UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF FATTY ACID PROFILE IN *SCORTUM BARCOO* (JADE PERCH)

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DETERMINATION OF FATTY ACID PROFILE IN

SCORTUM BARCOO (JADE PERCH)

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Determination of fatty acid profile in *Scortum barcoo* (jade perch)", by Amirul Nazhan Bin Ilias and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Project.

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DEDICATION

I dedicate this thesis to:

Ilias Yusoff

He gave me the greatest gift anyone could give: he believed in me.

Roseliza Abdullah

The only love that I really believe in is a mother's love for her children.

Sakinah Sultan Mohamed

Grandmothers always have time to talk and make you feel special.

Abdullah Yusof

See you soon and I love you, always…

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CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATION

%	percentage
FAME	Fatty Acid Methyl Ester
GLC	Gas-Liquid Chromatography
µl	microlitre
µm	micrometre
ml	mililitre
^{o}C	degree celcius
S. barcoo	*Scortum barcoo*
v/v	volume/volume
UFA	unsaturated fatty acid
SFA	saturated fatty acid
TSFA	total saturated fatty acid
TUFA	total unsaturated fatty acid
PUFA	polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
n-3	fatty acid with 3 carbon chain
n-6	fatty acid with 6 carbon chain

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek.

Penentuan profil asid lemak dalam *Scortum barcoo* (jade perch)

Oleh

Amirul Nazhan Ilias

2018

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Ikan marin adalah sumber terbaik bagi asid lemak tak tepu omega-3 (n-3 PUFA) terutamanya asid dokosaheksaenoik (DHA) dan asid eikosapentaenoik (EPA). Asid lemak omega-3 ini juga terdapat dalam spesies ikan air tawar, dalam komposisi yang mungkin berbeza. Dalam ikan air tawar, asid lemak omega-3 perlu diperolehi melalui sumber makanan kerana ikan air tawar tidak dapat menghasilkannya. Oleh itu, objektif kajian ini adalah untuk menentukan hubungan antara sumber makanan dan profil asid lemak dalam ikan air tawar, *Scortum barcoo* (jade perch). Profil asid lemak daripada enam ekor ikan dewasa *Scortum barcoo* (n=6) dan sampel makanan ditentukan dengan merangkumi tiga bahagian iaitu; 1) penyarian lipid keseluruhan, 2) penyediaan ester metil asid lemak (FAME) dan 3) kromatografi gas-cecair.

Keputusan menunjukkan bahawa hanya asid lemak mono tak tepu seperti asid oleik (C18:1n-9) dapat dikesan dalam makanan (0.38%) melalui kaedah kromatografi gas. Walaubagaimanapun, dalam daging ikan, jumlah asid lemak poli tak tepu (PUFA) adalah lebih rendah (4.24%) daripada jumlah asid lemak mono tak tepu (MUFA) (8.53%). Selain itu, jumlah keseluruhan omega-3 PUFA adalah 1.70% manakala jumlah omega-6 PUFA adalah 2.54%. Di samping itu, nisbah omega-3:omega-6 adalah 1:2 dalam daging ikan. Menariknya, DHA dan EPA didapati dalam daging ikan walaupun komponen asid lemak ini tidak dalam sumber makanan, masing-masing pada kadar 1.45% dan 0.25%. Kesimpulannya, kajian ini menunjukkan bahawa ikan air tawar *Scortum barcoo* boleh menukar karbon rantai pendek seperti asid oleik (C18:1n-9) yang terdapat dalam makanan, kepada karbon rantai panjang iaitu DHA (C22:6n-3) yang terdapat dalam daging ikan melalui proses pemanjangan enzim dan desaturasi rantaian hidrokarbon asid lemak. Ini dapat menunjukkan bahawa ikan air tawar *Scortum barcoo* berkemungkinan bergantung pada makanan yang diberikan yang menyumbang kepada profil asid lemak.

Kata kunci: *Scortum barcoo*, profil asid lemak, omega-3, asid dokosaheksaenoik

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

Determination of fatty acid profile in *Scortum barcoo* (jade perch)

By

Amirul Nazhan Ilias

2018

Supervisor: Dr. Hafandi Ahmad

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Marine fishes are better sources of omega-3 polyunsaturated fatty acids (n-3 PUFAs) especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These omega-3 fatty acids can also be found in freshwater fish species, but the composition may be varied. In freshwater fish, omega-3 fatty acid need to be supplemented in the diet as the fish cannot be produced it. Thus, the aim of this study was to determine the relationship between feed and fatty acid profile in freshwater *Scortum barcoo* (jade perch). The fatty acid profile of six adult *Scortum barcoo* (n=6) and feed sample were determined by involving three parts; 1) total lipid extraction, 2) fatty acid methyl esters (FAME) preparation and 3) gas-liquid chromatography. Results showed that only monounsaturated fatty acid such as oleic acid (C18:1n-9) was detected in the feed (0.38%) by the gas chromatography. However, in the

fish meat, the total of PUFA was lower (4.24%) than the total SFA (6.96%). Apart from that, the total omega-3 PUFA was 1.70% and the total of omega-6 PUFA was 2.54%. In addition, the ratio of omega-3 and omega-6 was 1:2 in the fish meat. Surprisingly, the DHA and EPA were found in the fish meat but not in the feed at 1.45% and 0.25%, respectively. In conclusion, this study revealed that the freshwater *Scortum barcoo* is capable to convert from short chain carbon such as oleic acid (C18:1n-9) found in the feed to long-chain carbon DHA (C22:6n-3) found in the fish through the enzymatic process of elongation and desaturation of the fatty acid hydrocarbon chain. This could indicate that the fresh water *Scortum barcoo* may depends on the feed given which contributes to the fatty acid profile.

Keyword: *Scortum barcoo*, fatty acids profiles, omega-3, docosahexaenoic acid

1.0 INTRODUCTION

1.1 Study background

Omega-3 polyunsaturated fatty acid (PUFA) is essential to human and animal. Eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) are examples of omega-3 PUFA. According to Swanson *et al.*, (2012) these fatty acids (EPA and DHA) are long-chain PUFA that have countless of benefits to human health. In the same study, they also stated that EPA and DHA promote proper neuronal, retinal and immune function in fetal development.

In animals study, dietary omega-3 supplementation improved cognitive function and brain gene expression (Hafandi *et al.,* 2014; Sopian *et al.,* 2015). In addition, these fatty acids help in enhancing cardiovascular functions and involving in anti-inflammatory actions in humans (Bruno, 2009). Thus, this could indicate that dietary omega-3 fatty acid is very important especially for human and animal health.

Omega-3 PUFA is an essential fatty acid (EFA) to mammals, which mean the only way to gain omega-3 is through diet. The major source of EPA and DHA can be found in marine fish and fish oils (Omega-3 Health Claims, 2009). According to Axe (2017), human can earn fish oil by eating the fish itself or a readily made fish oil supplement. Besides that, omega-3 PUFA can also be found in plant-based source such as flax seed oil (Bruno, 2009).

Numerous studies had been conducted to evaluate the essential fatty acid composition in both marine and freshwater fish species (Muhamad and Mohamad, 2012). It has been established that previous studies conducted to evaluate the essential fatty acid composition of aquatic animal started in the late 1960s (Nakagawa *et al.,* 2007).

In general, omega-3 PUFAs can be found in both marine and freshwater fish. However, the amount of these fatty acids might differ among these fish (Rahman *et al.,* 1995). According to Muhammad *et al.,* (2012), marine fishes has a higher amount of omega-3 PUFAs, EPA, and DHA. This was confirmed by Ugoala *et al.,* (2014) reported that in contrast to marine fishes, freshwater fish species are actually good sources of omega-6 PUFAs instead of omega-3 PUFAs. Over the past two decades, more studies were conducted with interest to evaluate omega-3 fatty acids level in the freshwater fish species.

Recently, fatty acid profile and metabolism in *Scortum barcoo*, a freshwater fish species is of interest due to increase in market demand. The reason behind this is that the fish was reportedly to be high in omega-3 fatty acid (Romano *et al.,* 2017). Apart from that, as mentioned by Kamaruddin (2015), *S. barcoo* is potentially marketable as it is meaty and can grow very fast. In addition, previous study also reported that this fish is a high-priced species that has good taste and fewer bones (Li *et al.,* 2009).

There are several factors that contribute to the fatty acid profile in the *S. barcoo.* Apart from the sources of feed and the environment of fresh water which consist of high polyunsaturated fatty acid (Geoff, 2008), freshwater fish species could have the ability to elongate the carbon atoms and double bonds to the fatty acid chain, which results in longer hydrocarbon chain. Thus, this study was conducted to determine the relationship of fatty acid profile in the feed to the fatty acid profile found in the freshwater jade perch.

1.2 Objective

To determine the relationship between feed and the composition of fatty acid profile in the *Scortum barcoo* (jade perch).

1.3 Hypothesis

Null Hypothesis: The feed given contributes to the composition of fatty acid profile in the *Scortum barcoo* (jade perch) meat.

Alternative Hypothesis: The feed given does not contribute to the composition of fatty acid profile in the *Scortum barcoo* (jade perch) meat.

1.4 Justification

Fatty acid profile and metabolism of *Scortum barcoo* (jade perch) is of interest due to increase in consumption and market demand for the fish. In Malaysia, there are limited findings reported on the fatty acid profile of this

species in terms of the fatty acid profile and the metabolism of the fish. Thus, this study was conducted to determine the fatty acid profile of a *Scortum barcoo* (jade perch).

2.0 LITERATURE REVIEW

2.1 *Scortum barcoo* (jade perch)

Jade Perch is a family of Terapontidae, genus *Scortum,* and species is known as *Scortum barcoo* (McCulloch and Waite, 1917). The fish is also known as Barcoo grunter, Jade Perch or in Malay, they called it puyu kukum. The species can be found abundantly in tropical freshwater (Jarau and Don, 2011). According to Romano *et al.,* (2017) jade perch is a freshwater omnivorous fish species endemic to Australia and in countries such as China, Malaysia, and Singapore, the fish is cultured in the recirculating aquaculture system and intensive ponds.

The species is known highly resistant and can grow rapidly when reared in aquaculture ponds (Jarau and Don, 2011). Romano *et al.,* (2017) mentioned that the fish can be identified with a stocky body, small-sized head and the body is brownish black coloured with few black spots, while the fins are darker compared to the body. In addition, the species is named jade perch due to the jade coloured displayed on the scales at the dorsal area as it reflects light (Sambell, 2017).

Al-Khafaji *et al.,* (2017) stated that the fish has gained much attention due to their rapid growth and hardy. Apart from that, according to a study conducted by Australia's Commonwealth Scientific and Industrial Research Organization (1998) jade perch possess a very high amount of omega-3

PUFA oil when compared to other fish species tested, made the fish become a popular choice among consumer. In agreement to that, Khoo (2016) stated that the fish gained much attention due to its high amount of omega-3 (PUFA) oil and people started to consume it due to less cost compared to marine fish such as salmon.

According to Khoo (2016), the production cost of rearing Jade perch is depending on the stage of production. In Malaysia, the current production cost of the species range from RM12 to RM14 per kg of body weight and the market price is at RM10 to RM11 per kg of body weight.

2.2 Fatty acid

Fatty acid is defined as an important component of lipids (fat-soluble components of living cells) that can be found in plants, animals, and microorganism. According to Ching and Lobb (2008), the elements of fatty acids are composed of carbon, hydrogen and oxygen atoms that are arranged in a linear carbon chain skeleton of variable length with the presence of carboxyl group (COOH) at one end. The general formula of fatty acids is CH_3-(CH_3) n-OOH (Athithan *et al.,* 2012).

The classification of fatty acids is depending on the number of carbon atoms and the number of double bonds presented or absent in the chain. As stated by Kelly and Ching (2008) saturated fatty acid (SFA) has no double bond, monounsaturated fatty acid (MUFA) has only one double bond and

polyunsaturated fatty acid (PUFA) may have either two or multiple of double bonds.

2.2.1 Saturated fatty acid

In general, fatty acids in nature are divided according to the presence of double bond termed as saturated fatty acid (SFA) and unsaturated fatty acid (UFA) (Ching and Lobb, 2008). As mentioned previously, SFAs has no double bonds and the general formula of this fatty acid class is R-COOH in which R- group stands for a straight-chain hydrocarbon of varying length with a range from short chain to longer chain composed of 30 or more carbon atoms (World Health Organization, 2008).

SFA is denoted as CN: M in which C; is designated for a carbon atom, N; is the number of carbon atoms presented in the hydrocarbon chain and M; the number of double bonds presented in the hydrocarbon chain, but in SFA, double bonds are not presented (Ching, 2008). Other characteristics of SFAs are higher melting points, solids at room temperature and the chemical chain are saturated with hydrogen atoms (Rustan and Devon, 2005).

According to Ching and Lobb (2008), SFAs can be found in animals milk fats (butanoic acid; C4:0) and in few seed fats (lauric acid; C12:0 and myristic acid; C14:0).

2.2.2 Unsaturated fatty acid

Unsaturated fatty acid is further divided into monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The difference between these UFAs is the number of double bonds presented in the carbon chain. According to Matthews (2018) in MUFA, only one double bond is presented whereas PUFA can have either two double bonds or more than that.

The most common MUFA found in animals is oleic acid (C18:1). Other than that, palmitoleic acid (16:1n-7) is also an example of MUFA, which can be found abundantly in animals, plants, microorganism and in some seed oils (Rustan and Drevon, 2005).

The main concern of PUFA is omega-3 (n-3) and omega-6 (n-6). Both omega-3 and omega-6 PUFA are physiologically and metabolically distinct (Danijela *et al.,* 2013). According to Ching (2008), the location of the first double bond for omega-3 and omega-6 appeared between the 3^{rd}=4^{th} and 6^{th}=7^{th} carbon atom from the methyl end of the fatty acids respectively. The Figure 1 shows the fatty acid metabolism in mammals.

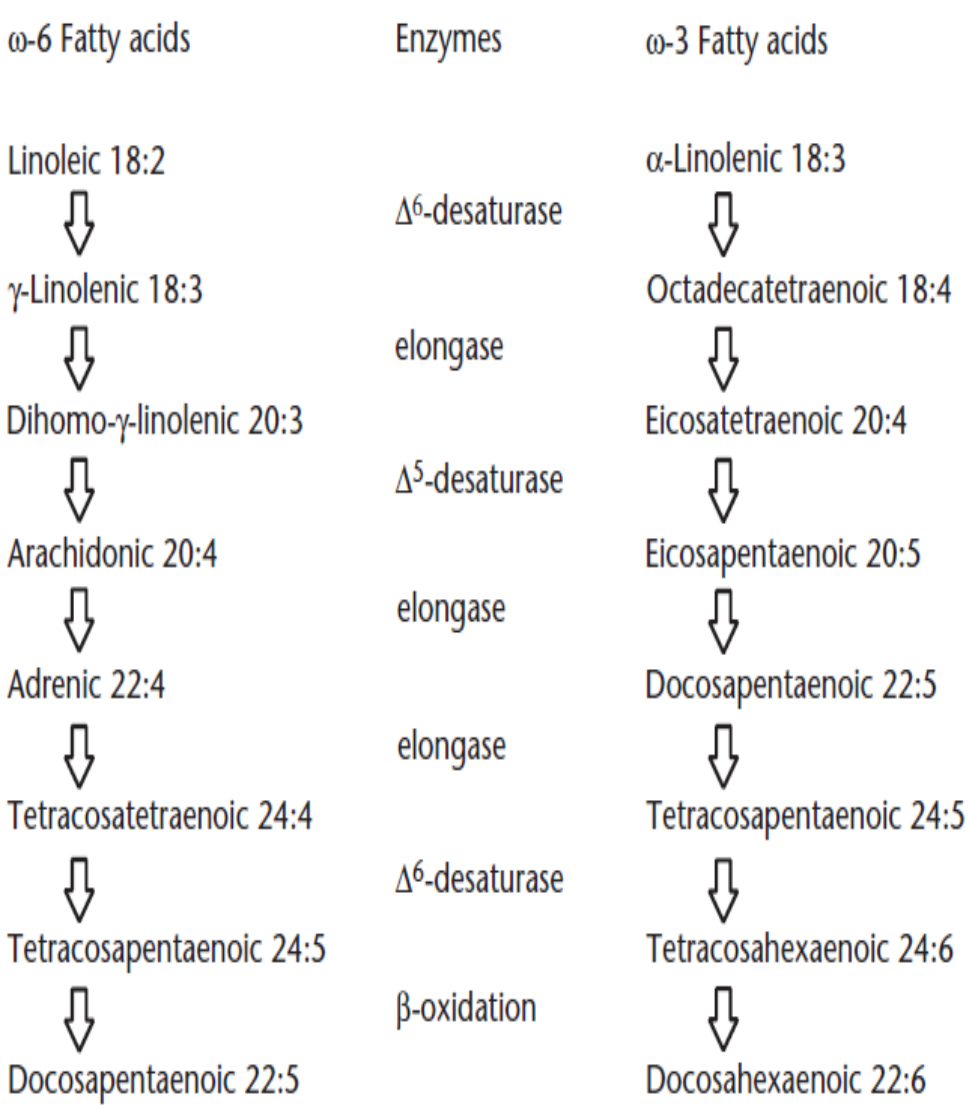

Figure 1: Synthesis of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs).

2.3 Sources and benefits of omega-3 and omega-6 PUFAs

Most fish oil supplements are derived from cold water fish species such as salmon, sardines, herring and mackerel (Bruno, 2009). In addition to that, according to American Heart Association (2015) omega-3 fatty acids can also be obtained from plants such as flax seed oil, walnuts, canola oil, and soybean oil. Omega-3 fatty acid promote many health benefits to human.

Dietary omega-3 fatty acid supplementation increased brain gene expression associated with brain cognitive function in mice (Sopian *et al.,* 2015; Hafandi *et al.,* 2014). Bruno (2009) mentioned that the other benefits of omega-3 fatty acid are reducing risk of heart disease, modifying cholesterol levels in the body and prevent the initiation of inflammatory process. Apart from that, this fatty acid also helps to prevent Alzheimer's disease (American Heart Association, 2015).

The sources of omega-6 fatty acids are numerous and can be found in refined vegetables oils, fast food, snacks and cookies (Weil, 2007). According to Stokel (2011), omega-6 fatty acid helps to fight chronic inflammation, eczema, dermatitis and many more. In addition, the application of this fatty acid includes lowers the low density lipid (LDL) and raising high density lipid (HDL) which has benefits in reducing the risk of heart disease (Serb *et al.,* 2013).

2.4 Fatty acid profile in fishes

Fish lipids contents are rich in long chain omega-3 PUFA (α-linolenic acid; C18:3, eicosapentaenoic acid; C20:5, and docosahexaenoic acid (C22:6) (Luczynska *et al.,* 2014). Rahman *et al.,* (1995) stated that the composition of these omega-3 PUFAs varies in both marine and freshwater fish species.

In marine fish, the most abundant PUFAs are eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Ackman, 2008). However, as mentioned by Kinsella *et al.,* (1997) there are some similarities of these PUFAs between freshwater fish and marine fish. Based on a study conducted by Luczynska *et al.,* (2014) freshwater fish, is actually a good source of omega-3 PUFA especially DHA and EPA with the exception of pangasius and tilapia, are good sources of n-3 fatty acids, especially EPA (C20: 5n-3) and DHA (C22:6n-3).

In contrast, Ugoala *et al.,* (2014) revealed that freshwater fish are actually good sources of omega-6 PUFAs rather than omega-3 PUFAs. In the same study, they concluded that because of the high content of linoleic acid (C18:2n-6) and arachidonic acid (C18:2n-), freshwater fish possess a good oil quality.

3.0 MATERIALS AND METHODS

3.1 Experimental design

The study was conducted on adult freshwater *S. barcoo* collected from Shahab Aquaculture Sdn. Bhd. located in Alor Star, Kedah. The type of feed and feeding regime were similar for all fish. All fish was separately wrapped using aluminium foil and labelled accordingly. These fish samples were placed into an icebox and immediately transported to the Physiology Laboratory in Faculty of Veterinary Medicine, UPM. Fish samples were then kept in a freezer at a temperature of -60^{o}C.

For feed sample, approximately 50 grams of pelleted feed was collected, kept inside a zip-lock bag and transported to the Physiology Laboratory in Faculty of Veterinary Medicine, UPM. The feed sample was kept in the zip-lock bag at normal room temperature.

3.2 Fish meat analysis

3.2.1 Sample preparation

In this study, six (n=6) randomly selected adult freshwater *S. barcoo* were used. For fatty acid profile of fish, 100 g of fish meat was filleted by carefully cutting the fish lengthwise along the backbone to gain the maximum amount of meat. The fillet was then wrapped in an aluminium foil and kept in the freezer at -60^{o}C prior to fatty acid profile analysis. For proximate analysis of fish, 50 g of fish meat was obtained, grind and dried in the oven at 60^{o}C for two days.

3.2.2 Proximate analysis of *Scortum Barcoo* meat

Proximate analysis was conducted on the fish meat in order to evaluate the percentages of crude protein and crude fat contents. All analysis was carried out according to certification procedures outlined by the Manual of Nutrition Laboratory Technique, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

3.2.3 Crude protein of *Scortum barcoo* meat

Crude protein of fish meat sample was determined by using the Kjeldahl method which comprises of three steps; digestion, distillation, and titration. In digestion step, 1 g of dried fish meat sample was weighed and placed into Kjeldahl flask together with Kjeldahl catalyzer tablet (3.5g K2SO4 + 0.4G CUSO4 X 5H20). Next, 20 ml of sulphuric acid (98%) was poured into a 250 ml Kjeldahl flask and was shaken gently. The flask containing sample was then fixed into Kjeldahl digestion set (Gerhart Malaysia). The temperature of the heating block was gradually increased to maximum and the digestion was continued until the solution colour become clear green.

The chemical reaction occurring during digestion process can be summarized as follow:

$$\text{Sample} = H_2SO_4 \longrightarrow (NH_4)_2SO_4 + H_20 + CO_2$$

Whilst the samples were being digested, acid standardization was done to determine the acid normality of a previously prepared 0.1 M hydrochloric acid.

After the digestion process completed, all flasks were allowed to cool down prior to distillation process by using a Kjeldahl distillation set (Vapodest 20® Gerhardt Malaysia). A solution made of 75 ml of 2% Boric acid and 8 drops of indicator (Methyl red & Bromhexdiol green) was earlier prepared at the distillation platform. 15 ml of distilled water was poured slowly into digester and then transferred into distillation platform. Another 15 ml of distilled water was used to rinse the Kjeldahl flask to clear any remaining of digester. The distillate was then fixed to the distillation tube. During this process, 50 ml of distilled water and 32% of NaOH were added.

The process took 3 minutes to complete where the entrapped sulphate salt of ammonium was released thus producing ammonium, which is collected by the Boric acid (2%) at the distillation set via following reaction:

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow NH_2SO + 2NH_3 + H_20 + 2NH_3 + H_20 \longrightarrow NH_4OH$$

The mixture in the Erlenmeyer flask was then titrated with 0.1 M hydrochloric acid to determine the nitrogen content by using the formula below:

Nitrogen (%) = (Vol. of titrant - Blank Value) x Acid Normality x 14.0067 x 100/ Weight of Sample

Crude protein (%) = % Nitrogen x Protein Factor

Where; Blank Value = %Nitrogen x Protein Factor

Acid Normality = 0.0955

Protein Factor = 6.25

3.2.4 Crude fat of *Scortum barcoo* meat

Empty round bottom flask (RBF) was labelled and dried in the oven at 105°C for 1 hour. Then, the empty RBF was weighed and 250 ml of petroleum benzene was poured into the flask. 3 g of fish meat sample was weighed and recorded, transferred into the extraction thimble and covered with fat-free cotton wool. The thimble and flask containing petroleum benzene were fixed into the Soxhlet apparatus. Water was let to flow gently so that condensation process can take place. The temperature of the heating plate was increased gradually to boil the mixture for 4 hours. After boiling had completed, the flask was detached and dried in the oven at 80°C overnight.

After drying, the flask was allowed to be cooled inside desiccator before weighing. The crude fat determination was calculated as follow:

Crude Fat (%) = Dried RBF after evaporation weight – Empty RBF weight x 100/ Weight of sample

3.3 Fatty acid profile determination on feed and *Scortum barcoo* meat

3.3.1 Total lipid extraction

Fatty acid extraction was done from the tissue sample obtained from both fish meat and feed sample using chloroform:methanol 2:1 (v/v), based on the method described by Folch *et al.,* (1957) modified by Rajion *et al*., (1985). First, 100 grams of fish meat pulled from all six fish were homogenized with 40 ml of C:M (2:1,v/v) for 1 minute by using Ultra-Turrex mixer.

Then, fatty acids were extracted with 40ml of C:M (2/1, v/v) for overnight. On the next day, the extracts were filtered into a 250 ml separating funnels using a filter paper (No. 1 Whatman paper, Whatman International Ltd. Maidstone, England).

Leftover residue on the filter paper was rinsed with 5 ml of C:M (2/1, v/v). Then, 10 ml of normal saline solution (0.9% NaCl) was poured into the separating funnel and the mixture was shaken vigorously.

The mixture was left for 4 hours.Approximately after 4 hours, the lowest phase in the separating funnel was collected into an RBF and evaporated by rotary evaporation (Labota 4000-efficient, Heidolph,

Germany) at 70°C. Then, 5 ml of C:M (2:1, v/v) was added to the dried residue and transferred into a capped methylation tube.

3.3.2 Fatty acid methyl esters (FAME) preparation

Methylation of the previously extracted fatty acids from flesh and feed sample was performed by adding potassium hydroxide (KOH) and 14% methanolic boron trifluoride (BF_3) according to AOAC (1990) methods. 100 µl of the internal standard, heneicosanoic acid (21:0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation to determine the individual fatty acid concentrations in the sample. The mixture was then heated in a water bath at 70°C for 5 minutes. Later, the mixture was dried on a heating block at 40°C under a constant and mild flow of pure nitrogen gas. Next, saponification process to saponify the lipid was done by adding 2 ml of 0.66N of methanolic potassium hydroxide (KOH).

Following saponification, the mixture was heated in a boiling water bath for 10 minutes, and shaken every 5 minutes. Next, the mixture was left to cool down and 2 ml of 20% methanolic boron trifluoride (BF_3) was added to initiate trans-esterification. The mixture was heated for another 20 minutes in a boiling water bath. Next, an equal amount of 4 ml each from petroleum ether and distilled water were added. The mixture was vortexed at 200 rpm for 30 seconds and centrifuged at 300 rpm for 5 minutes to facilitate separation.

Finally, the upper petroleum phase was transferred to a 4 ml screw-capped vial (Kimble Glass Inc., USA), flushed with nitrogen, the cap was closed tightly, and store at 4°C prior to gas-liquid chromatography.

3.3.3 Gas-liquid chromatography (GLC)

The methyl esters were quantified by GC (Agilent 7890N) using a 30m x 0.25mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). 1µ was injected by an auto-sampler into the chromatograph. High purity nitrogen was used as the carrier gas at 40 ml/min. High purity hydrogen (Dominick Hunter, Parker Hannifin Ltd., UK) and compressed air were used for the flame ionization detector in the GLC. The injector temperature was programmed at 250°C, while the detector was at 300°C. Initially, the column temperature was programmed at 100°C for 2 minutes, warmed up to 170°C at 10°C/min, held for 2 minutes, then warmed again to 220°C at 7.5°C/min, and finally held for 20 minutes in order to facilitate optimal separation.

Fatty acids identification was carried out by comparing relative FAME peak retention times of samples to standards obtained from Sigma (St. Louis, MO, USA). Gravimetric calculations and normalised percentage (%) of total fatty acids were used to determine the composition differences of fatty acids. Peak area was determined and calibrated using a personal computer integrator (Hewlett-Packard, Avondale, PA). Automatic expression of the peak areas as absolute and percentage amount of a detected fatty acid was

obtained with a programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

The amount of fatty acid is determined by their relative proportions (normalised percentages to total fatty acids) (Huerta-Leidenz et al., 1991; Alfaia et al., 2006). The normalised percentages describe the interactive and comparable relationship among fatty acids regarding lipid quality, while the gravimetric concentration can show the actual amount of fatty acids in tissues, which relates to nutritional intake.

3.4 Data analysis

The fatty acids values for both fish meat and feed were expressed in percentages of total fatty acid. The mean percentages of total fatty acid of *Scorum barcoo* meat and feed were calculated using Microsoft Excel 2013. The dominant fatty acid in both samples were identified and compared to determine the relationship between feed and fish meat.

4.0 RESULTS

4.1 Proximate analysis of *Scortum barcoo* meat

From the proximate analysis, both crude protein and crude lipid composition of *Scortum barcoo* meat were obtained. The results were tabulated in Table 4.1 and expressed in percentages.

Table 4.1: Total crude protein and crude lipid in *S. barcoo* meat

Sample	Crude Protein (%)	Crude Fat (%)
Fish meat	25.21	10.89

From table 4.1, the result of proximate analysis revealed that the total crude protein in the fish was higher than the total crude lipid content. The amount of crude protein found was 25.21%, while for crude lipid, 10.89%.

4.2 Fatty Acid Profile of Feed

The percentages of fatty acid profile in feed is presented in Table 4.2. The total fatty acids obtained from the feed comprised of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA).

Table 4.2: Total fatty acid profile of feed

Fatty Acid	Total Fatty Acids (%)
Palmitic (C16:0)	0.35
Oleic acid (C18:1n-9)	0.38
Total Saturated Fatty Acid	0.35
Total Unsaturated Fatty Acid	0.38
Total Monoenes	0.38
Ratio UFA : SFA	1.09

*All values were expressed in percentage

The feed sample fatty acid profile has only two types of fatty acids which were palmitic acid (C16:0) and oleic acid (C18:1n-9). The only component of unsaturated fatty acid found in the feed was oleic acid (C18:1n-9) at 0.38% which was higher compared to palmitic acid (C16:0) at 0.35%. In addition to that, there is no PUFA found in the feed sample.

4.3 Fatty acid profile of *Scortum barcoo* meat

The percentages of total fatty acids composition of *S. barcoo* is showed in Table 4.3. The total fatty acids obtained from the fish meat comprised of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA).

Table 4.3: Total fatty acid profile of *Scortum barcoo* meat

Fatty acids	Total Fatty Acids (%)
Caproic acid (C6:0)	0.05
Lauric acid (C12:0)	0.10
Myristic acid (C14:0)	0.44
Pentadecylic acid (C15:0)	0.02
Palmitic acid (C16:0)	5.09
Palmitoleic acid (C16:1)	0.72
Marganic acid (C17:0)	0.05
Ginkolic acid (C17:1)	0.02
Stearic acid (C18:0)	1.13
Elaidic acid (C18:1n9t)	0.06
Oleic acid (C18:1n9c)	7.57
Linoleic acid (C18:2n-6)	1.98
$_{\gamma}$-Linolenic acid (C18:3n-6)	0.30
Arachidic acid (C20:0)	0.05
Eicosanoic acid (C20:1)	0.14

Eicosadienoic acid (C20:2)	0.06
Eicosatrienoic acid (C20:3n-6)	0.23
Arachidonic acid (C20:4n-6)	0.03
Eicosapentaenoic acid (C20:5n-3)	0.25
Behenic acid (C22:0)	0.03
Docosahexaenoic acid (C22:6n-3)	1.45
Nervonic acid (C24:1)	0.02
Total SFA	6.96
Total UFA	12.77
Total MUFA	8.53
Total PUFA	4.24
Total PUFA n-3	1.70
Total PUFA n-6	2.54
Ratio n-3:n-6	0.70
Ratio UFA : SFA	1.80
Ratio PUFA : SFA	0.60

*All values were expressed in percentage

From Table 4.3, the total amount of UFA is 12.77% and the total amount of SFA is 6.96%. The total UFA is composed of total MUFA and PUFA, both at 8.53% and 4.24%, respectively.

The SFA is dominated by palmitic acid at 5.09% and in the UFA, oleic acid is the most dominant fatty acid found at 7.57%. Whereas, in PUFA families, both omega-3 and omega-6 fatty acids are presented.

Highest omega-3 found in the fish meat is docosahexaenoic acid (DHA; C22:6n-3) at 1.45% followed by eicosapentaenoic acid (EPA; C20:5n-3) at 0.25%. In addition, highest omega-6 found in the fish meat is linoleic acid (LA; C18:2n-6) at 1.98%. The ratio between total UFA and SFA is 1.8, while the ratio of omega-3 and omega-6 PUFA is 0.7.

5.0 DISCUSSION

In this study, the proximate analysis was done to determine the crude protein and crude lipid composition in the fish meat. According to Sutharshiny and Sivashanthini (2011), proximate analysis provides the nutritional value and quality of the fish.

The proximate analysis on *S. barcoo* meat revealed that the total crude protein was higher than total crude lipid. The results are comparable to the percentages of crude protein and crude lipid found in Jade perch muscle (Romano *et al.,* 2017). However, according to Love (1970), the principal component of protein is 16-21% and for fat is 0.2-5% in fish. Thus, the differences between the nutritional composition found from this study and the principal nutritional composition in fish suggested by Love (1970) might be due to the type of feed given to the fish.

In addition, there are several factors that influenced the nutrient composition of the fish such as water temperature, season, time of day, dissolved oxygen (DO), physiological of the fish and other water quality variables (Craig, 2009).

In this study, both SFA and UFA were presented in the fish meat with UFA appeared to higher than SFA. The UFA found in the fish is 12.79%, while SFA is 6.96%. Similarly, UFA is the major fatty acid component found in the feed with amount of 0.38%. The high amount of UFA found in the fish

might be a reflection of the fatty acid content found in the feed. This finding was in line with previous study who reported that the fatty acid in fish meat may reflect the fatty acid found in the fish feed (Ching, 2008).

In the fish meat, the total of MUFA is higher when compared to the total PUFA. In addition, oleic acid is the only dominant fatty acid of MUFA found in the fish meat.

Interestingly, DHA which is the longest chain of PUFA can be found in the fish meat but not in the feed. According to Craig (2009), freshwater fish possess enzyme systems that promote an elongation of fatty acid. It is believed that *S. barcoo* possess the capability to elongate the fatty acid in their body. The enzyme systems help to add a carbon atoms to the fatty acid chain and double bonds by further desaturase the chain, which resulted in a longer fatty acid chain. Thus, through these enzyme systems, EPA and DHA can be found in *S. barcoo* although the feed given does not contain long-chain fatty acids. Previous study also reported that freshwater fish cannot produced these omega-3 PUFAs and stated that freshwater fish needs the linoleic acid (C18:3n-3) in the diet to produce the long-chain PUFA (Craig, 2009). In fact, freshwater fish has lower amount of omega-3 PUFA than marine fish (Mohsen, 1985; Vlieg & Body, 1988).

The fatty acid found in the feed can influenced the fatty acid profile in the *S. barcoo*. In agreement to that, those jade perch found in wild

especially to those habitat in the open lake, is high in omega-3 content due to the natural foods such as algae or plankton that contained abundant sources omega-3 PUFA (EPA and DHA) (Romano *et al.,* 2017). On the other hand, the advantage of freshwater fish is that, feed with long-chain fatty acid is not necessary, instead they have the ability to elongate short-chain fatty acids found in their feed through the enzymatic process mentioned earlier.

6.0 CONCLUSION

In conclusion, omega-3 PUFA can be found in *S. barcoo* meat. Being a freshwater fish species, *S. barcoo* possesses the ability to elongate short-chain fatty acid found in the feed to a longer-chain fatty acid such as EPA and DHA in the body. The capability to further metabolized short-chain fatty acid to long-chain PUFA in this species is due to the possession of enzyme systems that help to add on carbon and double bond in the fatty acid chain found in the feed.

7.0 RECOMMENDATION

For future study recommendation, a larger sample size should be included to obtain more reliable data. Besides that, the physiological system of *S. barcoo* should be discovered further in terms of the fatty acids metabolism and mechanism, which may be related to other factors such as the stage of production, breeding, genetics and environmental effects.

Last but not least, comparison study on types of feed given to the fish should be conducted, which will provide a better understanding of fatty acid metabolism in the fish.

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