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Proximate Composition of Selected Freshwater Fish from Kampung Peta, Johor, Malaysia

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Abstract: Four species of freshwater fish from Kampung Peta, Johor namely *Osteochilus flavicauda* (Rong batu sirip), *Labiobarbus festivus* (Kawan), *Barbonymus gonionotus* (Lampam Jawa) and *Puntioplites bulu* (Tenggalan) were investigated in this study. The selected freshwater fishes from the Cyprinidae family were analyzed for their physical properties and proximate composition. The fishes exhibited weight of less than 400 g and have body length between 16 to 23 cm. Proximate analysis revealed that all fish species had comparable (p > 0.05) crude protein (13.40 - 13.75%) and ash content (2.76 - 3.27%) with each other. The moisture content was found to be between 77.02-82.65% where *B. gonionotus* exhibited a significantly (p < 0.05) higher percentage than the other species. The freshwater fishes were identified to be a lean fish containing crude fat of less than 2%. The highest (1.09 %) and lowest (0.48 %) amount of fat were recorded for *O. flavicauda* and *B. gonionotus* respectively. Meanwhile, the carbohydrate value ranging from 0.46 % to 5.26 % was inversely related to the protein content. Significant differences in carbohydrate value (p < 0.05) were observed between *O. flavicauda* (5.26%), *L. festiva* (2.91 %) and *B. gonionotus* (0.46%) while *L. festiva* and *P. bulu* (3.57%) showed insignificant differences (p > 0.05). These values are useful as a baseline data on the nutritional quality of freshwater fish from Kampung Peta, Johor.

Keywords: Freshwater fish, moisture, protein, fat, ash, carbohydrate, kampung peta

1. Introduction

Globally, fish is recognized as one of the healthiest foods contributing towards the nourishment of the body. It is an important source of lean protein providing all the essential amino acids for humans. Protein plays an important role in formation of body tissues, transport of oxygen and regulation of physiological activity. Besides having low caloric density, fish is also known as an excellent source of polyunsaturated fatty acids (PUFA) compared to land animals [1]. This nutritional quality is valuable in reducing risk of cardiovascular diseases and helps in the development of the brain. The overall health benefits of fish have significantly increased the demand for fish and fish products. According

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to the Food and Agricultural Organization (FAO) report on The State of World Fisheries and Aquaculture 2020, [2] the global fish consumption from 2000 until 2018 rose by 4.5 kg based on per-capita consumption with Asia accounting for a major proportion at an annual rate of 2%.

While the majority of commercial fish and fish products come from marine sources, about 51% of all known fish species are found in the freshwater [3]. Freshwater habitats include lakes, reservoirs, ponds, rivers, streams, brooks, springs, and groundwater exhibiting salinity environments of less than 1000 mg/l. In addition to playing an important role in the stability of stream ecosystems [4], freshwater fish are also the primary source of animal protein to the indigenous people in tropical Africa, Asia and Latin America. Moreover, farming of freshwater fish or aquaculture has been described to increase food security and dietary nutrition especially in developing countries [5-6].

Catfish (*Clarias* sp.), Tilapia (*Oreochromis niloticus*), Patin (*Pangasius* sp.), and various carp species (*Puntius gonionotus, Cyprinus carpio* and *Ctenopharyngodon idella*) are among the most well-known freshwater fishes in Malaysia. Despite all of these fish species, *Cyprinidae* is the largest family of freshwater fish having over 200 genera and 2000 species. Cyprinid fishes constitute around 27.8% of fish species composition in Malaysia [7]. Several studies have shown that freshwater fish is a good source of high quality protein [8-9], beneficial PUFA [10-13] and essential micronutrients [8] which are sometimes comparable with marine fish.

Although nutritional composition of freshwater fish has received significant attention among researchers, the available data may still be lacking. The different geographical areas, ecosystems and climate between freshwater bodies can influence nutritional quality [11]. Moreover, the diversity of fish species and sub-species also contribute to the variation of nutrient content among freshwater fishes. Therefore, this study aimed to evaluate the proximate composition of selected freshwater fish species from Kampung Peta, Johor, Malaysia. This area is known to be a settlement for the indigenous people of the Jakun tribe located near the Endau-Rompin National Park. Because this area is endowed with pristine rivers and ranging waterfalls, the local population has a long tradition of utilizing the freshwater fishes as food source. Considering the dependency on the aquatic animal for their daily diet, it is necessary to evaluate the nutrient content of the freshwater fishes from this area. Four freshwater fish species specifically *Osteochilus flavicauda (Rong batu sirip), Labiobarbus festiva (Kawan), Barbonymus gonionotus (Lampam Jawa)* and *Puntioplites bulu (Tenggalan)* were collected, identified and analyzed for their physical characteristics as well as proximate composition.

2. Material and Methods

2.1 Sample Collection

Four freshwater fish samples namely *Osteochilus flavicauda* (*Gerong*), *Labiobarbus festiva* (*Kawan*), *Barbodes gonionotus* (*Lampam Jawa*) and *Puntioplites bulu* (*Tenggalan*) were collected from Kampung. Peta, Johor, Malaysia. The fish samples were kept at 0°C in ice boxes before their weight and length were recorded. Scientific and common names of each species were also identified upon collection.

2.2 Sample Preparation

Prior to the proximate composition analysis, the fish samples were thawed and their skin was removed. The flesh of each fish was cut from the upper part below the dorsal fin down to the abdomen. Then, the fish samples were washed and cleaned to remove the remaining blood and impurities before being pat-dried with tissue paper [14]. Finally, 10 - 20 g of each fish sample was weighed and blended into a paste-like texture.

2.3 Determination of Proximate Composition

The chemical composition of fish samples was analyzed following the standardized Association of Official Analytical Chemists (AOAC), 2012 analysis.

2.3.1 Moisture

Oven drying method was used to determine the moisture content of the freshwater fish samples. 5g of each fish sample was placed into a pre-weighed aluminium dish prior to dehydration in an oven (Memmert, Germany) at $105 \pm 2^{\circ}$ C for 3 hours. Then, the dried samples were cooled in a desiccator for 15 minutes and weighed. The following formula was used to determine the moisture content of each sample on wet basis [15]:

$$Moisture (\%) = \frac{Initial \ weight \ of \ sample \ (g) - Weight \ of \ dried \ sample \ (g)}{Initial \ weight \ of \ sample \ (g)} x \ 100$$
(1)

2.3.2 Crude Protein

The crude protein content of the fish samples was determined using Kjeldahl method [15]. 2g of fish samples were placed into a standard 250 ml digestion tube and sample digestion was done at 420°C for 60 minutes in a sealed digestion system (VELP Scientifica, Italy). Distillation process in a Kjeldahl Distillation Units UDK 159 (VELP Scientifica, Italy) was followed after cooling the digested samples. Then, samples were titrated with hydrochloric acid (HCl) for estimation of nitrogen content. The percentage of nitrogen was converted to crude protein by multiplying with the conversion factor 6.25 (Eq. 2)

$$Protein(\%) = \% Nitrogen x \ 6.25$$
(2)

2.3.3 Crude Fat

Determination of the total fat content was based on Bligh and Dyer's method [16]. In this method, 4g of each fish sample was initially homogenized with 10 ml chloroform and 20 ml methanol solutions before another 10 ml chloroform solution and 8 ml of sodium chloride (NaCl) were added to the mixture. Then, the homogenate was vacuum filtered through Whatman filter paper. The filtrate was transferred into a 50 ml graduated cylinder and allowed to separate. After complete separation, the volume of the chloroform layer was recorded and the alcoholic layer was removed with a Pasteur pipette. Next, 5 ml of chloroform layer was transferred onto a pre-dried and weighed aluminum dish. The chloroform layer was allowed to be evaporated on a hot plate until a thin layer of lipid coating formed. The trace amount of chloroform solution was removed through oven dehydration at 105°C for 15 minutes. Finally, the aluminum dish was cooled and weighed. The fat content was calculated as:

$$Fat (\%) = \frac{initial \ weight \ of \ aluminium \ dish \ (g)}{final \ weight \ of \ aluminium \ dish \ (g)} x \ 100$$
(3)

2.3.4 Total Ash

Total ash represents the amount of total minerals present in fish samples was determined using dry ashing method. Approximately 5g of each fish sample was placed in a pre-weighed porcelain crucible. The fish samples were incinerated in a muffle furnace at 550°C for 6 hours until greyish white residue was obtained. The remaining inorganic residues were cooled and weighed. The ash content in each sample was calculated based on the following formula for calculation of the ash content:

$$Ash(\%) = \frac{weight of ash(g)}{weight of sample(g)} \times 100$$

(4)

2.3.5 Total Carbohydrate

The total carbohydrate was estimated by difference method as shown in the following equation [15]:

$$Total \ carbohydrate \ (\%) = 100 - (moisture + protein + fat + ash)$$
(5)

2.4 Statistical Analysis

Samples were tested in triplicate and one way ANOVA using SPSS software were performed to statistically analyse the results. All results were expressed as means with the standard deviation.

3. Results and Discussion

3.1 Physical Characteristics

Table 4.1 details the scientific, local and common names as well as morphometric data of the collected freshwater fish species from Kg. Peta, Johor. The scientific names for each species reveal that the selected fishes are from the same order, *Cypriniformes (Cyprinidae* family). According to FAO (2020), Cyprinid fishes represent the largest orders (29%) of freshwater fish followed by *Siluriformes* (24%) and *Characiformes* (14.5%). In general, the four fishes can be categorized as small fishes in which they weigh less than 400 g and have body length between 16 to 23 cm. This range of length is comparable to the length (17.5 to 20.2 cm) measured for *O. mossambicus (Tilapia)* inhabiting the River Indus of Mianwali district [9]. Among the selected freshwater fish in this present study, *Labiobarbus festivus* (*L. festivus*) or locally known as *Kawan* represent the smallest fish weighing at not more than 100 g and body length of only 16.57 cm. Nonetheless, previous morphometric characterisation of this fish species collected from the Sumatra Utara Province, Indonesia revealed much smaller size. Desrita *et al.*, (2021) reported weight ranges from 4-73 gram and standard length between 0.63-1.55 cm [17]. Meanwhile, *Puntoplites bulu (P. bulu)* or *Tengalan* is the biggest fish species identified with its weight approximately tripled the signal barb. *Barbonymus gonionotus (B. gonionotus)* which has been previously reported to have an average length of 40.5 cm [18] and between 10-12 cm [19] exhibited a body length around 20 cm.

Scientific name	Local name	Common name	Weight (g)	Length (cm)
Osteochilus flavicauda	Rong batu sirip	-	200.00 ± 0.37	16.78 ± 0.84
Labiobarbus festivus	Kawan	Signal barb	87.30 ± 0.22	16.57 ± 0.96
Barbonymus gonionotus	Lampam Jawa	Java barb	166.67 ± 0.26	19.77 ± 0.69
Puntoplites bulu	Tengalan	Crossbanded barb	333.30 ± 0.46	23.23 ± 0.39

Table 1- Scientific names, common names, length and weight of four freshwater fish species

3.2 Proximate composition

Moisture, crude fat, crude protein, total ash and carbohydrate content of the investigated freshwater fish samples are presented in Table 2.

Species	Moisture content (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Total carbohydrate (%)
O. flavicauda	$77.02\pm0.05^{\text{b}}$	$13.40\pm0.33^{\text{a}}$	$1.09\pm0.23^{\text{a}}$	$3.23\pm0.13^{\rm a}$	$5.26\pm0.70^{\rm a}$
L. festiva	$79.40\pm0.25^{\text{b}}$	13.57 ± 0.412^{a}	$1.03\pm0.07^{a,b}$	$3.09\pm0.09^{\text{a}}$	$2.91\pm0.46^{\text{b}}$
B. gonionotus	82.65 ± 1.09^{a}	13.75 ± 0.94^{a}	$0.48 {\pm}~ 0.08^{ ext{b}}$	$2.76\pm0.13^{\rm a}$	$0.46\pm0.06^{\circ}$
P. bulu	$78.92\pm0.17^{\text{b}}$	13.61 ± 0.33^{a}	$0.64\pm0.25^{a,b}$	$3.27\pm0.45^{\rm a}$	$3.57\pm0.05^{\text{b}}$

Table 2 - Nutritional composition of the selected freshwater fishes from Kampung Peta, Johor

3.2.1 Moisture Content

On average, moisture constituted about four-fifth of the proximate composition of the selected fish species, which varied between 77-82%. This range of moisture content corroborates with the moisture identified in fish species from the Serrasalmidae family and a mackerel-liked fish species collected from the freshwater of the South American region [10, 20]. However, at 82.65 % \pm 1.093, *B. gonionotus* exhibited significantly (p < 0.05) higher moisture content than the other species. This value is 6% higher than the amount of moisture analyzed by [21]. Previously, variation of moisture content between fish species was reported to be affected by the osmoregulation process that helps fish to maintain internal balance of salt and water [21]. Besides being essential for survival of fish, the presence of moisture in aquatic animals is also an important indication on the microbial stability, sensorial quality and physical characteristics.

3.2.2 Crude Protein

Unlike moisture content, the freshwater fish species does not show significant differences (P > 0.05) for the amount of crude protein. In Table 2, it can be seen that the difference between the highest (13.75 ± 0.935 %) and lowest (13.40 ± 0.327 %) level of protein recorded is only 0.35%. The range of protein content recorded indicates that the investigated fishes belong to the moderately high protein fish species [8, 2]. The recorded crude protein is slightly lower with (approximately 4-7% differences than the protein value reported for the freshwater fish collected from the Indus River [22]. The feeding habits of each fish species could have influenced the protein level observed in the present study. For example, *L. festiva* is known as a detritivores species that primarily feeds on decaying or dead animal and plant origin [23]. In addition, omnivorous fish such as *O. flavicauda*, *B. gonionotus* and *P. bulu* depend on submerged aquatic plants, algae, insects on the plants, crustaceans and small fishes. Previous studies have also highlighted that variation in morphology and physiology may indirectly influence nutritional composition [11, 24].

3.2.3 Crude Fat

The percentage of crude fat listed in Table 2 clearly indicates that the collected freshwater fishes are lean fish with fat content less than 2% [25]. *O. flavicauda* and *B. gonionotus* each with the highest $(1.09 \pm 0.230 \%)$ and lowest amount $(0.48\pm 0.080 \%)$ of fat exhibited significant differences (P < 0.05) where the fat in Java or silver barb is half

of the fat in the common barb. The low-fat concentration recorded corroborates the previous study on comparison of fatty acid composition between several freshwater and marine fishes [12]. It was reported that freshwater fish generally exhibited lower fat content than marine fish due to lack of storage mechanism and fat utilization during spawning [1]. Also, it can be noted that the fish species with the highest level of fat exhibited the lowest protein level showing the inverse correlation between fat and protein content in these freshwater fishes.

3.2.4 Total Ash

Total ash which represents the amount of mineral in the freshwater fishes showed insignificant difference between the investigated species. On average, the ash value for all species is around 3% which is higher than the overall fat content (Table 2). *B. gonionotus* recorded the least amount of ash at only $2.76\pm0.13\%$. This value is smaller by approximately 24% than the ash value reported for *B. gonionotus* collected from Jatigede reservoir in West Java, Indonesia [21]. Moreover, the total ash identified in this study was much lower by between 40-60% than the ash value reported for Cyprinid fishes of Manipur, India [8]. In the previous study, the high ast content for some Cyprinid fish species has been associated with their bony consistency in the edible parts. The higher ash content in some species might be due to their bony consistency in edible parts [8]. Freshwater fish has been highlighted as one of the dietary sources to meet the mineral requirement of humans especially on potassium, phosphorus and trace mineral iron.

3.2.5 Carbohydrate

The total carbohydrate shown in Table 2 exhibited quite a wide range of value compared to other nutrient composition. Significant differences (P < 0.05) were observed between *O. flavicauda, L. festiva* and *B. gonionotus* while *L. festiva* and *P. bulu* showed comparable value (P > 0.05). It is also noticeable that the trend of carbohydrate value is inversely related to the protein content. In the present study, fish with the highest protein content recorded the lowest carbohydrate and vice versa. This trend could be explained by the eating habits of the fish species. *O. flavicauda* with the most carbohydrate content ($5.26 \pm 0.70\%$) is herbivorous which relies on submerged vegetation to survive [26]. This fish species usually feeds on submerged weeds that are rich in cellulose and fibrous carbohydrates that could have contributed to the high level carbohydrate in this fish. Interestingly, an almost similar level of carbohydrate has been observed in gammarid ($5.8\pm0.9\%$) and caprellid $5.7\pm1.2\%$) of amphipod species [27]. On the other hand, *B. gonionotu* exhibited lower carbohydrate content (by 77%) than the similar fish species found in Jatigede Reservoir, Indonesia [21] which could be due to environmental factors. It is expected for fish to exhibit low carbohydrate content as this macronutrient does not represent a significant nutritional reserve for aquatic animals. Fishes utilize protein and lipids more efficiently than carbohydrates for energy production which leads to low levels of carbohydrate in the fish muscle. This is one of the reasons that lead to the growing demand for fish and fish products especially among society that chose a high protein diet or keto-diet lifestyle.

4. Conclusion

This study reveals that the collected freshwater fishes from Kampung Peta, Johor are from the Cyprinidae family. The different fish species identified are rather small and exhibited variation in certain proximate composition. Overall, the investigated fish species are considered as lean fishes with low fat content and moderately high protein levels. This finding shows that the freshwater fishes are a good source of essential nutrients for the local population. In addition, these fish species have the potential to be explored as alternatives to marine fishes and mitigating the food security issue in rural areas as well as developing countries. The analyzed proximate composition will be helpful in providing a baseline data on the nutritional quality of freshwater fish from Kampung Peta, Johor.

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