it is not significantly differ in a* value ($P>0.05$) with T5. After cooking, prawns obtained greater values of L*, a* and b* than raw samples. Prawn samples of T1, T2 and T3 showed significantly higher ($P<0.05$) L* values than T4 and T5. However, samples T4 and T5 showed much greater ($P<0.05$) values of a* than samples from the other treatment groups.

In the present study, prawns fed with PPFO have the strongest red-orange colour than other treatment groups, indicating a positive effect of its carotenoids content on prawns

colour. The colour parameter (L*, a* and b*) are one of the important indices to evaluate the colouration of crustaceans (Smith et al. 1992). Additionally, Supamattaya et al. (2005) also mentioned that the content of astaxanthin in tissues that has been converted by prawn from dietary carotenoids in PPFO was directly affects the colorations of prawns (Zhao et al., 2022).

Table 1. Color analysis of raw and cooked giant freshwater prawn samples fed with different oil palm carotenes.

Parameter	**Raw prawn samples**				
	T1	**T2**	**T3**	**T4**	**T5**
L*	52.99±5.69	56.80±7.07	51.80±2.43	52.71±7.12	51.15±11.04
a*	-3.28±0.81^{b}	-2.55±0.93^{b}	-2.78±1.20^{b}	-0.62±1.44^{a}	-1.96±1.02^{a}
b*	6.32± 2.76^{b}	6.97±2.91^{b}	6.51±2.14^{b}	10.08±3.92^{a}	7.04±3.43^{b}
Parameter	**Cooked prawn samples**				
	T1	**T2**	**T3**	**T4**	**T5**
L*	64.24±5.26^{a}	64.34±5.37^{a}	65.41±5.79^{a}	61.01±6.75^{b}	61.53±3.84^{b}
a*	1.94±3.60^{b}	0.99±2.10^{b}	2.64±3.58^{b}	9.75±3.46^{a}	7.75±3.61^{a}
b*	17.11±4.28^{b}	15.22±1.94^{b}	19.94±4.89^{b}	32.68±5.79^{a}	23.28±3.31^{ab}

Note: T1 – Commercial; T2 – Control; T3 – CPO; T4 – PPFO; T5 – commercial oil palm-mixed carotene. L* - lightness; a* - redness; b* - yellowness. Means in the same row with different superscripts were statistically different ($P<0.05$)

In general, regarding to consumer preference by observation through subjective analysis, orange colour after cooking is preferred for prawns (Tume et al. 2009). However, studies reporting colour preference for raw prawn have not been found. Therefore, it is suggested to conduct a study on sensorial analyses in order to observe the acceptance of the prawns supplemented with dietary oil palm carotene especially PPFO.

Conclusion

This study suggests that supplementing the giant freshwater prawn diet with PPFO improves colour intensity in terms of redness and yellowness, which potentially creates premium prawns for the market.

Acknowledgement

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HORSHOE CRAB CARAPACE AS FUNCTIONAL FOOD

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Introduction

Over the 450-million-year-old, the horseshoe crab has played an important role in the safe production of vaccinations, injectable pharmaceuticals, and some medical gadgets (Reference?). It is considered the oldest living fossil since the animal does not show any significant phenotypic changes after millions of years (Maloney et al., 2018). Of the four extant species namely, *Limulus polyphemus*, *Tachypleus tridentatus*, *Tapinauchenius gigas* and *Carcinoscorpius rotundicauda*, the latter three are found in Asian waters from India to Japan including East Indies and the Philippines (John et al., 2018). Interest in the horseshoe crab has grown over the last half-century due to the distinctive nature of its blood and popular publications articulating the species' link to migratory shorebirds. The animal's main commercial value is based on a substance found within its light blue blood (Krisfalusi-gannon et al., 2018). The horseshoe crab possesses a tough exoskeleton that offers protection from predators. Interwoven strands of cellulose-like material called chitin. Millions of tightly interwoven cellulose-like strands called chitin are orientated as tough and flexible glue and adhere to each other to form a shell. The chitin of horseshoe crab is favored for research and sought over other arthropod chitin because of its comparatively better purity (Pati et al., 2020). Chitin is converted into a soluble polymer called chitosan in a process that involves alkaline hydrolysis. The polymer stimulates antimicrobial activity and is a significant crystalline of N-acetyl glucosamine renewable sources. Diet is closely linked with a range of disease conditions and with significant age-related chronic disease states, therefore functional foods containing beneficial health-related compounds have become a major focus for researchers and the food industry. A portion of food can be considered functional if it has been properly demonstrated to have a good effect on one or more target functions in the body, in a way that is relevant to either increased health and well-being and/or a reduction in disease risk (Birch and Bonwick, 2019). The unusual activity of chitin, chitosan and their derivatives against a different group of microorganisms has received considerable attention in recent years. Therefore, the purpose of this study is to determine the antibacterial activity of chitin and chitosan from the horseshoe crab.

Materials and Methods

Sample Preparation

Horshoe crab was obtained from the cultivation tank of the Biodiversity Lab. The flesh,

appendages including their book gills and egg were removed leaving its exoskeleton. The exoskeleton was washed thoroughly and dried at 60°C until constant weight was obtained. Then, it was pulverized using a commercial mill until a size of ≤ 2mm was obtained and sieved with a 20 -50 µ sieve to increase uniformity.

Preparation of Crude Extract

Crude from powdered carapace was extracted with 100 mL of solvent; Methanol, Ethanol and Chloroform (1:2) each for 72 hours at room temperature. Each extract was filtered through filter paper and was concentrated using a rotary evaporator at 40°C, dried and kept at -20°C until utilization.

Production of Chitin

Chitin is produced through deproteinization and demineralization process. Deproteinization was achieved by adding powdered carapace into 3.5% NaOH and stirring for 2 hours until a solid to solvent ratio of 1:10 (w/v) was achieved. The demineralization process was achieved by adding 1M HCl, and soaking for 45 minutes at room temperature until a solid to solvent ratio of 1:15 (w/v) was achieved. The sample was rinsed with distilled water and dried in a forced air oven at 60°C until completely dry.

Production of Chitosan

Chitosan is produced through the deacytelation process by autoclaving at a pressure of 15 psi, 30 minutes at 121°C using 50% concentrated NaOH; 1:10 (w/v). the resulting chitosan was neutralized with distilled water and dried in a forced air oven at 60°C until completely dry.

Antibacterial Assay

The antibacterial activities of chitin and chitosan were determined by Disc Diffusion Method and Well Diffusion Method. The concentration of crude extract ranging from 12.5 - 100% was used for the assay. A 50 µL of each crude extract was delivered on the plate containing approximately 2×10^5 CFU/mL bacterial (*Streptococcus aureus, Streptococcus uberis, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Salmonella sp*.). The inoculated trays were incubated for 20 h at 37 °C in an inverted position. The minimum inhibitory concentration (MIC) was determined. Gentamycin was used as the positive control. All experiments were done in triplicate.

Results and Discussion

Crude Extraction

Table 1. Weight and color of crude extract obtained from different extraction solvent.

Solvent	Colour of Crude	Weight of Crude (g/100g)
Methanol	Yellow	0.389
Ethanol	Pale Orange	0.620
Chloroform	Bright Orange	0.492

Antibacterial Activity of Carapace

Table 2. The minimal inhibitory concentration (MIC, µg/mL) of carapace extract against bacteria was tested using Disc Diffusion Method.

Microorganism	Methanol	Ethanol	Chloroform
Gram-positive bacteria			
Streptococcus uberis	12.5	12.5	12.5
Streptococcus aureus	12.5	12.5	12.5
Gram-negative bacteria			
Salmonella sp.	-	-	12.5
Escherichia coli	-	-	-
Pseudomonas aeruginosa	12.5	100	12.5
Klebsiella pneumonia	-	25	50

Antibacterial activity of Chitin and Chitosan

Table 3. The minimal inhibitory concentration (MIC, µg/mL) of chitin and chitosan against bacteria tested using the Disc Diffusion Method.

Microorganism	Chitin	Chitosan
Gram-positive bacteria		
Streptococcus uberis	25	12.5
Streptococcus aureus	75	50
Gram-negative bacteria		
Salmonella sp.	-	25
Escherichia coli	12.5	12.5
Pseudomonas aeruginosa	100	25
Klebsiella pneumonia	50	12.5

From the MICs obtained, Gram-positive bacteria are more sensitive toward horseshoe crab carapace, chitin, and chitosan compared to Gram-negative bacteria. The differences in sensitivity among these two groups of bacteria could be related to the morphological differences in their cell wall. Gram-negative bacteria have two layers of wall structures with periplasmic space between them. The presence of the outer membrane in Gram-negative bacteria makes it less sensitive to antibiotics due to the polysaccharide component, which makes the cell wall impermeable to lipophilic solutes while Gram-positive bacteria have only an outer peptidoglycan layer which is not an effective permeable barrier. Both chitin and chitosan from horseshoe crab carapace have potential antibacterial activity against pathogenic bacteria tested. Among bacteria tested, Escherichia coli has shown the highest sensitivity toward chitin and chitosan. Since E. coli were commonly involved in food poisoning and urinary tract infection, these findings can be very interesting for developing functional food from horseshoe crab carapace.

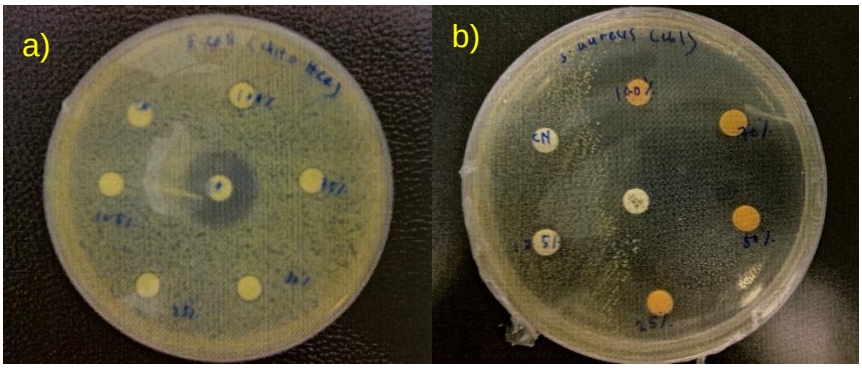

Figure 1. a) Inhibition zone against Gram-negative bacteria *Escherichia coli* against chitosan; b) Inhibition zone of chloroform crude extract against Gram-positive bacteria *Streptococcus aureus*.

Conclusion

Antibacterial activity of crude extract, chitin and chitosan obtained from the carapace of horseshoe crab were determined. Overall, Gram-positive bacteria are more susceptible to crude extract, chitin and chitosan compared to Gram-negative bacteria.These findings highlighted the potential of the marine-derived product as a potential functional food as the horseshoe crab carapace contains antibacterial agents against human pathogenic bacteria.

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DIVERSITY, AND DISTRIBUTION OF FRESHWATER GASTROPODS (MOLLUSCA: GASTROPODA) IN THE SELECTED FRESHWATER STREAM BETONG, SARAWAK, MALAYSIAN BORNEO

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Introduction

Gastropoda is one of the most important groups of Mollusca that has successfully overrun most of the continent. They may be found in a wide range of environments across the world, ranging from a defined aquatic environment to a more indeterminate terrestrial one. It was found in aquatic habitats, such as lakes, rivers, marshes, streams, reservoirs, drainage, and other seasonal water bodies, among other places (Al-Asif et al., 2021; Hamli and Al-Asif, 2021). The word "stream" is often used to refer to a small channel of freshwater that contains moving water (Riley et al., 2018). Freshwater streams may be divided into two categories: natural and artificial. While it's obvious that the narrow canal was discovered in the wild, the majority of natural stream definitions include continuous streams in which water flows continuously from the high altitudes of our continental system until it reaches the main destination or low altitude continental water such as the ocean. In Sarawak, most of the research focusing the diversity of marine and brackish gastropod (Idris et al., 2021) and less study on freshwater gastropod. Considering the knowledge gap, the current investigation was carried out to determine freshwater gastropod populations and their diversity from the selected stream Betong division.

Materials and Methods

Study location

The study was conducted in Betong and Saratok, Sarawak, Malaysia. There were 3 sampling stations that had been selected. Sampling point 1 (N 01°36’31.5”, E 111°41’58.7”) was located in Penebak, Layar. Sampling point 2 (N 01°36’27.3”, E 111°42’13.6”) was Sungai Nanga Tiga, Layar which located at same place but origin of the stream was different while both streams flows to Batang Layar, Betong. The sampling point 3 (N 01°46’39.4”, E 111°28’27.4”) was located in Saratok which nearby the Nanga Budu, Saratok border.

Field sampling technique

At each sampling sites, a 50m sampling transact was laid on the riverbank. Once it's set up, the existed of specimens were collected from each sampling point and preserved in the zip bags for species identification. The most popular approach of collecting freshwater conspicuous gastropods was simply to grab them using our hands from the ground and along the riverbank. Gastropod that attached to vegetation and floating debris also collected slowly to avoid the destruction of their habitats.

Statistical analysis

The data of a total number of individual and the species were tested for the Shannon-Weiner diversity index (H'), Pielou's evenness index (J'), and Margalef's richness index (D) using Paleontological Statistics (PAST) version 4.03.

Results and Discussion

Diversity Indices

A total of four Gastropoda species were recorded from all three stations Table 2. Where station 1 and 2 comprised of *Pila ampullacea* (Linnaeus, 1758), *Clea nigricans* A. Adams, 1855, *Brotia costula* (Rafinesque, 1833) and *Sulcospira pageli* (Thiele, 1908) species, while station 3 comprised of *Clea nigricans* A. Adams, 1855, *Brotia costula* (Rafinesque, 1833), *Sulcospira pageli* (Thiele, 1908) and *Vittina pennata* (Born, 1778) species respectively.

Table 1. The checklist of freshwater gastropod species from three different sampling station in Betong and Saratok, Sarawak, Malaysia

Order	Family	Species	Sampling Stations		
			S1	S2	S3
Architaenioglossa	Ampullariidae	*Pila ampullacea* (Linnaeus, 1758)	+	+	-
Neogastropoda	Nassariidae	*Clea nigricans* A. Adams, 1855	+	+	+
Caenogastropoda	Pachychilidae	*Brotia costula (*Rafinesque, 1833)	+	+	+
		Sulcospira pageli (Thiele, 1908)	+	+	+
Cycloneritida	Neritidae	*Vittina pennata* (Born, 1778)	-	-	+

Indicator: Present (+) Absence (-)

A total 234 of individuals were collected from all sampling stations. The station that consisted highest number of individual was Sungai Nanga Tiga which had 87 individuals (station 2). Followed by Sungai Penebak recorded 76 individuals (station 1) and Sungai Kabo recorded 71 individuals (station 3) (Figure 1). Result on diversity index indicated

that Shannon-Weiner's diversity, Pielou's evenness and Margalef's richness indices were found higher in station 3 compared with other stations.

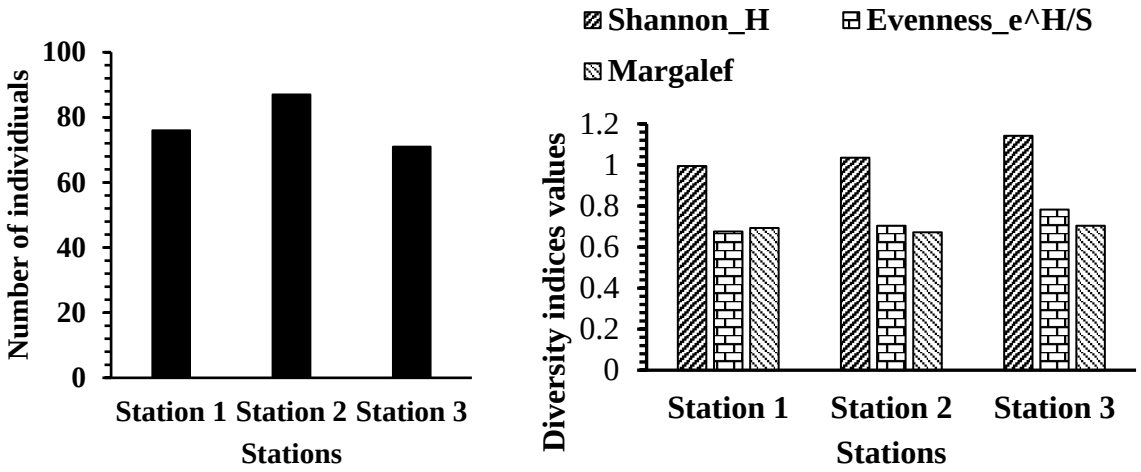

Figure 1: Number of individuals and diversity indeces in different stations.

It showed the *highest* value of evenness index was 0.78 followed by 0.7034 *(*station 2*)* and 0.676 (station 1). The highest value for the richness index was 0.703 (station 1) followed by 0.6927, 0.6718 which is station 1 and station 2 respectively. Previous studies from different localities of Sarawak referring more than four families reported by (Hamli, et al., 2020). It seem few species were found, it may be due to certain factors such as water quality and also the species habitats requirements. According to (Ekau et al., 2010), oxygen is one of the most essential components in aquatic organism's metabolism. Hypoxia will be caused by a low concentration of DO which affects the numerous types of biological functioning in gastropods.

Conclusions

The freshwater gastropod of Betong and Saratok was low diverse in term of richness but has a heterogeneous abundance. Further studies on the interrelation between biotic Gastropoda and the abiotic factors can be conducted to evaluate the interaction between them. Along with trophic structures, and ecological niche studies on the Gastropoda species in different streams in different location are recommended.

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SOCIO-ECONOMIC IMPACTS ON FISHING INCENTIVE RECIPIENTS AMONG FISHERMEN

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Introduction

According to the National Agro-Food Policy (DAN, 2010 - 2020), the number of marine fisheries landings in Malaysia is estimated from 1.32 million tons in 2010 to 1.76 million tons in 2020, with a growth of 2.9 % per year. Of this amount, inshore fisheries are expected to account for 65% of total sea catches, compared to 35% for deep-sea fisheries in 2020. Latest statistics from the Department of Fisheries Malaysia (DOF) conclude that in 2018 it was nearly 1,452,862 Tons of marine fish have been landed by Malaysian fishermen.The demand for fish in the country is expected to increase in line with population growth based on the Supply and Consumption Accounts Reports issued by DOSM (2020). To help with these problems, the government through the Department of Fisheries (DOF) and the Fisheries Development Authority Malaysia (LKIM) has introduced many initiatives and programs aimed at improving the socio-economy of fishermen in Malaysia. Programs, aid, and existing incentives focus more on improving the living standards and socio-economic aspects of fishermen in general (LKIM, 2020). Therefore, examining the effectiveness of the program is important to understand the issues and challenges faced by fishermen. The programs and incentives examined in this study include the tables below. The aim of this study was to assess fishermen's satisfaction with overall government programs and incentives and to measure the effectiveness and impact of these programs.

Table 1. Government-initiated programs and incentives

No.	Programs And Incentives
1.	Fishermen's Living Allowance (*Elaun Sara Hidup,* ESH)
2.	Fishing Incentives (*Insentif Hasil Tangkapan*, IHT)
3.	Natural Disaster Relief
4.	Housing Assistance
5.	Diesel Subsidies
6.	Petrol Subsidies

Materials and Methods

This study used primary data obtained via a field survey of 467 respondents consisting

of fishermen who were members of 25 Fishermen's Associations (PNK) across Malaysia between 2018 and 2019. The respondents were selected according to the stratified random procedure. Quantitative analysis of descriptive analysis was used to analyze the data using the Statistical Package for Social Sciences (SPSS) version 23. The descriptive statistical method was used to describe the basic characteristics of the data by providing simple summaries of the data.

Results and Discussion

The demographics of the fishermen studied showed that 97.7% of the fishermen were men but 2.3% were women. Most of them are Malays (80%), followed by Chinese (7.1%), Indians (2.5%) and others (10.3%). Many respondents have primary and secondary education at 46.8% and 48.1% respectively and on average each respondent has six people in the family with an average monthly income of RM1,707.20, which exceeds the minimum wage set by the Malaysian government 1,200 RM per month (Federal Gazette, 2020). Many of the fishermen surveyed were among the fishermen in Zone A (87.9%), an inshore fishing community, followed by Zone B at 9.1% and C and C2 at 2.4% and 0.4% with an average of 26 years of experience in the serving.

Table 2. Socio-economic profile of the respondents

Category		**Percentage (%)/ Mean**
Gender	Men	97.7%
	Women	2.3%
Race	Malay	80.0%
	Chinese	7.1%
	Indian	2.5%
	Others	10.3%
Education level	Primary school	46.8%
	Secondary school	48.1%
	Diploma/certificates	4.9%
	Degree	0.2%
No. of household		6 persons
Household income (RM)		RM 1,707.20
Fishing zone	Zone A	87.9%
	Zone B	9.1%
	Zone C	2.4%
	Zone C2	0.4%
Fishing experience (Years)		26 years

Effectiveness of government programs for fishermen in Malaysia

The overall effectiveness of government programs for fishermen in Malaysia shows that

diesel subsidy support is the most effective program based on the mean effectiveness score, with a mean score of 4.31. In addition, the recipients of housing assistance consider the assistance provided to be effective, with an average grade of 4.25. In addition, the natural disaster relief is rated with an average effectiveness rating of 4.24. As for the petrol subsidy, the petrol subsidy recipients felt that the petrol subsidy effectiveness was 4.09. The results of the study also found that recipients of fishermen's living allowance and fishing incentives gave average effectiveness ratings of 3.72 and 3.67, respectively, among the lowest compared to the other four grants, and showed that possibly some improvements are needed in terms of effectiveness to better support fishermen. (Table 3).

Table 3. Average effectiveness of government programs for fishermen in Malaysia

	Recipient	Mean	Std. Deviation
Diesel Subsidies	78	4.31	0.726
Housing Assistance	76	4.25	0.755
Natural Disaster Relief	24	4.24	1.091
Petrol Subsidies	249	4.09	0.984
Fishermen's Living Allowance (ESH)	362	3.72	1.157
Fishing Incentives (IHT)	157	3.67	1.103

Skala Likert: 1 = Very Ineffective, 2 = Ineffective, 3 = Neutral, 4 = Effective, 5 = Very effective

Impact of the program on the socio-economics of the fishermen

Overall, 45.1% of respondents experienced a decline in income and a total of 44% of fishermen stated that their local economy also declining. The burden of increased operating costs can be felt by 81.1% of fishermen. Most fishermen think that their standard of living (42.1%) and employment opportunities (53.5%) have not changed (Figure 1). Based on the chi-square test conducted, there is a significant difference between the recipients of each initiative/assistance with each socio-economic impact studied. However, there are only two impacts that have a significant impact on the recipients of each initiative/ assistance, namely the impact on the income and living standards of fishermen. Please discuss your results with references support.

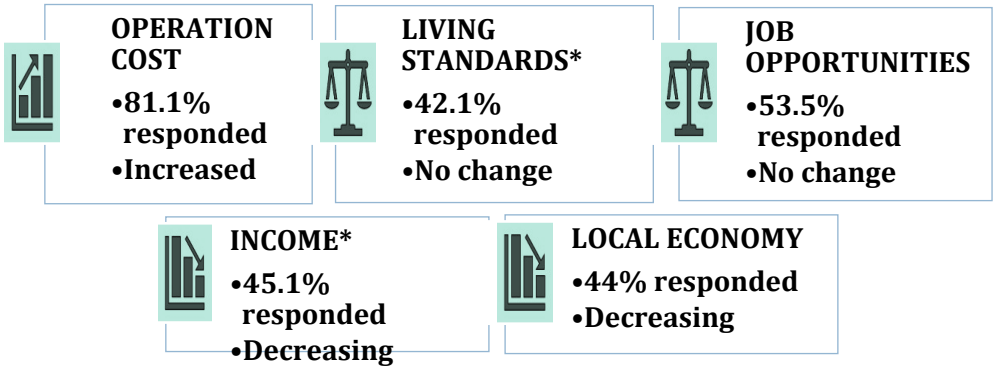

Figure 1: Impact of the program and incentives on the socio-economics of the fishermen.
*significant at 95% confidence level

Conclusion

The results of this study show that the administration of the programs was quite effective. The programs and initiatives implemented by the government are for the fishing community in general and have benefited the fishermen in this way. But not all are satisfied and have felt the benefits of the programs while the rest were not satisfied. Therefore, the government must review all the programs and initiatives that have been implemented and redesign special programs that can bring more benefits and sustain the fishermen's livelihoods. The results of this study suggest that there is tremendous opportunity to improve the management of the programs and that all parties should support governments' efforts to help the fisheries sector develop this industry.

Acknowledgement

The authors would like to thank the directors and study group members of the Socio-Economy, Market Intelligence and Agribusiness Research Center (ES) for their dedication and hard work. Recognition also to other MARDI Centers of Responsibility (PTJ) from headquarters, states, and participating stations. Special thanks to the Malaysian Ministry of Agriculture and Food Industry (MAFI) for funding this special project. Acknowledgments and thanks also go to the agencies and departments namely the Policy and Strategic Division (DPS), the Malaysian Development and Fisheries Authority (LKIM), the Department of Fisheries (DOF), the Area Fishermen Association (PNK) and the fishermen involved in the success ensure this study.

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FISH ASSEMBLAGES IN ARTIFICIAL DRAINAGE SYSTEM ACROSS OIL PALM PLANTATION DEVELOPMENT PHASES ON PEATLAND

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Introduction

The freshwater habitat of peat swamp is a habitat for diverse and interesting fish fauna with narrow niches and a restricted range. Fish inhabiting peat swamp forest (PSF) exhibit the highest endemicity and possess interesting characteristics whereby they are known to be tolerant to low oxygen and acidic water condition that are rich in decaying organic matter. Sule et al. (2016) stated that a total of 198 fish species have been recorded in Malaysia whereby 114, 49, 13, 58, 31, and 40 fish species were recorded from North Selangor PSF, PSF of Perak, PSF of Johor, PSF of Pahang, East Peninsular Malaysia, and East Malaysia (Sarawak and Sabah), respectively. Over the past few decades, the conversion of PSF for oil palm agriculture is common, particularly in the Southeast Asia region (Miettinen et al., 2016). Peatland has become the potential land for oil palm cultivation since the land of mineral soil has become scarce. Fewer studies related to the fish community are being carried out in oil palm plantations in Malaysia. Dosi et al. (2019) conducted a study in Sungai Kulak in Tinbarap Estate Miri, Sarawak and documented 13 fish species. A study by Chong (2014) was conducted in drainage systems and streams or rivers within conservation areas within the oil palm plantations had recorded 35 fish species. Both studies recorded the dominance of Cyprinidae in the conservation area in oil palm plantations. Being a waterlogged forest with a high-water table under the natural condition, the removal of excess water to lower the water table as required by oil palm is essential before cultivating oil palm on peatland (Lim et al., 2012). Therefore, the drainage network is often established for water table regulation, creating a new artificial habitat for aquatic inhabitants, particularly fish. This study was conducted to determine the fish assemblages inhabiting the artificial habitat in response to the conversion of drained peat swamp forests to oil palm monoculture was conducted.

Materials and Methods

The study was focused on the fish assemblages throughout the oil palm development phases in Ladang Lingga 2, Betong, Sarawak within N01°23.571' and E111°22.199'. Fish was caught from three different drainage systems: collection drain, perimeter drain, and main drain. Sampling was conducted from July 2013 to July 2019: i) before oil palm planting namely, drained peat swamp forest (DPSF) and cleared land (CL) that was converted to oil palm plantation; and ii) after planting involved five phases of the oil palm development phase, namely, 1-year oil palm (YOP), 2-YOP, 3-YOP, 4-YOP, and 5-YOP.

Fish were sampled using 3-layers gill nets, monofilament gill nets (mesh size of 5.5 cm and 2.5 cm), and kick nets. The gill nets were deployed for 3 days and 2 nights. Fish species were identified *in-situ* while fishes that were unable to be identified were preserved in formalin (10%) and brought back to the laboratory for further identification. Fish identification was carried out at the species level with reference to Kotellat et al. (1993), Inger and Chin (2002), and Froese and Pauly (2016).

Triplicates reading of water temperature, dissolved Oxygen (DO), pH and conductivity were measured at the subsurface of all sampling stations using YSI 6600 V202 Multi-parameter Water Quality Sonde (YSI Inc., Ohio USA).

One-way analysis of variance (ANOVA) was used to determine the significant differences in species richness and abundance of fish community among development phases, with a *posteriori* Tukey HSD test at a significance level of 95%. These analyses were computed using SPSS 17.0 Software. Variation in the fish community of different oil palm plantation development phases was investigated using Non-metric multidimensional scaling (NMDS) via metaMDS function from the vegan package in R version 3.6.3. Sites that were most similar to one another in species composition are closest together on the NMDS plot (Oksanen et al., 2015). Subsequently, statistical difference between groups was tested using the function "ANOSIM" from the vegan package.

Results and Discussion

A total of 1546 fish represented by 12 species belonging to 6 families were recorded throughout this study. The most dominant fish family was Anabantidae with 51.4% of the total number of individuals caught and followed by Helostomatidae representing 22.8%, Osphronemida representing 17.0%, Clariidae representing 5.1%, and Channidae representing 3.8%. The least dominant fish family in this area was Siluridae, with 0.1% of the total number of fish caught. Of the 12 species caught, *Anabas testudineus* was the most abundant comprising 51.4% of the total number of fish caught, followed by *Helostoma temminkii* (22.8%), *Trichopodus trichopterus* (16.8%), *Clarias leiacanthus* (2.3%), C*. meladerma* (2.0%), *Channa striata* (1.8%), and *C. lucius* (1.5%). Meanwhile, *Channa bankanensis*, *Clarias nieuhofii*, *Trichopodus pectoralis*, *T. vittata*, and *Ompok leiacanthus* were least dominant as they only contributed less than 1% of the total number of individuals caught. The NMDS ordination showed that the variation in fish species assemblages across different oil palm development phases was significant at $p<0.05$. The composition of fish assemblages differs as DPSF was converted to oil palm plantation. There is no significant difference ($p>0.05$) in fish species richness at artificial drainage systems along the oil palm development phases. The 5YOP recorded high number of species (mean = 6.75) followed by 2YOP (mean = 6.25), DPSF (mean = 5.50), 4YOP (mean = 5.25), 3YOP (mean = 5.00), 1YOP (mean = 4.75) and CL (mean = 4.33). After planting, during 1YOP, species richness shows an increment by at least 10 % compared to the CL phase but remains lower by at least 14 % as compared to DPSF. Meanwhile, fish abundance increased by at least 48 % as compared to DPSF and double that of those recorded during the CL phase. As it reached 2YOP, fish species richness was comparable to the DPSF phase and more than those recorded during the CL phase. During the CL phase, more areas were dredged to extend the drainage network.

The abundance of fish was significantly greater (p<0.05) in 2YOP (mean = 81 individuals), 3YOP (mean = 75 individuals) and 4YOP (mean = 80 individuals) than CL (mean = 21 individuals), DPSF (mean = 50 individuals), 1YOP (mean = 43 individuals) and 5YOP (mean= 61 individuals). Fish abundance was double as compared to the DPSF phase and was fourfold greater than in the CL phase. The presence of additional fish species in this study might be due to the drainage system connected to the adjacent mature oil palm plantation and Saribas River. Fish composition of each development phase of oil palm plantation shows that Anabantidae was dominant during each phase. Helostomatidae constitutes a very small percentage of the population before oil palm planting (i.e., during DPSF & CL phase). After oil palm planting (1YOP-5YOP) the proportion increased. Likewise, Osphronemidae which was only recorded after the forest was cleared showed increments in the community composition as palms aged. All of these species are air-breathing species where their organ enable them to obtain oxygen from atmospheric air and also when in water (Nelson, 2014). The dominance of *Anabas testudineus* could be related to its ability to tolerate low oxygen levels and turbid water conditions, thus enabling its succession in the new habitat, especially after forest clearance (Habib and Hassan, 1994; Bhattacharjee et al., 2009).

Trichopodus trichopterus has the ability to colonize anthropogenically disturbed habitats, tropic opportunism, and fast growth rate (Pinter, 1986). Meanwhile, the composition of Channidae was lower after conversion and oil palm planting. *Channa bankanensis* was recorded during the DPSF and 2YOP phases but was not recorded as the forest was being cleared. Similarly, the composition of Clariidae was lower compared to DPSF and CL phase after oil palm planting. Siluridae, represented by *Ompok leiacanthus* was only recorded during the 5YOP which suggests that it might have entered the plantation drainage network from the nearby river. In Borneo, catfishes are the most abundant component of freshwater fish fauna together with Cyprinidae (Kottelat et al., 1993). Catfishes of the *Ompok leiacanthus* recorded in this study were listed as Near Threatened species under IUCN Red List.

The *in-situ* water quality parameters indicate that there are significant differences (p<0.05) in water temperature, pH, dissolved oxygen, and conductivity throughout the oil palm development phases. After planting with oil palm, the pH of the water becomes more acidic, water temperature and dissolved oxygen are lower, and conductivity is higher. This may be the reflection of agricultural activities in the oil palm plantation. These results indicate that fish inhabiting the water habitat in oil palm plantation were able to tolerate the acidic, low dissolved oxygen and water temperature and higher conductivity.

Conclusion

This study assesses the response of fish assemblages in artificial drainage systems as the drained peat swamp forest was being converted to oil palm monoculture. Species richness and abundance decreased as forest area was cleared. However, after oil palm planting, fish abundance shows an early recovery as palm reached one-year-old meanwhile fish species richness recovers as palm reached two years old. Letting natural

plants grow along with the drainage system for better water quality hence creates habitats for aquatic and wetland carnivorous fauna

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EFFECTS OF ENGKABANG BUTTER OIL (*Shorea macrophylla*) AS LIPID SOURCE ON GROWTH PERFORMANCES OF JAVAN MAHSEER FINGERLINGS (*Tor tambra*)

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Introduction

Mahseers diversity is most eminent in Southeast Asia, particularly in the Indo-Burma and Sunda-land biodiversity hotspot (Walton et al. 2017). One of the other 10 valid species in this region is known as *Tor tambra* (Hoàng et al., 2015), which is reported as "data deficient" on the IUCN Red List of Threatened Species (Kottelat, 2018). Many Tor spp fish farmers face difficulty in getting suitable feed as there are no specific feed for their optimal growth (Iehata et al. 2021; Redhwan and Komilus 2021). Fish oil in fish feed at different stages namely larvae, fry and fingerlings has been used rapidly until today. The rapid growth of aquaculture coupled with the decreasing trend of marine fish landings has pressured nutritionists to look for alternative lipid sources (Han et al., 2015) that are suitable for each fish stage. As a consequence, immediate action should be taken to reduce the use of fish oil as the main source of lipids in the fisheries feed manufacturing industry. To date, scientific reports on the effects of using *Shorea macrophylla* butter oil (Sm) as feed ingredients for freshwater fish, *Tor tambra*, are still limited, especially on the fingerling stage. *Shorea macrophylla* is commonly known as meranti, engkabang or tengkawang hantelok in Malaysia and is a part of the natural diet consumed by Tor spp. (Kamarudin et al. 2018a). Therefore, this research was conducted to evaluate the potential of *Shorea macrophylla* butter oil in fish feeds that could be a alternative lipid source for upstream freshwater fish.Thus, these study aims to analyse the proximate composition of *Shorea macrophylla* oil as a lipid source and to determine the efficacy of *Shorea macrophylla* oil as an alternative lipid source on the growth performance of *Tor tambra*.

Materials and Methods

Diet Preparation

Experimental Diets and proximate analysis were prepared for each phase based on five different treatments as shown (Table 1). Fatty acid profile of the experimental diets has been done by using Total Fat Soxhlet with Hydrolysis.

Experimental animals and conditions

The 30 days feeding trial conducted at Hatchery Faculty of Faculty of Bioresources and Food Industry (UniSZA). An application for approval from the Animal and Plant Research Ethics Committee (UAPREC) was submitted prior to the experiment. The fishes were weighed and selected in triplicates for each treatment used for feeding trials These were 3 phases of treatments applied to fishes; phase 1 was starter diet (0-10 days), phase 2 was continuous diet (11-20 days) and phase 3 was finisher diet (21-30 days). Fishes were fed twice daily at 7-8 a.m in morning and 5-6 p.m in the evening. The following productive performance parameters were evaluated: Body Weight Gain (BWG); Feed Conversion Ratio (FCR); Feed Intake (FI); Survival Rate (SR). Parameters were obtained according to the following formulas based on the previous method (Komilus and Mufit, 2021). Water quality parameters like Temperature, Ammonia, Dissolved Oxygen (DO), pH were checked on 0 days, 10 days, 20 days and 30 days respectively by using YSI multiparameter.

Results and Discussion

Fatty acid profile for *Shorea macrophylla* shown in fat (%) Lauric acid,0.0088; Myristic acid,0.0545; Pentadecanoic acid,0.0192; Palmitic acid,16.8075; Palmitoleic acid 0.0518; Heptadecanoic acid,0.1541; Stearic acid,45.8739; Oleic acid 33.5900; Linoleic acid (cis),0.7297; α-Linolenic acid,0.1297; Arachidic acid,1.8836; Behenic acid,0.1253; Lignoceric acid,0.3378; and Nervonic acid, 0.2340.

Table 1. Feed composition of the experimental diets (% as fed basis) for *Javan Masheer* that utilized *Shorea macrophylla*.

Ingredient	**Shorea macrophylla**				
	Control	**T1(1.25%)**	**T2(2.5%)**	**T3(3.75%)**	**T4(5%)**
Fish Meal[1]	30.00	30.00	30.00	30.00	30.00
Krill Meal	20.00	20.00	20.00	20.00	20.00
Wheat Meal	14.58	14.58	14.58	14.58	14.58
Sago Starch	10.42	10.42	10.42	10.42	10.42
Fish Oil	5.00	3.75	2.50	1.25	0
Shorea Oil	0	1.25	2.50	3.75	5.00
Vitamin Premix[2]	5.00	5.00	5.00	5.00	5.00
Mineral Premix[3]	5.00	5.00	5.00	5.00	5.00
Vitamin C	5.00	5.00	5.00	5.00	5.00
Cellulose	5.00	5.00	5.00	5.00	5.00
Proximate Composition					
Crude protein	29.47±0.28^{ab}	30.12±0.15^{b}	30.95±0.93^{b}	27.76±0.99^{a}	28.24±0.49^{a}
Lipid	8.52±0.42^{a}	10.38±0.83^{ab}	10.78±0.50^{b}	8.66±0.08^{a}	10.22±0.29^{ab}
Crude Fiber	2.97±0.07^{a}	3.18±0.14a	3.40±0.23a	3.4±0.21^{a}	3.42±0.22^{a}
Ash	19.24±011^{a}	14.75±0.10^{a}	12.15±6.58^{a}	14.37±0.72^{a}	15.78±0.18^{a}
Moisture	7.52±0.18^{a}	10.09±0.19^{d}	7.99±0.01^{b}	11.19±0.21^{e}	9.60±0.14^{c}

[1]FishmealFishmeal with a crude protein, fat, moisture and salt at 71.5%,10%,7% and 3.7% respectively.

[2]Vitamin Premix (g kg^{-1}premix): vitamin A,0.05; vitamin D3 0.01; vitamin E 1.30; vitamin K3 0.01; vitamin B1 0.01; vitamin B2 0.016; vitamin B6 0.1; vitamin B12, 0.5; Niacin 0.2; C9H17NO5, 0.056;C19H19N7O6 0.008; biotin 0.5; Anticake 0.02. [3]Mineral Premix (g kg^{-1} premix): Copper,7.5; Iron,125; Manganese,25; Zinc,125; Cobalt,0.5; Iodine,0.175; Selenium,0.3; and Anticake,10.
Values are means ± SD of duplicate measurements. Treatments sample followed by a, b and c in row were significantly different ($p<0.05$)

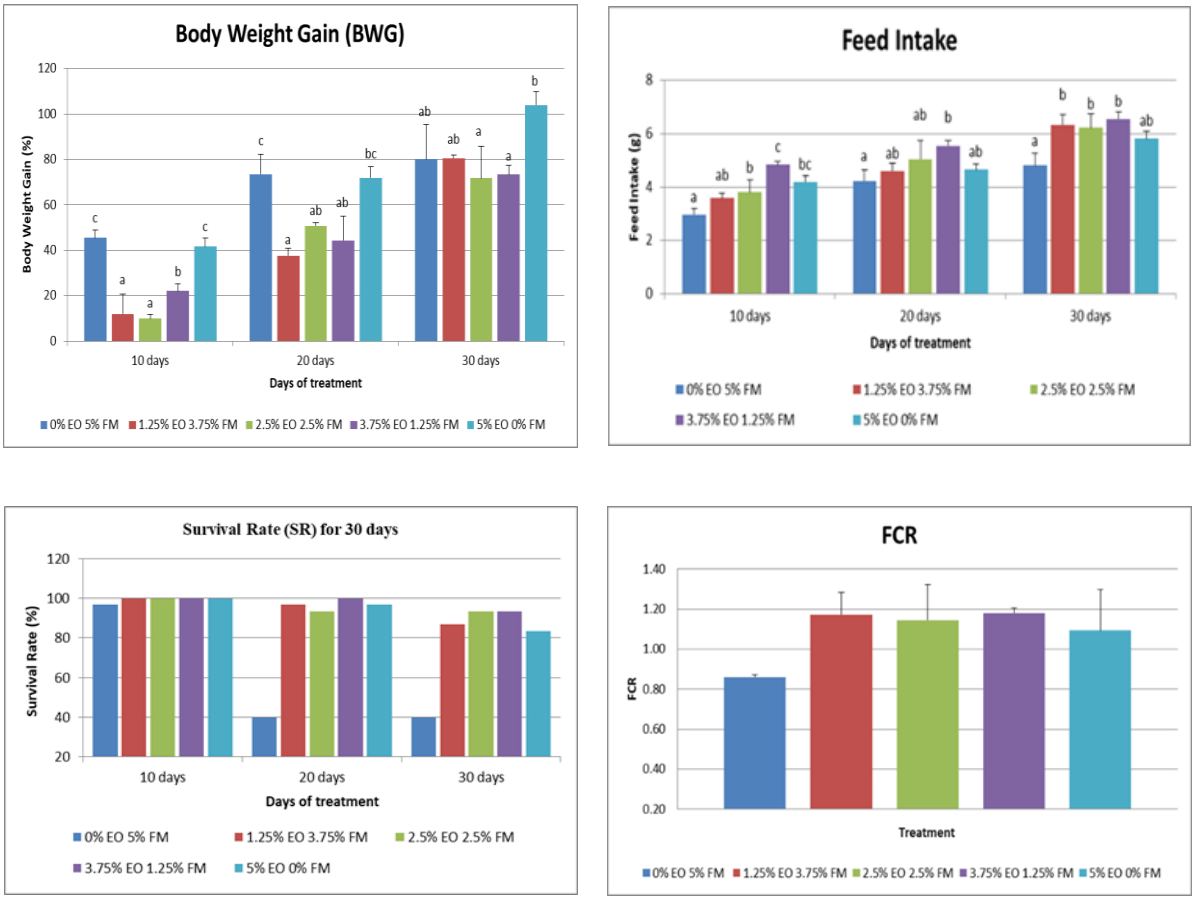

Figure 1. Growth Parameter index (BWG,FI,SR,FCR) *Javan mahseer* for 30 days.

T4 (5% of *Shorea macrophylla* oil) shows a similar efficiency to T0 (5% of fish oil) that gave good growth performance for *Tor tambra* fingerlings. T4 gave the best BWG (2.49 g) and FCR (1.09) meanwhile T0 gave the best FI (4.81 g) and T3 gave the best SR (93.3 %).

Conclusion

It can be concluded that *Tor tambra*, prefer diets with supplementation high n-6 oil source. Although, 5% of *Shorea macrophylla* oil in as lipid sources showed positive effects towards *Tor tambra* fingerlings as an alternative lipid source. It is recommended that a similar dietary feeding trial with elongated trial period to be conducted in future so that the effects of *Shorea macrophylla* oil as feed ingredients can be fully understood.

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SHORT-TERM STORAGE OF JAPANESE KOI (*Cyprinus carpio*) SPERM AND ITS EGG FERTILIZATION ABILITY

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Introduction

The ornamental fish industry is currently rising in demand and is estimated to be growing, with more than 60% being of freshwater origin (Dey, 2016). Malaysia is one of the world's top exporters and producers of ornamental fish, after Japan, Singapore, Thailand, and the Netherlands. Cyprinids are the major group that contributes 36% of the national ornamental fish production (DOF, 2020). Of these, the Japanese koi (*Cyprinus carpio*) is one of the ornamental fish from the Cyprinidae family with high economic value. It is appreciated because of its beauty, unique colour, and representation as the symbol of prosperity and abundance (Ng, 2017). Induced breeding has long been practised in aquaculture to maximize fish egg and larval production. This reproductive technique is one of the methods used to produce Japanese koi fish. Nevertheless, the success rates of induced breeding are inconsistent despite the extensive usage of this technique worldwide, with abundant literature available on the subject (Chapman, 2019). Good sperm quality and adequate sperm volume are the keys to success in the breeding program. However, the lack of mature male broodfish, unsynchronized maturation periods, and inadequate sperm have been the challenges of the induced breeding program for Japanese koi at the Koi Nucleus Breeding Centre (NBC), FRI Glami Lemi, Jelebu. Therefore, preserving Japanese koi sperm via short-term storage and cryopreservation is the alternative to resolve this breeding program problem (Perez-Cerezales et al., 2009; Magnotti et al., 2016). As such, the objectives of the present study were to determine the suitable extender solution, dilution ratio, and storage temperature for short-term sperm storage and, subsequently, to evaluate the effectiveness of short-term storage sperm in egg fertilization in *C. carpio*.

Materials and Methods

Study samples

Mature *C. carpio* broodfish of the Koi NBC collection at FRI Glami Lemi, Jelebu, were used for the study. Clove oil was used to anaesthetize the selected broodfish before milt and egg collection.

Short-term storage

Milt samples were collected from mature male *C. carpio* broodfish by gently abdominal stripping. Milt samples were pooled from 5-6 male broodfish. Three extender solutions, i.e., Extender B, M and F, were tested at sperm to diluent ratios of 1:1 and 1:5. Extender B comprised NaCl, KCl, $MgSO_4.7H_2O$, Na_2HPO_4, KH_2PO_4, $NaHCO_3$ and $C_6H_{12}O_6$; Extender M comprised NaCl, KCl, NaH_2PO_4, $MgSO_4$ and $CaCl_2$; while Extender F comprised NaCl, KCl, $CaCl_2$, $MgCl_2$ and $NaHCO_3$. Storage temperature was tested at 4 °C and room temperature (RT). Sperm samples without extender solution were used as control. All treatments were tested in triplicates. Sperm motility percentages were quantified daily for a week. At day 0, sperm motility was quantified in all treatments four hours after milt sample collection.

Egg fertilization

Mature female and male *C. carpio* broodfish were selected for the induced breeding trial. Ovaprim® was administrated on the selected broodfish at the concentration according to the manufacturer's recommendation. The broodfish were then kept overnight in the 3-tonne fibreglass tank. Eggs were collected from the female broodfish the following morning by gently stripping at the abdomen. For the egg fertilization trial, 3 mL of *C. carpio* sperm after storage at 4 °C for 2 days in Extender F at sperm to diluent ratio of 1:1 was used to fertilize approximately 120 g or 145,000 eggs. Sperm/egg mixture was gently mixed well using a sterile chicken feather. Subsequently, hatchery water was added to the sperm/egg mixture to activate the sperm, gently mixed well and left to fertilize the eggs. The sperm/egg mixture was then rinsed three times with hatchery water before evenly distributing it on the aquarium filter brush used as the substrate material for egg attachment. These aquarium filter brushes were placed in the 3-tonne fibreglass tank, which was set as the egg incubation tank. The egg incubation tanks were set with proper aeration with the water temperature maintained between 23 – 28 °C throughout the incubation period. A freshly collected sperm sample without adding any extender solution was used as a control in this egg fertilization experiment. A total of 20 eggs were sampled in triplicates from each tank to observe and quantify the fertilization percentages. The hatching percentages were also estimated for each tank.

Data analysis

All data were expressed in treatment mean $\pm$ standard deviation (SD) and then subjected to statistical analysis using STATISTIX version 8.0. Results of all treatments were compared with control. $P<0.05$ was considered significantly different.

Results and Discussion

Short-term storage

On day 0, sperm motility in three treatments stored at 4 °C (i.e. treatments with Extender F at sperm: diluent ratios of 1:1 and 1:5, and treatment with Extender B at a sperm: diluent ratio of 1:1) and a treatment stored at RT (i.e. treatment with Extender F at a sperm: diluent ratio of 1:1) showed no significant ($p>0.05$) difference compared to the

control treatments stored at 4 °C dan RT (Table 1). For all treatments stored at RT, disregarding the addition of extender solution, sperm motility decreased drastically to <20% after 24 hours, as quantified on the following day (day 1) and became immotile after 48 hours (day 2). For treatments stored at 4 °C, starting day 1, only treatment with Extender F at sperm: diluent ratio of 1:1 and control possessed sperm motility >90%, and both treatments showed no significant difference ($p>0.05$). However, on day 2 and day 3, *C. carpio* sperm diluted with Extender F at sperm: diluent ratio of 1:1 and stored at 4 °C showed significantly ($p<0.05$) higher sperm motility than all other treatments, including control treatment. On day 3, sperm motility still retained at 70-80% in this treatment, while it was significantly ($p<0.05$) lower, i.e. 50-60% in the control treatment (Table 1).

Table 1. Daily changes in motility percentages of *C. carpio* sperm stored at room temperature (RT) or 4 °C with the presence of different extender solutions (Extender B, D and F) at different sperm to the diluent ratio (1:1 or 1:5). *Treatments without the addition of an extender were used as controls.

Storage Temperature	Treatment	Day							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
RT	**W/O Ext***	91.67 ±3.51^{ab}	15.67 ±4.04^{def}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext B (1:1)	83.33 ±2.89^{bc}	10.00 ±2.00^{ef}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext B (1:5)	23.33 ±7.64^{gh}	6.00 ±1.73^{f}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext D (1:1)	75.00 ±5.00^{cd}	9.00 ±1.73^{ef}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext D (1:5)	40.00 ±5.00^{f}	5.67 ±2.08^{f}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext F (1:1)	91.67 ±2.89^{ab}	17.67 ±2.52^{de}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext F (1:5)	28.33 ±7.64 ^{fg}	9.33 ±1.15^{ef}	0.00 ±0.00^{d}	0.00 ±0.00^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
4 °C	**W/O Ext***	98.67 ±1.15 ^{a}	96.67 ±1.53^{a}	59.33 ±7.51^{bc}	54.67 ±5.03^{b}	23.33 ±2.89^{cd}	13.33 +2.89^{b}	3.00 ±1.00^{bc}	0.00 ±0.00^{b}
	Ext B (1:1)	91.67 ±2.89 ^{ab}	66.67 ±6.11^{b}	54.00 ±5.29^{c}	34.33 ±4.04^{c}	26.67 ±7.64^{bc}	16.67 +7.64^{ab}	5.33 ±1.53^{b}	0.00 ±0.00^{b}
	Ext B (1:5)	61.67 ±7.64 ^{de}	21.00 ±6.56^{d}	11.00 ±3.61^{d}	1.33 ±0.58^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext D (1:1)	56.67 ±5.77 ^{e}	56.00 ±4.00^{c}	55.00 ±5.00^{bc}	25.33 ±2.52^{d}	15.00 ±5.00^{d}	11.67 ±2.89^{b}	4.67 ±1.53^{bc}	0.00 ±0.00^{b}
	Ext D (1:5)	11.67 ±1.53 ^{h}	14.67 ±2.52^{def}	2.00 ±1.00^{d}	1.33 ±0.58^{e}	0.00 ±0.00^{e}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{b}
	Ext F (1:1)	98.33 ±2.89 ^{a}	96.33 ±2.08^{a}	87.33 ±6.43^{a}	76.00 ±5.29^{a}	43.33 ±5.77^{a}	23.33 ±5.77^{a}	15.00 ±5.00^{a}	11.67 ±2.89^{a}
	Ext F (1:5)	88.33 ±2.89 ^{abc}	70.67 ±3.06^{b}	66.00 ±5.29^{b}	60.00 ±5.00^{b}	33.33 ±3.61^{b}	19.00 ±3.61^{ab}	7.67 ±2.52^{b}	0.00 ±0.00^{b}

Mean values in the same column with different alphabets (a>b>c>e>f>g>h) are significantly different at p<0.05 (ANOVA, Tukey' test).

Egg fertilization

In the breeding trial, egg fertilization percentages by using short-term storage sperm and freshly collected sperm (control) were 55.5±10.8% and 58.3±12.6%, respectively (p>0.05), as shown in Table 2. The fertilized eggs started to hatch after 48 hours. The hatching percentages of the fertilized eggs were also not significantly different (p>0.05) between the short-term storage sperm and freshly collected sperm.

Table 2. Comparison of egg fertilization and hatching percentages in *C. carpio* between short-term storage sperm and fresh sperm (control).

Variables	Short-term storage sperm	Fresh sperm (control)
Egg fertilization (%)	55.5±10.8[a] (40.0 – 70.0)	58.3±12.6[a] (45.0 – 70.0)
Hatching (%)	47.5±6.5[a] (40.0 – 55.0)	48.3±12.6[a] (35.0 - 60.0)

Mean values in the same row with different alphabets were significantly different at p<0.05 (ANOVA, Tukey test). Values are expressed in treatment means±SD, and values in the bracket are the range.

Short-term storage sperm possesses equivalent egg fertilization ability compared to fresh sperm. Using short-term storage sperm in egg fertilization has eased and simplified the breeding of *C. carpio* as more efforts can be focused on handling female broodfish, stripping eggs, and fertilization processes such as cross-breeding between selected koi varieties. This sperm storage procedure has provided a simple, practical, and inexpensive method for facilitating the management and reproduction programs in aquaculture (Contreras et al., 2020). Besides that, the suitable extender solution formulation and dilution ratio determined in this study can further be applied in developing the cryopreservation protocol for *C. carpio* sperm. Extenders or diluents that possess the ability to maintain sperm viability and fertilizing capacity are prerequisites in conserving fish gametes for later use (Chapman, 2019).

Conclusion

In conclusion, a short-term storage method for *C. carpio* sperm that still possesses the ability to fertilize eggs as good as fresh sperm was developed in the present study. On top of that, the appropriate extender solution for cryopreservation of *C. carpio* sperm was also successfully determined in the current study.

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DIVERSITY AND DISTRIBUTION OF INDIGENOUS BETTA FISH IN SARAWAK

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Introduction

The genus *Betta*, locally known as *ikan laga*, consists of small freshwater fighting fish that belongs to the Osphronemidae family. They are native to Southeast Asia and are found within various types of water bodies, thus leading to their diversity. There are currently 27 *Betta* species documented in Malaysia, with seven species originating from Sarawak (Tan and Ng, 2005; Schindler and Schmidt, 2008; Tan, 2009; Tan and Ahmad, 2018; Kamal et al., 2020). *Betta* fish are potential ornamental fish with economic and ecological importance (DoF, 2018). They are usually caught by traders or hobbyists. However, the population of these Sarawak *Betta* fish are currently being threatened by various factors (Giam et al., 2012). Furthermore, two of these indigenous species are listed as "Data Deficient" under the IUCN Red List (Low, 2019a; Low, 2019b).

Therefore, the objectives of this study are (1) to determine the diverse *Betta* species of Sarawak and their distribution, as well as (2) to assess their current habitat. This information is crucial for the documentation of Sarawak ichthyofauna and to plan a better conservation strategy in the future.

Materials and Methods

Field sampling

The survey was conducted from March 31st 2022 until July 24th 2022 within Sarawak, namely Matang, Lundu, Sematan and Serian. The fish sample were collected using either the scoop nets or the fishing rods, depending on the species and habitat. The samples were then temporarily kept in labelled plastic bags and transported back to the laboratory in the Faculty of Resource Science and Technology, UNIMAS.

The water quality parameters recorded during this study include temperature (°C), pH value, dissolved oxygen (mg/L) and turbidity (NTU) using suitable equipments. The general habitat of the sites was observed and recorded. The location was also photographed and videographed for future reference.

Fish identification

Fish identification followed Tan and Ng (2005) and Kamal et al., (2020). The total length was measured using the measuring board and a ruler to the nearest 0.1 cm. The samples were also weighed with an electronic balance (add brand).

Results and Discussion

A total of six species of wild *Betta* were collected in this study (Table 1). However, Tan (2005; 2009) recorded seven species in Sarawak. His finding includes *B. akarensis* and *B. macrostoma*, but these species has not yet been sampled in the current study.

Table 1. The diversity of *Betta*, locality and brief description of their habitats.

Species	Locality	Brief Description Of Habitat
B. ibanorum *B. brownorum*	Matang	The waters are brownish cloudy with muddy substrates
B. lehi	Lundu	The waters are clear with muddy substrates
B. midas *B. rutilans*	Sematan	The waters are brownish cloudy with gravel substrates
B. taeniata	Serian	The waters are clear with gravel substrates

In this study, two samples of *B. rutilans* were discovered in Sematan. *B. rutilans* has only been previously studied as an indigenous species to Kalimantan Barat (Kottelat, 1991). However, this is not the first record of this species in Sarawak because *Betta* hobbyists have found *B. rutilans* within Sarawak throughout the past decade and has since shared their discovery online.

Table 2. The ranges of temperature (°C), pH value, dissolved oxygen (mg/L) and turbidity (NTU) of the respective *Betta* habitats.

Species / Parameters	***B. ibanorum*** ***B. brownorum***	***B. lehi***	***B. midas*** ***B. rutilans***	***B. taeniata***
Temperature (°C)	26.37±0.37 (25.9 – 27.1)	27.5±0.61 (26.5 – 28.6)	27.7±0.36 (27.2 – 28.4)	25.57±0.18 (25.0 – 26.3)
pH Value	5.30±0.02 (5.27 – 5.34)	6.03±0.27 (5.71 – 6.56)	5.37±0.01 (5.36 – 5.39)	7.74±0.04 (7.59 – 7.87)
Dissolved Oxygen (mg/L)	6.20±0.21 (5.8 – 6.5)	3.6±0.40 (2.8 – 3.9)	1.0±1.52 (0.8 – 1.0)	6.65±0.07 (6.4 – 6.9)
Turbidity (NTU)	16.30±6.84 (7.49 – 29.77)	3.35±0.39 (2.84 – 4.12)	45.90±5.47 (39.77 – 56.83)	6.71±0.40 (5.92 – 7.23)

B. taeniata lives in flowing water (lotic), whereas the other five species were in relatively stagnant waters (lentic). These waters were found to range from slightly acidic to neutral

(Table 2). Aquatic vegetation was also aplenty, added with the coverage from the forest canopy. *B. ibanorum* and *B. brownorum* were both found within the same habitat, similarly to *B. midas* and *B. rutilans*. This is in contrast with a study by Nur et al. (2022), where the *Betta* species exhibited territorial behaviour leading to no two species living in the same habitat.

In this study, *B. brownorum* had the shortest total length while the longest total length goes to *B. ibanorum*. This is also true for their body weight, with *B. brownorum* being the lightest while *B. ibanorum* was the heaviest. Out of the 37 total sample collected, *B. lehi* was most predominant (29.73%) and the least being *B. rutilans* (5.41%).

Table 3. The ranges, average total length and body weight of wild *Betta* caught. NA – Not available.

Species	Total Length (cm)	Body Weight (g)	Total Sample
B. ibanorum	9.44±0.97 (5.6 – 10.8)	8.20±1.72 (1.61 – 11.74)	5
B. brownorum	3.04±0.10 (2.8 – 3.4)	0.28±0.03 (0.22 – 0.37)	5
B. lehi	7.10±0.26 (5.8 – 8.6)	4.24±0.41 (2.69 – 6.53)	11
B. midas	7.68±0.52 (6.7 – 9.0)	5.81±1.40 (3.26 – 8.90)	4
B. taeniata	6.26±0.15 (5.4 – 6.9)	2.53±0.15 (1.69 – 3.36)	10
B. rutilans	NA	NA	2

The low number of *Betta* sample collected could possibly be attributed to the presence of anthropogenic activities close to their habitats (Giam et al., 2012). For instance, the Pan-Borneo Highway project under construction was within reach of the site where *B. lehi* was found. Besides that, *B. taeniata* was found within a stream, not far from a nearby settlement. Furthermore, the habitat of *B. ibanorum* and *B. brownorum* was only adjacent to a durian plantation. As in the case of *B. midas* and *B. rutilans*, a fish trap was already set up at the site before we arrived.

Conclusion

There were six indigenous *Betta* species found, namely *B. ibanorum*, *B. brownorum*, *B. lehi*, *B. midas*, *B. rutilans* and *B. taeniata*. The discovery of *B. rutilans* requires further study and documentation. More samplings will be done in the future to update the checklist of *Betta* species in Sarawak. The findings in this study will aid relevant agencies in conservation efforts for these *Betta* species.

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PREFERENCES OF FISH KEEPING AMONG ORNAMENTAL FISH KEEPERS IN MELAKA, MALAYSIA

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Introduction

Ornamental fishes are known as living jewels, popular due to their beautiful colours, varying shapes, attracting magnifying behaviours, and able to live in confined spaces (Lokman et al., 2019). It is easy to get hypnotized by arowana's graceful gliding movement with its 180° turn, similar to a synchronized ballet movement of goldfishes in an aquarium (Ng, 2016). There were 229 registered ornamental fish exporters in Malaysia in 2017. The collection, breeding and marketing of ornamental fishes can generate income and job opportunities (Ng, 2016). Besides, the ornamental fish breeding sector has contributed to gross domestic products (GDP) and better foreign exchange (Lokman et al., 2019). Definitely, the ornamental fish sector can offer better livelihood and opportunities to many low-income families around the globe (Cagauan, 2007). Based on a report from the Department of Fisheries Malaysia (2020), Malaysia has generated RM506,447,789 in ornamental fish sector in 2019, which recorded a great increase when compared in 2017, at only RM326,953,262. By state in 2019, the state of Johor has led the economic value in ornamental fish productions with a value of RM243,470,349, followed by the state of Perak and Penang with values of RM2,160,168 and RM2,628,868, respectively. Some fishes have impressive characteristics that contributed to their fame in Malaysia, which include chocolate gourami (*Sphaerichthys osphromenoides*), bumble-bee catfish (*Leiocassis poecilopterus*), hangus fish (*Labeo chrysophekadion*), croaking gourami (*Trichopsis vittata*) and kissing gourami (*Helostoma temminkii*), while some fishes are admired for their transparency such as the glass catfish (*Kryptopterus* spp.) and glassfish (*Parambassis siamensis)* (Ng, 2016). Not to forget, *Betta* spp. and *Parosphromenus* spp. for their gorgeous and beautiful colorations. In general, there are four categories of fish keepers, which include the 'beginners', 'collectors', 'biotope enthusiasts' and 'fengshui practitioners' (Ng, 2016). Species that usually kept for 'fengshui' include arowana (*Scleropages formosus*), koi (*Cyprinus carpio*), blood-parrot cichlid, and flowerhorn (Ng, 2016). Besides all of the great contributions of ornamental fisheries and its activities, there are some issues that need to be highlighted. Issues related to ecological sustainability (Lokman et al., 2019) such as threats to reef ecosystem (Pandey and Mandal, 2017) and alien fish invasion becoming challenging to the industry (Chan et al., 2019). The introduction of new, alien, and invasive fish species may create negative ecological and environmental impacts (Chan et al., 2019). Furthermore, some of the fish supplies were obtain from wild-caught. For instance, almost all of the coral reef ornamental fishes, including clown fish and blue tang were collected from the wild (Biondo, 2018).

Likewise, fish keeper's practices and their demands in fish keeping need to be highlighted. Problems faced by fish keepers and traders include lack of government support, difficulties in maintaining aquariums, lack of marine fish supplies, varying hobbyist interests, difficulties in accessing fish species, and other issues that limiting the development of the ornamental fisheries sector (Sirajudheen et al., 2014). This includes overcrowding which can caused injury, stress, diseases, and infection of parasites to fishes (Ng, 2016). This can lead to high mortality and economic loss to the fish keepers and ornamental fish industry in general. The best solutions include the use of tropical disinfectants, antimicrobials, and probiotics (Ng, 2016). Lack of information that available about fish keeper's preferences is also crucial, specifically in terms of species preferences, originating sources, freshwater or marine types of fishes, and the value of the fishes (Biondo and Burki, 2020). Thus, the objectives of the study were to determine the basic-socio demographic background, fish preferences, and motivations of fish keeping among ornamental fish keepers in Melaka, Malaysia. This study would be able to understand factors that affecting fish selection among fish keepers based on their preferences. The study would also provide basic information for the management agencies to enhance ornamental fisheries industry in the near future.

Materials and Methods

The study was conducted online, using two applications for data collection. The Facebook and WhatsApp were the applications that being utilized as platforms, which include all known Facebook groups and WhatsApp groups associated to ornamental fish keepers in the state of Melaka, Malaysia. Permissions from the groups' administrators were obtained by contacting the administrators through personal messages. The questionnaire was distributed using Google Form as tool for data collection. The questionnaire was posted once a week, with the first posting was about the introduction and invitation to fill-in the Google Form. The second to the fourth postings, were about reminders of the survey questionnaire. The data collections were conducted from November to December 2021.The survey questionnaire has three sections. 'First', it was related to the basic socio-demographic background of the respondents, which include gender, age, education levels, and income. The 'second section' was dealing with the preferences of fish keeping among the fish keepers. This include questions related to fish color, combination of color, fish size, fish care, fish feeding habits, fish behaviour, and amount of money spent in an aquarium store. The 'third section' was measuring the motivations of fish keeping among the fish keepers. The questions were derived from items and variables developed by Manfredo et al. (1996) based on Recreation Experience Preference (REP) scale. There were nine variables utilized in this study which include 'reinforcing self-image', 'social recognition', 'family togetherness', 'general learning', 'being with friend', 'creativity', 'nostalgia', 'tranquillity', and 'meeting new people'.Multiple choice questions and open-ended questions were utilized for questions in section one and two. Meanwhile a five-point Likert scale was used for questions in section three, related to the motivations of fish keeping. The respondents were asked to rate their motivations on a scale of 1 (strongly agree) to 5 (strongly disagree), with 3 for neutral response. All of the data were analyzed using descriptive statistics, including mean, percentage, frequency, and standard deviation. Finally, comparisons between groups of the basic socio-demographic

background were conducted, to determine the REP scale. This was conducted using a one-way ANOVA, with a significant value of $p<0.05$. All data were analyzed using SPSS version 27.0.

Results and Discussion

A total of 125 respondents participated in the survey questionnaire and considered as samples of the study. Their basic socio-demographic background information, their preferences of fish keeping, and their motivations of fish keeping were measured and analyzed. The motivations of the respondents later were determined by comparing their basic socio-demographic background groups.

Basic socio-demographic background

Results for 'gender' among the respondents indicated that the male respondents comprised of 87.20%, while the female respondents comprised of 12.80%. The males were most likely than females to involve in ornamental fish activities, and visiting ornamental fish stores. Similar result has been observed in other ornamental fish studies where male was the most dominant (Pargunan and Alagappan, 2020). For the results of 'age', it was ranged from 18 years old to 56 years old, with a mode of 24 years old. Later, the respondents were divided into three age groups including '18-24 years old', '25-34 years old', and '35 years old and above'. The highest frequency was observed in the '25-34 years old' group (42.74%). This was followed by respondents in '35 years old and above' group and '18-24 years old' group at 29.03% and 28.23%, respectively. In terms of 'education levels', some of the fish keepers obtained a diploma or certificate (36.80%), and almost similar to those who have a bachelor's degree (36.00%). These were followed by those who completed high school or obtained a SPM (18.40%). Only 8.00% of the samples obtained a master or Ph.D., with one respondent from a primary school level of education (0.80%). For 'income', the data were categorized into seven categories, ranging from 'no income' to 'more than RM10,000'. Most of the respondents were in the category of 'RM1-RM2,000' which made up of 40.00% from the total respondents. This was followed by those who have 'no income' (17.60%) and those who earned 'RM2,000-RM4,000' (16.00%). Respondents who earned 'more than RM10,000' comprised of 11.20% from the total samples. The rest of the respondents' income fell within the income groups that were listed above. In general, the results revealed that, those who earned 'below RM4,000' comprised of 73.6% of the total respondents of the study. They fall within the below-40 group (B40). This study might missed the other groups of fish keepers, mainly from the middle-40 (M40) and top-20 (T20) groups.

Preference of fish keeping

Results revealed on the preferences of fish keeping among ornamental fish keeper in Melaka, Malaysia. Some of the characteristics in this study were also used in previous study by Tolon (2018) in determining the fish hobbyist preferences. In term of 'fish color', the highest percentage was observed in 'red color' with 32.00% of respondents. This was followed by 'golden color' with 22.40% of respondents, while 'yellow color' and 'blue color' were observed with 9.60% of respondents, each (Figure 1). Red color is bright,

attractive and vibrant which can be the reasons why many respondents selecting red as their favourite fish color. The industry is focusing on breeding strains that are more vibrant and vivid, where physical appearance, coloration, and pattern traits are the most appealing aspects of ornamental fish (Ng, 2016).

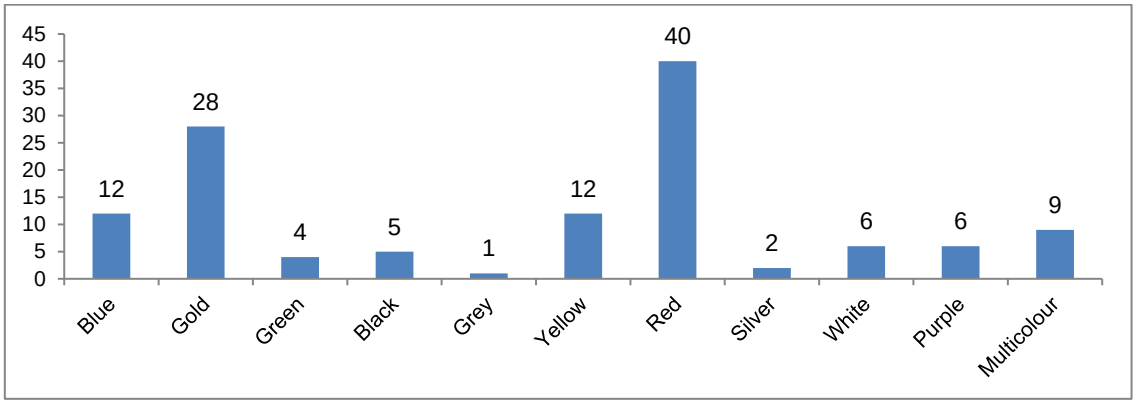

Figure 1. 'Fish color' selection among the fish keepers of the study.

For the results of question of 'combination of color', almost half of the respondents (43.20%) preferred 'multi-combination color', and this was followed by those who preferred 'two colors' (32.00%). The least selected option was 'one color' which comprised only 3.20% of respondents (Figure 2). The same results can be observed in a study conducted by Tolon (2018), where the multi-coloured fishes were the most preferred by a large number of fish hobbyists. Although in the earlier question, most of the respondents preferred red color, however, in this question a multi-combination color was the selection.

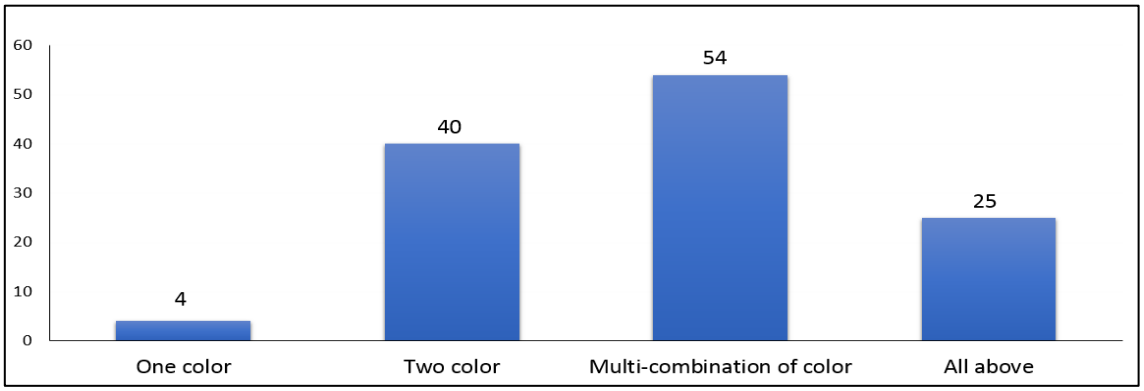

Figure 2. 'Combination of color' selection among the fish keepers of the study.

For 'fish size', a total of 35.20% of respondents claimed that they preferred 'all size of fish' in their tank or aquarium. However, detail information revealed that 24.80% of respondents preferred 'medium size of fish', while 20.00% of respondents (each) preferred 'small or nano size' and 'large or adult size' of fish. Probably, fish that is too small is less attractive, and fish that is too big may restricted fish keepers to keep it, due to limited space in aquarium. The same results can be observed in the study of Pargunan and Alagappan (2020), where small size of fish was the least interested when compared to the other sizes of fish. Fish size had the lowest average importance scored by the fish

keepers when compared to another attributes (Pargunan and Alagappan, 2020). In terms of 'fish care' a total of 39.52% of the respondents preferred 'easy' fish care. This was slightly higher than 'intermediate' fish care (37.10%), while the lowest was 'delicate' fish care (23.38%). This probably due to levels of care, where 'easy' requires less time and effort when compared to 'delicate' fishes. Sirajudheen et al. (2014) mentioned that the local fish keepers preferred fish with lower mortality rate, easy to take care of, and cheap price tag, such as angel fish and gobies.

Results for 'fish feeding habits' indicated that the highest percentage belongs to the 'omnivore' fish (54.17%). This was followed by 'carnivore' fish (25.83%) and 'herbivore' fish (20.00%) (Figure 3). In general, most of the captive-bred ornamental fishes are omnivore. Pandey (2013) mentioned that omnivore ornamental fishes are the most effectively produced and they have adapted very well in captive conditions, due to its wide selection of diets.

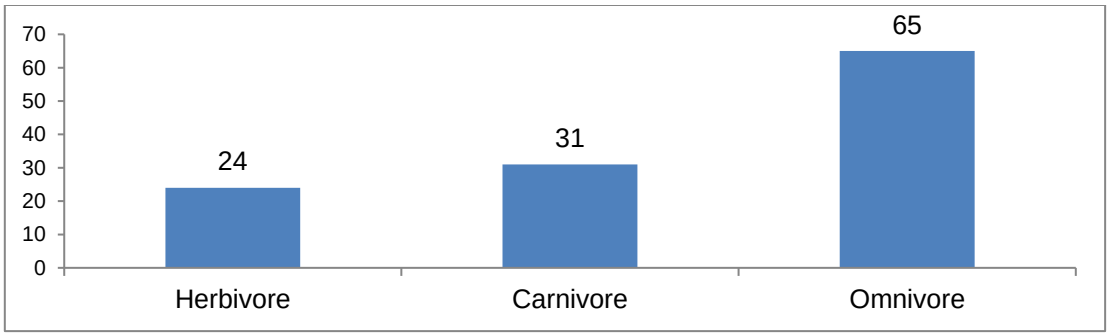

Figure 3. 'Fish feeding habit' selection among the fish keepers of the study.

For 'fish behavior', a total of 62.10% of the respondents chose 'community fish' category. This was followed by those who chose 'territorial fish' and 'schooling fish' categories with 24.19% and 13.71%, respectively. The same results can be observed in a study conducted by Arif et al. (2018), who found that majority of the fish keepers preferred community fish category. This would probably due to fish keepers always enjoy to mix-and-match their fishes in aquarium. There was evidence that having an aquarium at home was linked to tranquillity (Clements et al., 2019).

Finally, results for 'amount of money spent in an aquarium store' indicated that 24.80% of respondents spent 'RM11-50'. This was followed by those who spent 'RM101-500' (21.60%), and 'RM51-100' (19.20%). This could provide a basic idea that most respondents in this study spent from RM11 to RM500 in an aquarium store that they visited. Only some of them spent 'below RM10' (11.20%), and spent 'RM501-1,000' (12.00%) and 'more than RM1,000' (11.20%) (Figure 4). The relative importance of the attributes showed that 'colour' attribute of fish was the strongest factor in influencing respondents' purchase behaviour with the second important attribute was 'price, 'species' and 'size' (Tolon, 2018).

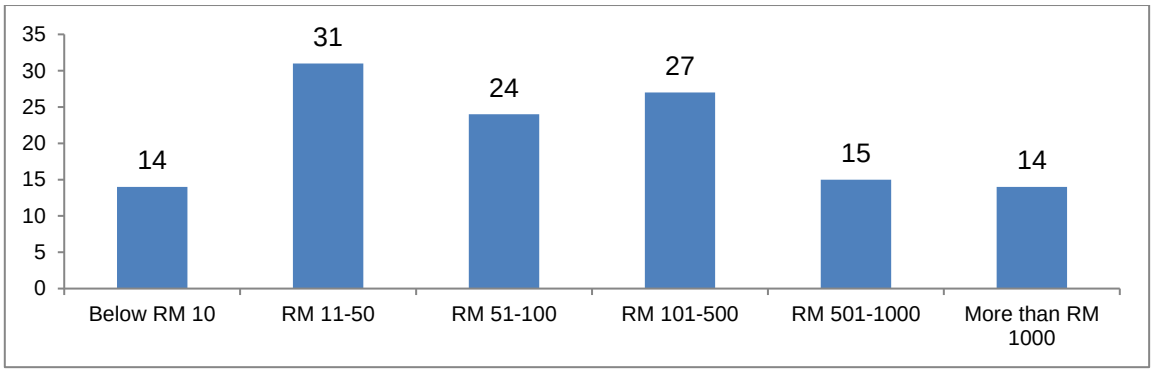

Figure 4. Amount of money spent in an aquarium store among the fish keepers of the study.

Motivations of fish keeping

The third section was measuring the motivations of fish keeping among the fish keepers. The highest variable agreed by most respondents was 'general learning' with a mean value of 4.60±0.71 (mean±SD). This was followed by 'creativity' with 4.25±0.87 and 'family togetherness' with 4.23±1.03. Meanwhile, the lowest mean value was observed in 'social recognition' with 3.18±1.27 (Table 1).

Table 1. The nine variables used in the study based on Recreation Experience Preferences (REP) scale.

Variables	**Mean ± SD**
Reinforcing self-image	3.80 ± 1.10
Social recognition	3.18 ± 1.27
Family togetherness	4.23 ± 1.03
General learning	4.60 ± 0.71
Being with friends	3.82 ± 1.17
Creativity	4.25 ± 0.87
Nostalgia	3.68 ± 1.31
Tranquillity	3.61 ± 0.82
Meeting new people	3.73 ± 1.21

This indicates that the fish keepers' motives for fish keeping, were to learn something new and to develop new knowledge in general. These were supplemented with motives to be creative and to do something creative, other than to do something with family members. Those three variables suggested the reasons why the fish keepers involved in fish keeping activities. On the other hand, it was obvious that the fish keepers did not sought for high impression or good perception from others towards them in fish keeping activities.

Comparison between 'education levels' and between 'income groups' based on REP scale

Almost all of the comparisons conducted for the socio-demographic groups did not show any significant difference ($p>0.05$) based on the REP scale measured. However, there were some significant differences observed ($p<0.05$) under variables of 'general learning',

'being with friends', and 'tranquillity'. Detail analysis found a significant difference ($p<0.05$) between 'education groups'. This was under the REP scale on variable of 'general learning'. Those who were in the 'primary and secondary school' group (4.85) was significantly higher ($p<0.05$) than those who were in the bachelor's, master, and PhD. groups (4.46) (Figure 5). This illustrates that the fish keepers at the level of primary and secondary schools were motivated to learn something general from fish keeping activities.

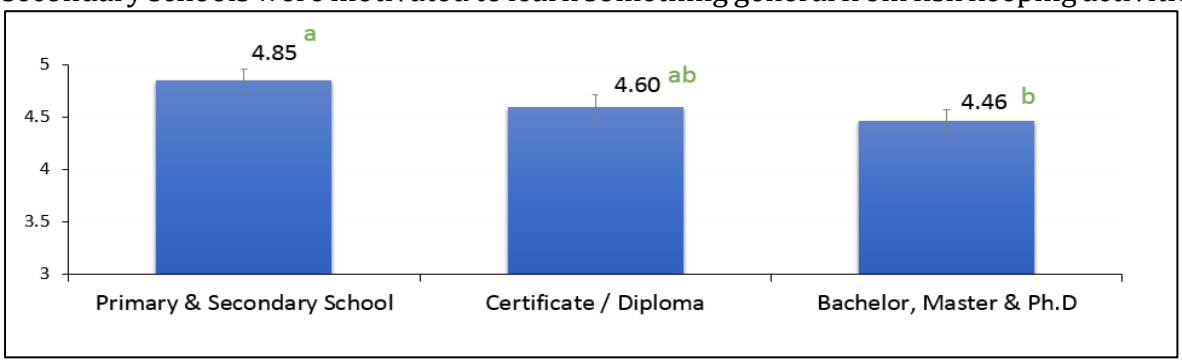

Figure 5. Different education levels were compared based on REP scale on variable of 'general learning'.

The next analysis revealed some significant differences ($p<0.05$) between 'income groups'. These were under the REP scale on variables of 'being with friends' and 'tranquillity'. For variable of 'being with friends', those in group of 'RM0-2000' (4.04) was significantly higher ($p<0.05$) when compared to those in group of 'RM4000 - more than RM10,000' (3.33) (Figure 6).

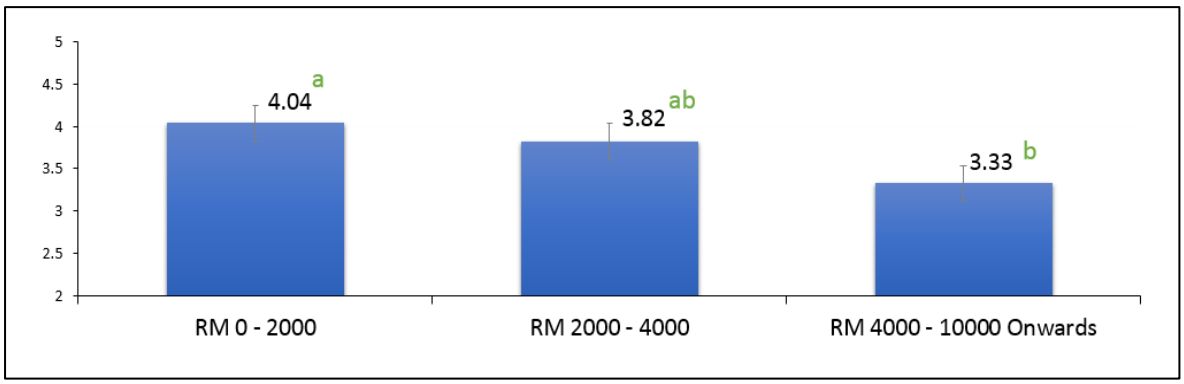

Figure 6. Different income groups were compared based on REP scale on variable of 'being with friends'.

Anderson and Fowers (2019) stated that friends with virtue regard their friendship, as well as their shared interests and aims, as worthwhile in and of themselves. Meanwhile, for variable of 'tranquillity', those in group of 'RM0-2,000' (3.76) was significantly higher ($p<0.05$) when compared to group of 'RM4000 - more than RM10,000' (3.33) (Figure 7). These indicate that the lower income group of respondents in this study tend to have high motives for being with friends and tranquillity when involved in fish keeping activities. Understanding on the motives of fish keepers, would help the government through its agencies to understand the stakeholders of the ornamental fisheries industry, better. This includes some strategies to control the wild-caught ornamental fishes, but at the same

time securing the income and livelihood of the people who entirely dependent on the stock.

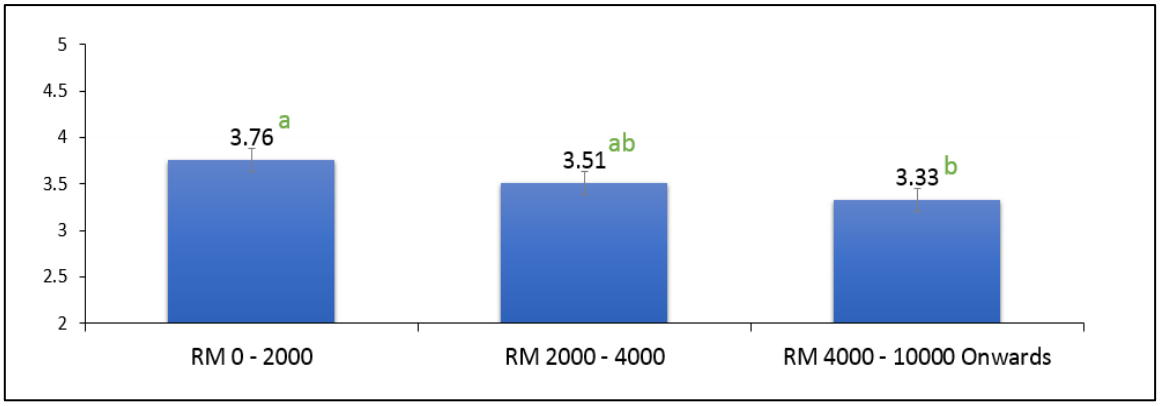

Figure 7. Different income groups were compared based on REP scale on variable of 'tranquillity'.

Hatcheries in aquaculture system can reduce the pressure on wild stock fish population. Developing and utilizing aquaculture techniques, such as brood stock management and captive breeding to supply fish for trade, can reduce the pressure on wild-capture and thus reducing overexploitation of the fishes (Pandey and Mandal, 2017). Malaysia is one of the well-known countries in the world, other than Thailand, Indonesia, China and Singapore, that is popular for its ornamental fish trade, who specialized in breeding and multiplication of freshwater ornamental fishes (Livengood and Chapman, 2007).

Conclusion

Ornamental fisheries is important to the country's economy and the social well-being of the people. A total of 125 fish keepers responded to the survey questionnaire. The basic socio-demographic background information of the fish keepers were explored. Most of the respondents are male, and age between '25 to 34 years old', and many of them obtained a diploma or certificate. On the other hand, fish keeper's preferences of fish discovered that they preferred red color fish and multi-combination color fish. They preferred all size of fish, easy to take care of, omnivore fish, and fish that able to live in a community. Many of them spent RM11.00 to RM50.00 each time at an ornamental fish store. Meanwhile, the fish keeper's motivation variables were derived from Recreation Experience Preference (REP) scale. The highest selections were recorded under 'general learning', 'creativity' and 'family togetherness'. Most of the variables did not show any significant results towards the motivations of fish keeping, except for some of the variables within the education levels and income groups. Definitely, more studies need to be done nationwide to understand further on fish keeper preferences towards ornamental fishes and their motivation towards fish keeping. This study revealed some important information about fish keepers in Melaka, for a better management of the ornamental fisheries industry in Malaysia.

Acknowledgement

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EFFECTS OF CRAB SHELL WASTE AS FEED ON GROWTH PERFORMANCE AND COLORATION OF SIAMESE FIGHTING FISH (*Betta splendens*)

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Introduction

The Siamese fighting fish (*Betta splendens*), commonly known as the *betta,* is a freshwater fish native to Southeast asia (Regan, 1910). *B. splendens* is native to Southern Asia such as northern Malay Peninsula, central and eastern of Thailand and Southern Vietnam (Witte and Schmidt, 1992). Malaysian *Betta* fish export value has reached at RM370 million a year even though Malaysia is not the biggest exporter of ornamental fish (Department of Fisheries Malaysia, 2020). This made Malaysia the fourth top exporter for Siamese fighting fish in Asia.t High priced fighting fish that sold to an amount of RM7000 had sparked interest among ornamental fish enthusiasts. Crab shells are often thrown away after consumption. Crab shell is rich with carotenoids that can serve as a natural coloring agent in food. Crab shell contain astaxanthin which gave red colour to the shells. Carotenoid components can be utilized to boost coloration of Siamese fighting fish. Objectives of this study are to analyze the proximate composition and carotenoid content in feed and to determine the effect of crab shell as feedstuff on growth performance and coloration of *Betta splendens*.

Materials and Methods

Experimental design

Portunus sanguinolentus were collected at Malaysian Fisheries Development Authority (LKIM) Fisheries Complex Kuala Besut. Crab purchased were 7 kg. All these sample was being processed to make it into powder form by using Waring Commercial Bar Blender HGB25EK and stored in 20˚C future use. 60 tails of one month old *Betta splendens* ranged weight between 0.1g to 0.4g were purchased from a local fish breeder located at Kuala Nerus, Terengganu. Adaptation period for one week (7 days). Feed were given daily and after a week, each 45 fishes were weighted and randomly divided into 45 individual aquariums with size 18cm x 12cm x 9cm. 1g of sample (astaxanthin, crab shell waste and krill) were dissolve with 10 ml of ethanol and then filtered by using Whatman No.42 filter paper repeatedly until the filtered became colorless and then was quantified by using UV-Vis Spectrophotometer at wavelengths of 400nm, 450nm and 500nm (de Carvalho et al., 2012). Proximate analysis were conducted for measuring crude protein, crude lipid, crude fibre, ash, moisture and nitrogen free extract. Feeding trial was

conducted for 40 days. Feed was given twice a day. Body sampling were taken every 10 days. All the data obtained were calculated by following these parameters: Body Weight Gain (BWG), Feed Intake (FI), Feed Conversion Ratio (FCR) and Survival Rate (SR) (Kim et al., 2016). Fabanjo and Abdullah, (2021) used Toca Color Guide method for qualitative data collection. All the data obtained from the experiment was interpreted by using One-Way ANOVA in IBM SPSS Statistics version 27 (Workagegn et al., 2014).

Results and Discussion

Proximate Composition

Table shows that T1 is highest protein content at 22.40%. It is also the closest protein percentage to the optimum protein needed by *Betta splendens* which is 35% (Watson et al., 2019). All treatments showed high lipid percentage except for T1 and T2.

Table 1. Proximate of Feed Treatment

Treatment	TC	T1	T2	T3	T4
CL	8.89 ± 1.36^{b}	6.66 ± 0.10^{ab}	6.10 ± 0.05^{a}	7.95 ± 0.08^{ab}	7.51 ± 0.11^{ab}
CP	20.95 ± 0.38^{d}	22.40 ± 0.29^{e}	18.04 ± 0.49^{c}	13.40 ± 0.62^{b}	11.04 ± 0.08^{a}
CF	5.67 ± 0.28^{a}	4.86 ± 0.06^{ab}	6.505 ± 0.19^{ab}	6.66 ± 0.30^{bc}	7.44 ± 0.35^{c}
Ash	21.29 ± 0.30^{b}	18.27 ± 0.43^{a}	23.07 ± 0.27^{bc}	25.10 ± 0.98^{c}	28.17 ± 1.29^{d}
Moisture	11.42 ± 0.42^{a}	12.15 ± 0.50^{a}	14.28 ± 0.12^{b}	13.90 ± 0.12^{b}	11.52 ± 0.20^{a}

High lipid consumed by fish can cause fatty liver, fat deposition and damage the health of the fish (Wang et al., 2021). Fibre in T1 is the lowest compared to the other treatments. Proximate composition for all treatment is significantly different ($P<0.05$).

Carotenoid Analysis

Krill and crab shell waste showed low carotenoid content (Table 2). However, crab shell waste appears to have lower carotenoid content compared to krill. The purpose of using different wavelength is because different research conducted used different wavelength that ranged between 450nm to 500nm (de Carvalho et al., 2012; Sindhu and Sherief, 2011)

Table 2. Carotenoid Content

Carotenoid Content (µg/g)	400	450	500
Astaxanthin	142.6312	108.5648	135.0694
Krill	7.407407	6.790123	4.899691
Crab Shell Waste	5.632716	3.433642	2.391975

Growth Performance

All treatments in Figure 1 show an increase in BWG with T1 showing a significant increase among all treatments. This can be influenced by the amount of protein in T1. Protein is important for good growth performance and development of the fish (Watson et al., 2019). The least BWG is TC with only 12.57% of increment on day 40. All of the fishes in all treatments were given feed amounting to 3% from the total weight of all fish in each treatment. (Folorunso et al., 2017).

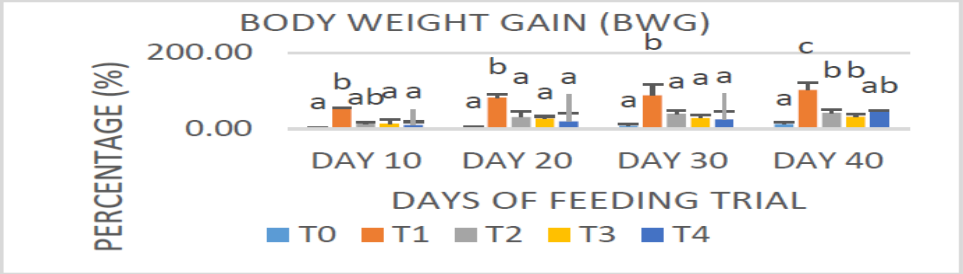

Figure 1. Body Weight Gain

Results in Figure 2 show that feed intake decreases with increase of crab waste in diet except for T1 without any significant differences among treatments.

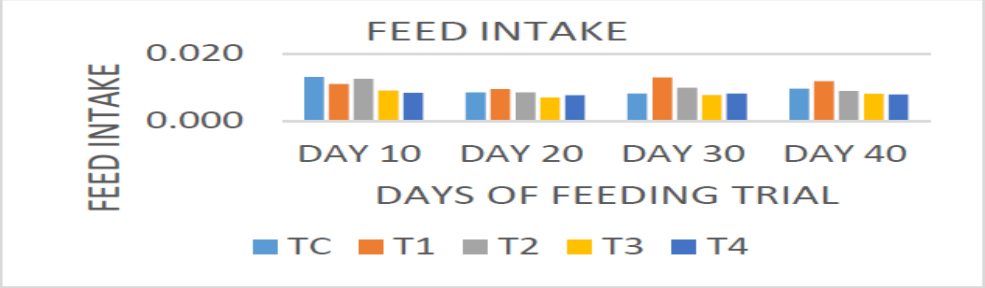

Figure 2. Feed Intake

FCR in Figure 3 are calculated to know the efficiency of diet given to fish as feed is one of the most expensive components in culturing and fish management (Craig & Kuhn, 2017; Fry et al., 2018). Lower FCR shows that it is efficient for the fish growth performance. T2 have the lowest FCR at 0.02.

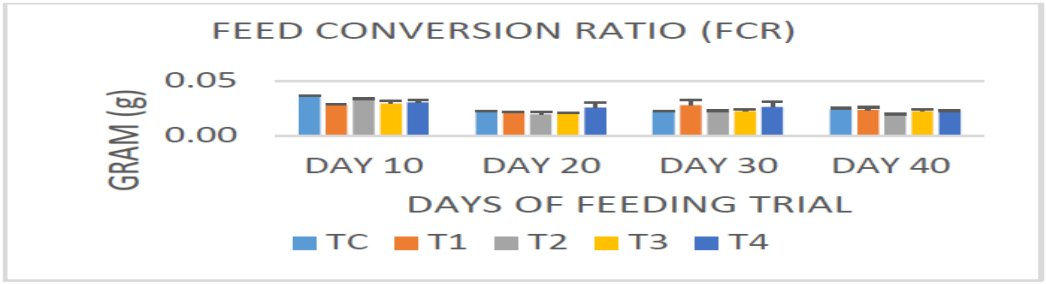

Figure 3. Feed Conversion Ratio

All of the treatment in figure 4 shows 100% SR except for two treatments, TC and T2.

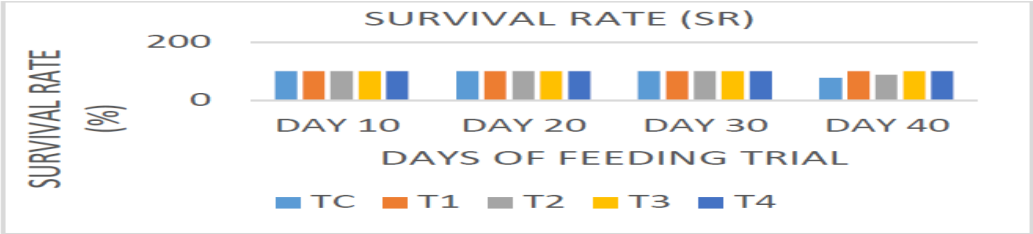

Figure 4. Survival Rate

Coloration

All treatment shows no changes in color. Previous research conducted on coloration of *Betta splendens* has a longer period of feeding trial at four month (Fabanjo and Abdullah, 2021). Color of fishes are determined by the genetics of the fish, the fish nervous system and glandular factors as well as the fish diet (Kaur Rajinder and Tarang Kumar Shah, 2017).

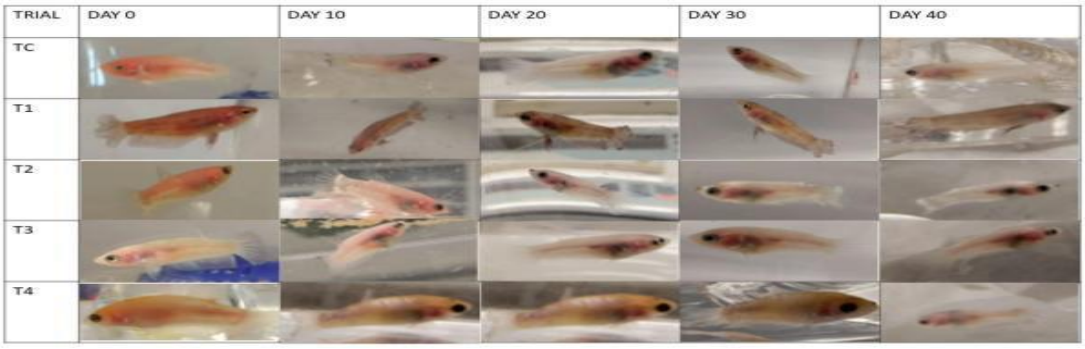

Figure 5. Changes in Fish Coloration during Feeding Trial

Conclusion

T1 indicated the best feed as it has the highest protein and low lipid content. For BWG, T1 shows the best result as it has the highest BWG among all treatments. Fish coloration showed no changes. This might be due to low reading of carotenoid content in crab shell waste.

Acknowledgement

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EFFECTS OF FEEDING THE HERB MISAI KUCING (*Orthosiphon stamineus*) ON THE MEAT QUALITY OF BROILER CHICKEN

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Introduction

Natural antioxidants have expanded on the grounds that they are thought to be safer than synthetic antioxidants and have more noteworthy application potential for consumer acceptability and the time span of usability of meat products. Misai Kucing (*Orthosiphon stamineus*; OS) has been specified as a good antioxidant. Traditional medicinal herbs have been proposed to livestock producers as alternatives to antioxidative growth promoters, including many that are rich in phenolic compounds, flavonoids and aromatic compounds which have been shown to improve growth performance of livestock through enhancing the immune status of the animals (Jang et al., 2008; Saleh et al., 2018). Poultry diets supplemented with antioxidants, such as Vitamin E, have been extensively studied to improve meat oxidative stability. Herbs with antioxidant capacity are being tested to improve animal performance and the quality and shelf life of meat products (Karami et al., 2011; Sugiharto et al., 2020).

Some herbs have been used to replace Vitamin E in animal diets (Karami *et al.*, 2011). Others improve the sensory characteristics of foods, besides extending their shelf lives, by reducing or eliminating pathogenic bacteria and enhancing overall quality through the inhibition of oxidative rancidity and the stabilizing meat color (Suliman et al., 2021). In the present study, *O. stamineus* leaf powder added as a supplement to the diet of broiler chickens was evaluated for its antioxidant potential, Vitamin E serving as the respective positive control.

Materials and Methods

Birds and Experimental Design

One hundred and fifty (150) one-day old male broiler chickens (Cobb 500) were sourced from a local hatchery. The broilers had *ad libitum* access to water and were fed a commercial broiler starter diet (0–6 days) and a grower diet (7 – 20 days). At the end of week 3, the broilers were weighed and reassigned to four different dietary treatments to achieve comparable average weights for each treatment. Each treatment had five replicates with eight broilers in each replicate. From the 21st to the 42nd days of the experimental period, the broilers were fed the following diets:

1) Basal diet (Control basal diet)
2) Basal diet supplemented with 8 g/kg *O. stamineus* leaf powder (8 g/kg OS + basal diet)
3) Basal diet supplemented with 200 mg/kg Vitamin E (Vitamin E + basal diet)

The basal diet was formulated according to the National Research Council recommendation (NRC, 1994). Fresh samples of cultivated Orthosiphon stamineus (OS) obtained from the Herbal Farm in Universiti Putra Malaysia. Vitamin E in the form of d-1-α-tocopherol acetate was supplied by Lutavit E 50 (BASF, Germany). Vitamin E served as a positive control for its antioxidant activity. On day-42, ten broilers from each treatment group were individually weighed to the nearest gram and slaughtered by severing the carotid artery and jugular veins (Islamic method).

Sensory Evaluation

A panel of twenty untrained students from Universiti Putra Malaysia were asked to evaluate their impressions of the aroma, tenderness, juiciness, flavor and overall acceptability of the samples using a nine-point Hedonic Scale.

Meat Color and pH Measurement

The color of breast meat samples was determined at 0, 3, 6 and 9 days after storage at 4 ºC. The lightness (L*), redness (a*) and yellowness (b*) of breast meat was measured using a ColorFlex® System (Hunter lab, Reston, VA, USA). Breast meat samples (10 g) were homogenized in 50 mL of double distilled deionized water to determine the pH 0, 3, 6 and 9 days after storage at 4ºC. A standardized combination electrode attached to a bench-top pH meter (Inlab® Expert Pro, UK).

Measurement of Antioxidative Potential

Free radical scavenging activity of meat was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Hatano et al., 1988), with slight modifications. Lipid oxidation was assessed on the basis of malondialdehyde (MDA) formed during storage. Malondialdehyde, the end-product of lipid peroxidation by reactive oxygen species, was evaluated using the TBARS assay kit (Oxiselect™, Cell Biolabs, U.S.A.). Breast meat samples were stored at 4 ºC and readings were taken after 0, 3, 6 and 9 days of storage.

Statistical Analysis

Statistical data analysis was carried out using the SPSS software (IBM SPSS version 21). Differences between means for all parameters were determined using one-way analysis of variance (ANOVA), with means compared using Duncan's test to show differences among treatments ($P<0.05$).

Results and Discussion

Sensory Evaluation

In the present study, human sensory acceptability to the meat of broiler chickens subjected to different dietary treatments was evaluated and results are shown in Table 1. All sensory parameters including overall acceptability score were found not to be significantly different among all the dietary treatments.

Table 1. Sensory scores of cooked breast meat of broiler chickens fed on different diets

Treatments	Aroma	Flavor	Tenderness	Juiciness	Overall Acceptability
Control	6.65 ± 0.34	6.65 ± 0.32	7.15 ± 0.33	6.50 ± 0.35	7.30 ± 0.31
8g/kg OS	6.75 ± 0.32	6.70 ± 0.34	6.80 ± 0.41	6.60 ± 0.35	7.10 ± 0.37
Vitamin E	6.55 ± 0.39	6.80 ± 0.37	7.25 ± 0.44	6.85 ± 0.26	7.55 ± 0.29

Treatment means ± std errors are presented. No significant differences among treatments were found (P>0.05).

Meat Color and pH

There was no significant difference in the yellowness values of raw breast meat among the experimental treatments. Raw breast meat from broilers fed OS (L* lightness 54.64± 1.64) and Vitamin E (L* lightness 55.25± 1.28) supplements were much lighter in coloration than that of broilers on the control (L* lightness 48.91± 1.48) diet. The direct antioxidant action of α-l-tocopherol on membrane lipids may indirectly delay oxymyoglobin oxidation and thus meat discolouration (Sugiharto et al., 2020). The pH differences among raw breast meat samples from broilers fed various diets emerged after 9 days of storage (pH values ranging from 5.75 to 6.09), with OS and Vitamin E supplementation giving significantly lower pH values after 9 days of storage.

Anti-oxidative Activity of Meat

Table 2 shows the DPPH radical scavenging activity and lipid oxidation potential of breast meat from broilers after storage at 4 °C for 9 days. The 8 g/kg OS + basal diet and the basal + Vitamin E diet, DPPH readings were already significantly different on Day-6 as compared to Day-0.

Table 2. Changes in the antioxidative potential of breast meat of broiler chickens in relation to duration of storage at 4 ºC.

Treatments	**Duration of storage** (Days) 0	3	6	9
	DPPH radical scavenging activity (%)			
Control	68.47 ± 0.64^{ay}	67.65 ± 1.51^{ay}	64.88 ± 0.95^{ay}	59.56 ± 1.44^{by}
8 g/kg OS	74.26 ± 0.85^{ax}	72.48 ± 0.69^{abx}	69.60 ± 1.11^{bx}	63.31 ± 2.10^{cy}
Vitamin E	76.17 ± 0.58^{ax}	73.50 ± 0.58^{abx}	72.90 ± 0.48^{bx}	70.52 ± 1.95^{bx}
	TBARS value (mg MDA/kg)			
Control	0.83 ± 0.02^{dx}	1.59 ± 0.07^{cx}	2.14 ± 0.05^{bx}	3.08 ± 0.10^{ax}
8 g/kg OS	0.68 ± 0.03^{dy}	1.24 ± 0.02^{cy}	1.71 ± 0.04^{by}	2.31 ± 0.07^{ay}
Vitamin E	0.75 ± 0.02^{dy}	1.32 ± 0.04^{cy}	1.69 ± 0.03^{by}	2.24 ± 0.06^{ay}

Treatment means ± std errors are presented.
Treatment means with different superscript letters (a - d) within the same column differ significantly (P<0.05). Treatment means with different superscript letters (x, y) within the same row differ significantly (P<0.05)

A comparison between the experimental treatments showed that supplementation of the basic diet with OS or with Vitamin E gave significantly higher DPPH readings in the breast meat on Day-6 as compared with readings from the controls. This advantage was maintained in the Vitamin E + basal diet treatment, but not in the OS + basal diet treatment, on Day-9. TBARS values in all dietary treatments rose significantly (P<0.05) over each 3-day interval from Day-0 to Day-9 of storage. Across the dietary treatments, supplementation with Vitamin E and with OS significantly lowered TBARS readings as compared with the basal diet alone from Day-0 onwards. Tetracycline supplementation did not alter TBARS readings significantly relative to the control. According to Nikmarama et al. (2018), many herbs contain active compounds, including polyphenols that have antioxidant activity, which could extend the shelf life of meat products and improve their quality. Phenolic compounds react with lipid and hydroxyl radicals to convert them into stable products (Hadidi et al., 2022). Moreover, crude fat of breast meat of broiler fed vitamin E and 8 g/kg OS supplementation was also reduced. In addition, natural antioxidants, in particular polyphenols, are the major plant compounds which have the ability to attenuate the oxidative damage of a tissue indirectly by enhancing the natural defenses of the cell and/or directly by scavenging the free radical species to combat pathological disorders generated by phytochemical Reactive Oxygen Species (ROS) (Nikmarama et al., 2018).

Conclusion

The inclusion of *O. stamineus* leaf powder at 8 g/kg in diet was comparable with 200 mg/kg Vitamin E supplementation in the diet. *O. stamineus* leaf powder has, therefore, the potential to replace conventional antibacterial and antioxidant compounds as broiler diet supplements in organic and sustainable poultry production.

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HYPO-OSMOTIC SWELLING TEST(HOST) ON BUCK SEMEN SUPPLEMENTED WITH STINGLESS BEE HONEY

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Introduction

Hypo-osmotic swelling (HOS) test is done by assessing the membrane integrity of the sperm cells morphologically. It is an important diagnostic tool for sperm motility and fertilization success. The ability to increase in volume in a hypoosmotic solution indicates that these cells are active biochemically. Swelling occur during the hypo-osmotic test and causes changes in both cell size and shape that can be evaluated using a contrast microscope (Cabrita et al., 1999). The swelling is at peak when the equilibrium between the fluid compartment of the spermatozoa and the extracellular medium is established. It can be observed at the coiled flagellum inside the membrane (Jeyendran et al., 1984). Fonseca et al. (2005) reported that 125 mOsm/l solution would be best for use in HOST in fresh goat spermatozoa. Stingless bee honey (SBH) in Malaysia has recently received commercial attention. The highest content of monosaccharides in SBH consist of two monosaccharides which are fructose and glucose and a small amount of disaccharide which is sucrose (Souza et al., 2006). The inclusion of trehalose and sucrose (disaccharides) in the semen extender was suggested as a non-penetrating cryoprotectant in goat sperm preservation (Khalili et al., 2009). Despite its usage as an energy source, it can reduce the damaging effects during sperm freezing (Purdy, 2006). The competency of the sperm membrane plasma treated with SBH is vague since less research had been done on it. Thus, this study was conducted to determine the effect of the stingless bee honey as a semen extender on membrane integrity and the quality of buck semen.

Materials and Methods

This study was carried out at the Goat Rearing Facility, Faculty of Sustainable Agriculture, University of Malaysia Sabah (UMS) Sandakan Campus. Semen samples were collected from two (2) Boer bucks using an artificial vagina (AV). Semen collected was pooled and diluted into five different concentrations of SBH at 0%, 0.5%, 1.0%, 1.5% and 2% volume per volume (v/v) respectively. TRIS was used as control. All treatments were replicated three times. Pooled semen samples were centrifuged and washed prior to dilution and the supernatant was removed. The remaining pellet was used for further dilution with the treatment in 1:8 ratio (v/v). For every treatment, 0.3 ml of semen sample was dispensed into a sterilized collection tube containing 3.0 ml of all treatment extenders each. Sperm individual progressive motility, sperm viability and sperm abnormality were assessed through microscopic observation and eosin-nigrosin staining procedure.

Throughout this period, the extended semen was kept in water bath set at 37°C. The HOS tests were performed by mixing the 10µl of treated semen with 2 mL hypo-osmotic solution and incubated at 37˚C for 1 hour. 20µl drop of sample was placed on a clean glass slide and covered with a cover slip for the HOS test analysis. Minimum of 200 spermatozoa were observed for the cells associated with swelling and curled tails using microscopic fields. The percentage of swollen and curled tails sperm was recorded (Jeyendran et al.,1984). All data were determined using one-way analysis of variance (ANOVA).

Results and Discussion

The effects of different concentration of SBH supplemented in TRIS on viability, individual progressive motility (IPM), hypo-osmotic swelling test (HOST) and abnormalities are presented in Table 1.

Table 1. Mean percentage of sperm viability, individual progressive motility and hypo-osmotic swelling test (HOST) with different concentrations of SBH in Tris extender.

Parameter	**Honey extender (%) (Mean±SEM)**				
	SBH 0 (C)	**SBH 0.5**	**SBH 1**	**SBH 1.5**	**SBH 2.0**
Viability	35.33±1.45^{a}	33.33±2.03^{a}	37.33±0.70^{a}	37.00±1.2^{a}	37.33±1.50^{a}
IPM	21.56±0.61^{a}	21.66±0.59^{a}	22.06±0.7^{a}	22.00±0.66^{a}	22.90±0.26^{a}
HOST	32.33±1.50^{b}	31.00±2.02^{b}	31.00±0.67^{b}	38.66±1.20^{a}	39.33±1.50^{a}
Abnormalities					
Damaged head	2.21±2.05 ^{a}	2.86±0.49 ^{a}	1.18±0.62 ^{a}	0.95±0.49 ^{a}	2.87±1.37 ^{a}
Detached head	0.99±0.44 ^{ab}	1.90±0.55 ^{a}	0.70±0.42 ^{b}	0.95±0.56 ^{ab}	1.91±0.28 ^{a}
Midpiece	5.63±0.56 ^{a}	3.33±0.28 ^{c}	2.82±0.45 ^{c}	4.29±0.41 ^{b}	2.87±0.26 ^{c}
Coiled tail	9.77±0.31 ^{a}	10.48±0.27 ^{a}	9.42±0.16 ^{a}	9.05±0.43 ^{a}	6.70±0.42 ^{a}
Bent tail	8.57±1.34 ^{a}	7.14±0.84 ^{a}	9.16±0.90 ^{a}	5.71±1.27 ^{a}	5.26±0.64 ^{a}

*Mean±SEM followed by different alphabets within the same column indicate significant difference at $p<0.05$. C (Control = TRIS); SBH (Stingless Bee Honey); SBH0.5 (TBS+0.5% SBH); SBH1 (TBS+1.0% SBH); SBH1.5 (TBS+1.5% SBH); SBH2 (TBS+2.0% SBH); IPM= Individual Progressive Motility; HOST= hypo-osmotic swelling test.

The present results reported that sperm viability and individual progressive motility were not significantly affected ($p>0.05$). These results were lower than reported by Khalili et al. (2009) when the sperm was supplemented with trehalose and sucrose at different osmolality. According to a review done by Rao et al. (2016), glucose, fructose, and sucrose content of SBH are generally lower compared to regular honey. The lower concentration of sugar in SBH may explain the reduced mean in percentage of viable sperm in the present study. Another factor should be considered are the stability of

semen pH (Weitze and Petrunkina, 2007, Hahn et al., 2019) and the examination (Roof et al., 2012). The present study showed significant ($p<0.05$) improvement in HOST when the concentration of SBH is at 1.5 and 2 %. However, these results were significantly lower than 40 % which were reported by Khalil et al. (2009) and Sundararaman et al. (2016). We are suggested that the membrane integrity of the sperm considered below the minimum requirement when at least 50% of swelling sperm is needed to increase the chance of pregnancy (Tartagni et al., 2002). Mean percentage of damaged head, coiled tail and bent tail were not significantly affected by the SBH. Detached head in SBH0.5 and SBH2 were significantly higher ($p<0.05$) when compared to SBH1. Midpiece abnormalities was higher ($p<0.05$) in SBH0 as compared to SBH0.5, SBH1, SBH1.5 and SBH2. Morphological abnormalities from a normal fertile buck are usually less than 5%, however it can reach more than 10% if the buck is below average or poor in fertility (Ott, 1978). Genetic-make up, physiological stage of the animal, nutrition, climate, or presence of underlying disease may be contributed to these abnormalities (Dana et al., 2000).

Conclusion

In conclusion, results suggest that inclusion of SBH1.5 and SBH2 in TRIS gained the best HOST in goat spermatozoa. However, the semen quality of all treatments was below the minimum requirement. We would suggest that another study must be done with the possible factors affecting the sperm quality.

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DIGESTIVE EVALUATION OF OIL PALM EMPTY FRUIT BUNCH TREATED WITH *Ganoderma lucidum* AS TOTAL MIXED RATION IN GOATS

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Introduction

Malaysia Palm Oil Board (MPOB) in 2021 stated a total of 27 million tonnes of oil palm products in the country were exported and huge amounts of waste were produced along the way. Utilizing these wastes is important to manage pollution and upcycling these resources into valuable and profitable products will be an added benefit. Oil palm empty fruit bunches (EFB) are a major biomass by-product from the industry and a potential feed for herbivore livestock for its source of fibre. It could also be one of the solutions to the feed shortage problem of ruminants. EFB makes up the highest percentage of waste generated in palm oil mills, which accounts for 23% of the fresh fruit weight with an amount of 15.91 tonnes/year/hectare (Derman et al., 2018). However, EFB is a lignocellulosic fibre material which is not favourable for livestock due to its high undegradable fibre content. On the other hand, ruminants are physiologically able to convert low-quality, fibrous material into high-quality end products (Ross et al., 2012) due to the symbiotic relationship between the ruminant and its microbial community. Nevertheless, the excessive fibre content in the feed will still hinder the overall digestibility of feed, reducing the feed intake of animals, and leading to lower growth and performance of animals. Studies reported the application of biological pre-treatment using white rot fungi (WRF) through solid-state fermentation (SSF) was effective in overcoming the high fibre content of a substrate. Nur Nazratul et al., (2019) used this theory on mycelia culture of *Ganoderma lucidum* (*G. lucidum*) on EFB for 12 weeks of SSF period and found effective degradation of the lignin structure of EFB. The study revealed that the now treated-EFB has the potential as a feedstuff for livestock. However, no further study has been done on the application of EFB treated with the mycelia culture of G. lucidum as feed for ruminants. The objectives of this study were to replicate the previous study, in larger-scale production, and to evaluate its potential as a feed, in terms of its digestibility, when the treated EFB is formulated as a total mixed ration and given to goats, in a feeding trial.

Materials and Methods

Preparation of oil palm EFB fibres, inoculation, and incubation with Ganoderma lucidum

Approximately 1 kg of shredded oil palm empty fruit brunch (EFB) fibres were filled into a 35 x 58 cm heat-resistant polypropylene bag. The EFB were previously soaked overnight, drained, and tossed to remove excess free-flowing water. Each bag which contained 1 kg of EFB fibres was sterilized using an autoclave machine (Tomy, SX-700) at 121 °C with 15 p.s.i. for 15 mins. The EFB was then left to cool to room temperature before inoculation. One bag of EFB was inoculated with 10 fully cultured plates (90 x 15 mm) of G. lucidum. The fermentation was left to occur in a dark room for 12 weeks. Finally, the EFB was harvested, dried in a 60 oC oven and ground to 1-5 mm to be used as feed.

Animal and feeding management

Fifteen male Boer cross goats aged approximately 6 months, with an average body weight of 17.4 kg were used in this study. The feeding of goats was conducted for 2 weeks, which included 7 days adaptation period. Animals were randomly assigned according to a completely randomized design (CRD) arrangement, into either one of the three dietary treatment groups. The three dietary treatment groups were: T1: Napier 50% + concentrate (control); T2: Untreated EFB 25% + Napier 25% + concentrate; and T3: Treated EFB 25% + Napier 25% + concentrate. Each group consisted of 5 goats as replicates. The diet was offered daily according to 3% body weight at 0900 and 1730 hours, with ad libitum access to water. Next, the digestibility study commences using the total collection method. A digestibility study was conducted for 7 days with feed intake and total fecal output recording and collection. For each animal, 10% of the total daily fecal production was sampled out and pooled to be one sample. The feed and fecal samples were then kept in a -20 ºC freezer prior to lab analysis.

Feed and fecal sample analysis

Feed and fecal samples were first partially dried at 60 °C for 3-5 days or till constant weight as sample preparation before further analysis. Proximate analysis for dry matter (DM), organic matter (OM) as well as crude fat (CF) were performed according to the method by the Association of Official Analytical Chemists (AOAC, 1995). Crude protein (CP) was analyzed using Dumas method. Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) followed methods by Van Soest et al. (1991).

$$\text{Apparent nutrient digestibility (\%)} = \frac{\text{Amount of nutrient consumed} - \text{Amount of nutrient excreted in feces}}{\text{Amount of nutrient consumed}} \times 100$$

Statistical analysis

All data obtained were analyzed using one-way analysis of variance (ANOVA) with means compared using Duncan's multiple range test (DMRT) at significance level of 5% ($p<0.05$). All statistical analyses were performed using the Statistical Analysis System (SAS) 9.4 (2007) or later.

Results and Discussion

Dry matter intake and fecal output

Table 1 shows DMI and FO of all treatments had no significant differences, and this indicates that the feed across all treatments was equally palatable and consumed by the goats. One of the reasons is probably due to the fact as feed was offered as TMR, where all the feed ingredients were ground to a similar size, and mixed to homogeneity for all the treatments, hence no bias in feed selection could be done by the goats. Size reduction, by grinding EFB treated or untreated, helps provide a uniform feed which improves nutrient digestibility and feed efficiency (Montoya and Leterme, 2011), which resulted in the feed being equally favorable, when compared to the control group.

Table 1. Dry matter intake and fecal output of goats fed with treatment

Parameters (g dry matter, DM)	Treatment Mean ± SE		
	T1	T2	T3
Average dry matter intake, DMI	323.53±48.93[a]	264.60±40.26[a]	355.65±22.01[a]
Fecal output, FO	83.70±21.82[a]	62.95±8.48[a]	92.19±10.3[a]

T1 (control): Napier 50% + concentrate, T2: Untreated EFB 25% + Napier 25% + concentrate, T3: Treated EFB 25% + Napier 25% + concentrate.
[a] Mean with same superscript within row does not differ significantly (p>0.05) using Duncan's Multiple Range Test (DMRT).

Apparent nutrient digestibility

Table 2 shows the apparent nutrient digestibility between treatments indicated no significant difference (p>0.05), except on ADLD%. ADLD% of T2 was lower compared to other treatments, and this is due to the higher lignin content of EFB in T2, as compared to T3, where the EFB was treated with *G. lucidum.*

Table 2. Apparent nutrient digestibility of treatment fed to goats

Apparent nutrient digestibility (%)	Treatment Mean ± SE		
	T1	T2	T3
Dry matter (DMD)	75.14±3.98[a]	74.65±1.35[a]	74.07±2.39[a]
Organic matter (OMD)	77.15±3.74[a]	76.40±1.21[a]	76.77±2.17[a]
Neutral detergent fibre (NDFD)	71.31±4.75[a]	72.49±1.26[a]	72.65±2.78[a]
Acid detergent fibre (ADFD)	63.32±6.35[a]	69.29±1.64[a]	69.62±3.04[a]
Acid detergent lignin (ADLD)	46.21±4.30[a]	20.95±1.86[b]	45.35±3.18[a]
Crude fat (CFD)	90.73±2.08[a]	94.44±0.97[a]	94.47±0.81[a]

T1 (control): Napier 50% + concentrate, T2: Untreated EFB 25% + Napier 25% + concentrate, T3: Treated EFB 25% + Napier 25% + concentrate.

[a,b] Mean with different superscript within row differ significantly ($p<0.05$) using Duncan's Multiple Range Test (DMRT). SE: Standard error.

Lignin causes lower fermentation and digestibility rate in the rumen, which leads to a slower disappearance through the digestive tract, thus limiting feed intake (Chanjula *et al*., 2019). This study showed that the pre-treatment with *G. lucidum* is effective in improving the quality of EFB as digestibility of T3 was higher as compared to T2.

Conclusion

The feeding of G. lucidum treated-EFB as a feed component in the TMR of goats had no significant effect on the feed intake and fecal output of goats. Apparent nutrient digestibility also showed no significant difference except for ADLD%, where the digestibility was higher for the treated-EFB group and comparable to the control, which had no inclusion of EFB. This proved that EFB treated with *G. lucidum* can be used successfully as one of the feed ingredients in the diets of goats as TMR, at the inclusion level of 25%, without posing a detrimental digestive effect towards the animal.

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PREPARATION AND NUTRITIONAL EVALUATION OF YEAST-FERMENTED HERBS FORMULATION FOR CHICKEN ADDITIVE

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Introduction

Herbs have extensive application and vital functions in curing various ailments; they can be alternatives to antibiotics for chicken production. Herbal antibiotics, which are safer than synthetic ones and feature bioactive components with antibacterial properties, may replace synthetic antibiotics. Malik et al. (2020) mentioned that herbs are rich in natural antioxidants needed for human health. Various techniques are utilised to turn herbs into feed additives; however, fermentation is likely the greatest technique for increasing nutritional and sensory properties as well as shelf life. The fermentation process is a purposeful conversion of a substrate through the activities of microorganisms to acquire the desired product completed through microbial actions (Adebo and Medina-meza 2020).

Fermentation improves nutritional value and quality, according to several research. Yang et al. (2021) found that quinoa fermentation boosted free and bound phenolic content and antioxidant and enzyme inhibitory properties. Yeast fermentation is a healthy approach to turn herbs into feed additives. Several studies have indicated that yeast plays a critical function in fermentation. Dai et al. (2020) concur that fermentation improves feed quality and gut ecology. Schlabitz et al. (2021) also believe adding *S. cerevisiae* yeast to animal diet increases health and performance. *Andrographis paniculata*, *Orthosiphon stamineus*, *Euphorbia hirta*, *Curcuma longa*, and *Syzygium polyanthum* were yeast-fermented for nutritional evaluation as demonstrated in the present study.

Materials and Methods

The local market provided dry samples of *A. paniculata*, *O. stamineus*, *S. polyanthum*, and fresh samples of *C. longa*. *E. hirta* samples were collected at Ladang Kongsi 2, Universiti Putra Malaysia Kampus Bintulu. Dry samples were oven-dried in the universal oven for 2 hours at 60 °C, and fresh samples for 72 hours at 60 °C. Fresh samples (*E. hirta* and *C. longa*) were cleaned with water and distilled water before being cut into 1-2 cm pieces and oven-dried.

The dried sample was ground to powder in a electrical grinder (Model SZ-1000A-3) and sieved through a one mm screen before being stored at 4 °C until further use. 200 mL of

distilled water, 40 mL of molasses, 40 mL of Lactobacillus, and 40 g of Baker's yeast (*Saccharomyces cerevisiae*) were fully dissolved in a 500 mL conical flask. The yeast suspension was gradually mixed into the 200 g of ground herb. The herb was placed in a zip-lock bag and kept for 14 days. The initial and final pH was measured. The proximate and mineral content of the fermented ground herb was also determined. The sample will be done in three replications and the results were analyzed statistically with Statically Analysis System (SAS) 9.4 Tukey test ($p< 0.05$).

Results and Discussion

Table 1 shows the contents of dry matter, moisture, protein, carbohydrate, fat, crude fibre, and ash in various herbs namely, *Andrographis paniculata*, *Orthosiphon stamineus*, *Euphorbia hirta*, *Curcuma longa* and *Syzygium polyanthum*. Analyses of the present study found that all herbs have variable amount of dry matter, moisture, protein, carbohydrate, fat, crude fibre, and ash. *S. polyanthum* recorded a significantly higher amount of dry mass than other herbs at 45.7%. This herbal species was also found to have a significantly higher amount of carbohydrate (36.7 g/100 g) and fat (2.3 g/100 g) as compared to other herbs tested in the present study.

Table 1. Proximate analysis of yeast-fermented herbs.

Parameters	***E. hirta***	***A. paniculata***	***C. longa***	***O. staminues***	***S. polyanthum***
Dry matter *(%)*	42.67±0.67^{b}	43.67±0.33^{b}	43.67±0.33^{b}	42.67±0.33^{b}	45.67±0.33^{a}
Moisture *(%)*	57.33±0.67^{a}	56.33±0.33^{a}	56.33±0.33^{a}	57.33±0.33^{a}	54.33±0.33^{b}
Crude Protein *(g/100g)*	6.32±0.50^{c}	6.61±0.13^{bc}	10.14±0.03^{a}	7.57±0.10^{b}	6.59±0.02b^{c}
Carbohydrate *(g/100g)*	32.18±0.48^{b}	24.77±0.03^{d}	26.54±0.39^{c}	30.59±0.44^{b}	36.66±0.47^{a}
Crude Fat *(g/100g)*	1.20±0.22^{c}	1.12±0.02^{c}	1.56±0.03^{b}	1.07±0.06^{c}	2.27±0.11^{a}
Crude fiber *(g/100g)*	10.21±0.14^{b}	13.19±0.19^{a}	8.70±0.44^{c}	12.33±012^{a}	9.96±0.29^{b}
Ash *(%)*	3.47±0.02^{c}	12.72±0.07^{a}	7.62±0.00^{b}	3.57±0.25^{c}	2.09±0.02^{d}

Values are means ± standard errors. a-e different letters show significant different at 5% level (Tukey HSD)

Andrographis paniculata, *Orthosiphon stamineus*, *Euphorbia hirta*, *Curcuma longa* shared similar amount of moisture at 56% to 57% which was significantly higher than *S. polyanthum* at 54%. C. longa had highest crude protein content at 10.14 g/100 g while *E. hirta* was the lowest at 6.32 g/100 g. *S. polyanthum* recorded the highest carbohydrate content at 36.66 g/100 g and *A. paniculata* was the lowest at 24.77 g/100 g. *S. polyanthum* contained the highest amount of crude fat at 2.27 g/100 g while *E. hirta*, *A. paniculata*, and *O. stamineus* were the lowest at 1.20, 1.12, and 1.07 g/100g, respectively. *A. paniculata* (13.19 g/100 g) and *O. stamineus* (12.33 g/100 g) contained the highest

amount of crude fibre while *C. longa* was the lowest, at 8.70 g/100 g. *A. paniculata* recorded the highest amount of ash at 12.72% and *S. polyanthum* was the lowest at 2.09%.

C. longa contained the highest amounts of primary nutrients such as nitrogen (3.25%), phosphorus (0.49%), potassium (5.14%), and a secondary nutrient, magnesium (0.41%) as compared to other herbs in this study (Table 2).

Table 2. Essential nutrients composition of yeast-fermented herbs.

Nutrient elements	***E. hirta***	***A. paniculata***	***C. longa***	***O. stamineus***	***S. polyanthum***
	percent				
N	2.43±0.026^{b}	2.14±0.010^{c}	3.25±0.011^{a}	2.46±0.031^{b}	2.18±0.014^{c}
P	0.27±0.000^{d}	0.28±0.00^{c}	0.49±0.003^{a}	0.36±0.003^{b}	0.25±0.003^{e}
K	1.62±0.006^{d}	2.48±0.003^{b}	5.14±0.007^{a}	2.27±0.003^{c}	0.78±0.003^{e}
Mg	0.19±0.000^{b}	0.41±0.004^{a}	0.41±0.005^{a}	0.19±0.003^{b}	0.11±0.003^{c}
Ca	1.81±0.004^{b}	2.40±0.008^{a}	0.76±0.009^{d}	0.72±0.010^{e}	0.97±0.007^{c}
	ppm				
Cu	7.20±0.06^{b}	8.53±0.21^{a}	6.00±0.30^{c}	7.80±0.12^{b}	3.33±0.03^{d}
Zn	57.05±0.07^{c}	128.50±5.21^{a}	68.73±0.37^{b}	46.58±0.34^{d}	45.43±0.01^{d}
Na	2082.25±28.72^{a}	330.00±1.73^{b}	255.00±5.77^{d}	275.50±6.06^{d}	312.50±4.33^{b}

Values are means ± standard errors. a-e different letters show significant different at 5% level (Tukey HSD)

A. paniculata demonstrated the highest amount of secondary nutrients of magnesium and calcium at 0.41% and 2.40%, respectively, where magnesium was similar as C. longa. *A. paniculata* also had the highest concentrations of copper and zinc, at 8.53 ppm and 128.50 ppm, respectively. Nutrient content of *S. polyanthum* indicated significantly lower amounts of most of the nutrients, except calcium and sodium, which were at moderate levels. In general, *C. longa* has the highest concentration of major nutrients especially on N, P, K, and Mg, making it essential for feed formulation for animal or livestock feeding. This agrees with the findings of Ikpeama et al. (2014), who claimed that Curcuma has a wealth of nutrients, including carbohydrates, proteins, and dietary fibre. However, when Curcuma is used into animal feed formulation, it may be necessary to supplement with certain minerals. This is demonstrated by the fact that the amount of minerals in *C. longa* detected in this study is relatively low (Table 2). The present study suggests that the *C. longa* might be one of the beneficial ingredients in formulating animal feed from which the diet could exhibit nutritional and medicinal properties for excellent animal growth performance.

Conclusion

In conclusion, this study found that yeast fermented from five herbs, hempedu bumi (*Andrographis paniculata*), misai kucing (*Orthosiphon staminues*), ara tanah (*Euphorbia hirta*), turmeric (*Curcuma longa*), and salam (*Syzygium polyanthum*) had varying proximate and mineral content. This study also suggests using C. longa to be included in the animal feed formulation. Fermented herbal yeast may contain antibiotics.

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IMPROVING THE VIABILITY OF LOCAL GRAIN CORN PRODUCTION FOR POULTRY FEED WITH RESEARCH AND DEVELOPMENT

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Introduction

Grain corn is the main ingredient for energy source and constitutes of at least 50 - 60% of total ingredients in animal feed especially for poultry. Malaysia imports almost all of nation's requirements for grain corn which costs the country RM1.72 billion in 2020. Producing own grain corn is a strategy to reduce dependency on imported grain corn and The Ministry of Agriculture and Food Industries (MAFI) has developed a Blueprint for nation's Grain Corn Industry. The target for the blueprint is to reduce importation of grain corn by 30% by 2032. Currently the productivity of local grain corn production is still low and needs to be improved. This can be improved by research and development (R&D) addressing critical issues across the supply chain. The limiting factor for local grain corn production is the high cost of production. In the production of local grain corn, the three highest costs are mechanization (46%); fertilizer (24%) and drying (16%) (Nor Amna A'liah Mohammad Nor et al 2020) To improve the viability of grain corn production, this production cost must be reduced or the yield must be increased.

Importance of good genetic materials

In order to improve the yield of any crop, good genetic materials are important precursors, the same applies to grain corn production. Hence, it is important to identify grain corn varieties that can adapt well and stable to be planted throughout Malaysia. This can be done by planting different varieties in different locations throughout Malaysia. Screening and identifying grain corn varieties were done where twenty- eight varieties were screened tor stability and yield in three separate locations in two different stages. These varieties are produced by International Seed Companies and the seeds are available to be imported for planting. Five locations have been selected which were Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai, Pulau Pinang (Northern area, mineral soil), MARDI Serdang, Selangor (Middle Western / Central area, mineral soil), MARDI Bachok, Kelantan (Eastern area, sandy soil), MARDI Kluang (Southern area, mineral soil) and Pertubuhan Peladang Kawasan (PPK) Bakri at Labis (Farmer plot-mineral). The performance of 28 imported grain corn varieties were evaluated in two phases for various characteristics including high yield and stability at

five different MARDI locations. In both studies, different environments or soil characteristics at different locations have affected the performance of the varieties. In terms of consistency, varieties that gave high yields (>8 tan/ha) in various locations are P4546 (Corteva/Pioneer) and S73 (Syngenta) from the first trial (Mohamad Bahagia et al, 2019) and P3875, P3582, P4554 and DK9979C (Bayer/Monsanto) from the second trial (Mohamad Bahagia et al, 2021). To ensure food security for grain corn in Malaysia, MARDI is in the process of developing local grain corn varieties, however, for now, these hybrid varieties can be recommended to the farmers to increase their production yield.

Development of Standard Operating Procedure (SOP) for Grain Corn Production

A Standard Operating Procedure (SOP) is currently being developed where all aspects of grain corn cultivation from planting to drying is being addressed. The SOP developed focused on planting of grain corn at mineral soil and specifically using variety P4546 as it has been found to have the best yield and most stable across several research plots in Malaysia. The SOP covers techniques for land preparation, planting, fertilizing, weed control, pest and disease control including Fall Army Worm (FAW) and harvesting that are fully mechanized. In order to reduce the land preparation cost, it is recommended to practise minimum, strip or zero tillage. These practices can help reduce cost up to $975.00 /ha. However, care need to be taken as it is not applicable to all types of soil. The ideal planting density for planting grain corn is 20cm x 75 cm giving 66,000 trees. In terms of pest and disease control, Integrated Pest Management (IPM) is recommended as it is more environmental-friendly. Weedicide rotation with different active ingredients is important for sustainable weed management in grain corn farms to reduce resistance. For harvesting, harvesting patterns which followed the planting rows recorded the lowest loss at 1.9%. Techniques that are included in the SOP have been tested in experimental plots and Local Verification Trials up to 2 hectares and have given yield up to 7 tonnes/ha. Currently, this SOP is being verified at bigger scale of >5 hectares.

Quality control of grain corn

Aflatoxin contamination is a serious issue in grain corn industry. Being hygroscopic, grain corn produced in wet tropical countries such as Malaysia often get contaminated with fungus during storage. Hence grain corn needs to be stored properly and treated with fungicide to reduce aflatoxin contamination. Not only aflatoxin contamination is dangerous for human and livestock consumption, grain corn that are contaminated will have difficulty to be sold to feed millers which will bring economic loss to farmers. To study the best environment for grain corn storage in Malaysia harvested grain corn were stored in three different packaging conditions commonly used by farmers in Malaysia which are 40 kg woven polypropylene bag (58 × 96 cm) ,1-tonne woven polypropylene jumbo bag (top duffle, bottom spout) (95 × 95 cm) and in a sealed container (49.5 × 96 cm, □ 39.5 cm). Two different ambient storage rooms in MARDI Serdang were used. In an attempt to further control fungal growth and aflatoxin contamination, grain corn that have been harvested were subjected to spraying by AgroZIDE™ a nano-fungicide based on nano-emulsion concept that contains cinnamon essential oil and surfactant. Studies on storage control revealed that for short term storage, three types of packaging conditions; 40 kg woven polypropylene, (PP) bag (58 × 96 cm) ,1-tonne woven polypropylene jumbo bag and in a sealed container are suitable for short-term storage of less than 3 months as the moisture content, composition of carbohydrate, protein and ash

remained unchanged throughout the storage period (Mohd Ramli SH et all ,2021). However, for longer term storage, treatment of grain corn with AgroZIDE™ is recommended as it can control growth of Aspergillus flavus to be less than 1% till 6 months while non-treated samples had A. flavus growth above 30% as early as during the second month and reached 100% by the fifth month (Noor Azlina Masdor, 2022)

Conclusion

In order to make local grain corn production in Malaysia more viable, recommendations based on Research and Development is vital. Various issues of grain corn production have been addressed and it is hoped the SOP developed based on the research findings will be a guide by farmers to increase their yield and make grain corn production in more viable and will bring economic benefits.

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ECO-SUSTAINABLE PRODUCTION OF BIOACTIVE SIALYLATED-MUCIN (SiaMuc) GLYCOPEPTIDE FROM EDIBLE BIRD'S NEST CO-PRODUCT

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Introduction

Edible bird's nest (EBN) is the dried salivary secretion of swiftlets during the breeding season. The widely consumed EBN for its highly unique nutrients has contributed to a high demand of international exportation (Hui Yan, Babji, et al., 2021). However, the processing of EBN to remove inedible materials (i.e. sand, dust, feathers) has contributed to a number of waste. The disposal of these waste is hazardous to environment and a waste of value due to abundant sialylated-mucin (SiaMuc) glycoprotein attached in the impurities (Noor et al., 2022). In fact, this "waste" is actually the processing co-product of EBN. Therefore, this study aimed to recover SiaMuc-glycoprotein in EBN co-product (EBNco-P) in the form of bioactive SiaMuc-glycopeptide hydrolysate. Through enzymatic hydrolysis, an eco-friendly alternative to transform insoluble SiaMuc-glycoprotein in EBNco-P into soluble bioactive SiaMuc-glycopeptide, valuable nutrients are separable from the impurities. Previously, through our own research, this biotechnology has been applied on cleaned-EBN (Hui Yan, Lim, et al., 2021). The research finds that the enzymatic hydrolysis of EBN that produce EBN glycopeptide has led to the bioconversion into high-grade product with enhanced nutritional bioavailability and functionality. Therefore, the eco-sustainable production of EBN bioactive SiaMuc-glycopeptide hydrolysate from its co-product not only provide a low-cost alternative for all levels of consumers to obtain valuable nutrients from EBN, but it also enhanced the bioavailability and bioaccessibility of EBN nutrients and functionality. This study may contribute to broaden the EBN product development in terms of nutraceutical functionality. With the availability of low-cost EBNco-P, this study is beneficial not only scientifically, but also the consumers, commercialization and industrialization

Materials and Methods

Sample Materials

Cleaned edible bird's nest (EBN) and co-product were provided by Mobile Harvester Sdn. Bhd, Selangor, Malaysia. Sample were grinded and stored in air-tight container.

Double-Boiling and Enzymatic Hydrolysis

Doubled-boiling and enzymatic hydrolysis of sample were carried out as described elsewhere (Hui Yan et al., 2022). Briefly, sample were double-boiled using waterbath for 30 minutes, followed by hydrolysis process using protease at pH 8.0 and 60°C. Sample was then filtered using a cloth strainer, followed by Whatman filter paper No.1 to remove inedible materials. Sample was then freeze-dried for further analyses.

Recovery Yield

Filtrate (i.e. EBN hydrolysates) was weighed after freeze-dried according to previous study (Noor et al., 2018). The weight of inedible materials, particularly the EBN co-product (EBN-coP) was recorded to determine the recovery yield of EBN in the form of bioactive SiaMuc-glycopeptide.

Proximate Analyses

Proximate analyses were performed according to method of Association of Official Analytical Chemistry (AOAC). Protein content of sample were determined using Kjeldahl method; Soxhlet method was used to determine fat content; ash or mineral content was determined by drying ashing method through combustion in a furnace; moisture content was determined by oven drying method; and carbohydrate was determined using subtraction method.

Physicochemical Analyses

Soluble protein content, peptide content, total polysaccharide content, reducing sugar content, sialic acid content, and glycoprotein/peptide content was determined based on Bradford assay, o-phthaldialdehyde (OPA) assay, phenol-sulphuric assay, DNS assay, resorcinol assay, and periodic acid Schiff (PAS) assay respectively (Hui Yan et al., 2022).

Antioxidative Activities

Antioxidative activities was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonic acid) (ABTS) radical scavenging activity, and ferric reducing antioxidant powder (FRAP) assays as described in previous study (Noor et al., 2022).

Statistical Analysis

All analyses were conducted in triplicates and one-way analysis of variance (ANOVA) was used to determine statistical difference of all means at a significance of $p \leq 0.05$.

Results and Discussion

Recovery Yield

The recovery yield of bioactive glycopeptide from cleaned-EBN and co-product was determined after enzymatic hydrolysis. The cleaned-EBN revealed a recovery yield of

92.0 ± 1.0 % with 8.0 ± 1.0 % of inedible materials such as dust, sands, and tiny feathers. The EBN co-product as well revealed a high recovery yield of 61.0 %, whereby the inedible materials (39.0 %) are successfully separated from the bioactive EBN glycopeptide (refer to Figure 1).

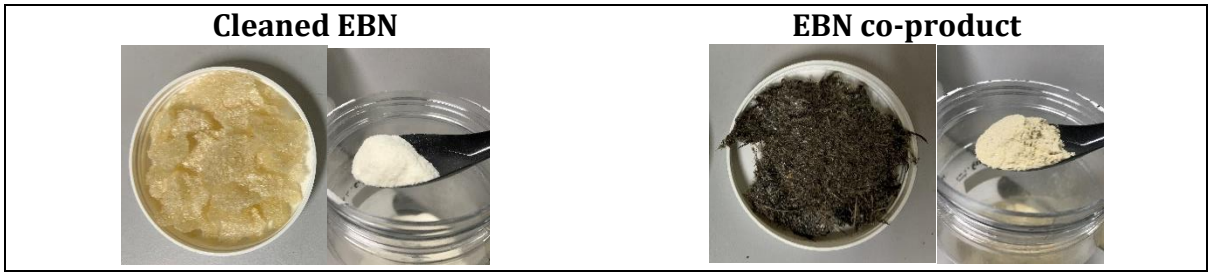

Figure 1. From left to right, the raw and hydrolysate of cleaned-EBN and co-product

Proximate and Nutritional Content

Proximate results of cleaned EBN and co-product were similar. Whereby, protein content stands the largest portion, followed by carbohydrate content, moisture content, and mineral content. Surprisingly, the fat content of EBN co-product revealed to be higher when compared to cleaned-EBN. This may be related to the presence of feathers attached in EBN co-product. Whereby, bird feathers contain residual grease (Tesfaye et al., 2017).

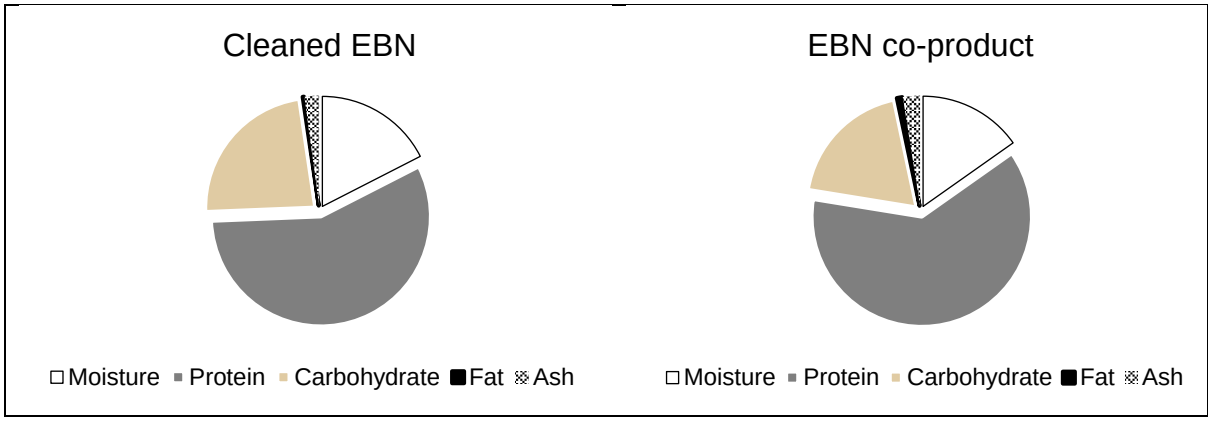

Figure 2. Comparison of proximate content in cleaned-EBN and co-product.
EBN = edible bird's nest.

Physicochemical and Antioxidative Activities

Similar findings were observed in terms of biochemical contents in cleaned-EBN and co-product. This indicates that bioactive SiaMuc-glycopeptide from both cleaned-EBN and co-product contains similar nutritional content. In details, soluble protein content of hydrolysed cleaned-EBN and co-product revealed at 1.91% and 2.31%; the peptide content at 55.78% and 42.81%; the polysaccharide content at 19.67% and 26.12%; reducing sugar content at 11.68% and 16.76%; sialic acid content at 22.98% and 31.09%; glycoprotein/peptide content at 88.94% and 72.63%. The EBN co-product showed a slightly higher quantity of biochemical contents, yet insignificant statistically.

The antioxidative activities of both cleaned-EBN and co-product disclosed similar results. The DPPH scavenging activities of cleaned-EBN and co-product hydrolysate was observed at 35.51% and 34.48%. However, the ABTS and FRAP activity showed a higher results antioxidative activity in EBN co-product. In details, cleaned-EBN has a ABTS value at 46.3% and 52.3%; the FRAP activity at 12.0 and 16.1 ug AAE/mg respectively. This is in line with previous studies (Gan et al., 2020).

Conclusion

The study finds that the bioactive sialylated-mucin (SiaMuc) glycopeptide from edible bird's nest (EBN) co-product has a physicochemical and nutritional properties similar and even better than those in cleaned-EBN. However, the eco-sustainable production method of EBN from co-product is a low-cost alternative for all consumers.

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