



**UNIVERSITI PUTRA MALAYSIA**

**BLOOD FATTY ACIDS ANALYSIS IN CAPTIVE FALSE GHARIAL  
(*Tomistoma schlegelii*)**

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**BLOOD FATTY ACIDS ANALYSIS IN CAPTIVE FALSE GHARIAL**

*(Tomistoma schlegelii)*

**NUR NABILA BINTI SARKAWI**

A project submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

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It is hereby certified that we have read this project paper entitled “Blood Fatty Acids Analysis in Captive False Gharial (*Tomistoma schlegelii*)”, by Nur Nabila binti Sarkawi 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**

“To the poorly known, now you are better known...”

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**LIST OF ABBREVIATION**

%	percentage
°C /min	degrees celcius/minute
µl	microlitre
18G	18 Gauge
ALA	Alpha linolenic acid
ARA	Arachidonic acid
BF <sub>3</sub>	Methanolic borontrifluoride
CITES	Convention of International Trade of Endangered Species
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EDTA	Ethylenediaminettetraacetic
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FID	Flame ionization detector
g	gram
GC	Gas chromatography
ID	Ionization detector

IUCN	International Union for Conservation of Nature
LA	Linoleic acid
LDL	Low-density lipoprotein
mL	millilitre
ml/min	millilitre/minute
mm	millimetre
µm	micrometre
MUFA	Monounsaturated fatty acid
N	moles
n=5	Sample size = 5
n-3	Omega-3
n-6	Omega-6
°C	degrees celcius
PUFA	Polyunsaturated fatty acid
rpm	revolutions per minute
SFA	Saturated fatty acid
<i>T. schlegelii</i>	<i>Tomistoma schlegelii</i>
v/v	volume/volume
α-linolenic acid	Alpha linolenic acid

**ABSTRAK**

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

**ANALISIS ASID LEMAK DALAM DARAH BUAYA JENJULUNG**

*(Tomistoma schlegelii)*

Oleh

**Nur Nabila Binti Sarkawi**

**2016**

**Penyelia : Dr. Tengku Rinalfi Putra Tengku Azizan**

**Penyelia Bersama : Dr. Hafandi Ahmad**

Asid lemak adalah alat fisiologikal yang penting untuk lebih memahami mekanisma badan haiwan. Sebarang data yang diperoleh daripada analisis sedikit sebanyak membantu meningkatkan pengurusan hidupan liar dalam kurungan dan sekaligus meningkatkan kualiti hidup terutamanya bagi spesis yang berada di ambang kepupusan. Kajian terhadap komposisi asid lemak dalam buaya jengjulong adalah terhad berbanding spesis buaya yang lain. Oleh itu, kajian asid lemak telah dilakukan ke atas lima ekor buaya jengjulong yang terdiri daripada dua jantan dan tiga betina. Komposisi asid lemak dikaji lebih lanjut dengan mengambil kira aspek pengurusan makanan. Darah diambil menerusi sisi vena ekor menggunakan jarum bersaiz 18G untuk kegunaan analisis asid lemak dalam plasma dan sampel makanan juga turut

diperolehi. Pengenalpastian dan penentuukuran komposisi asid lemak dilakukan dengan menggunakan gas kromatografi setelah melalui prosedur pengekstrakan lipid dan penyediaan metil ester asid lemak. Keputusan asid lemak dalam plasma menunjukkan kesemua buaya jenuh mengandungi asid lemak taktepu yang tinggi. Khususnya, asid lemak politaktepu adalah yang tertinggi, diikuti oleh asid lemak tepu dan asid lemak monotaktepu, masing-masing dengan kadar peratusan 47.30%, 29.24% dan 23.47%. Berdasarkan analisis kandungan asid lemak dalam makanan, ayam dan ikan dipercayai merupakan sumber omega-6 asid lemak politaktepu. Memandangkan terdapat kekurangan sumber omega-3 asid lemak politaktepu, nilai asid eikosapentaenoik (EPA, C20:5 n-3) dalam plasma adalah rendah. Berbeza daripada itu, asid dokosaheksaenoik (DHA, C22:6 n-3) didapati tinggi berbanding spesies buaya yang lain dan juga dengan nilai asid lemak yang diperolehi daripada makanan (ayam dan ikan). Oleh itu, beberapa faktor penyebab dapat menjelaskan punca peningkatan nilai DHA di dalam plasma. Sebagai kesimpulannya, komposisi asid lemak di dalam buaya jenuh bukan sahaja disebabkan diet, malahan juga bergantung kepada bagaimana mekanisme badan bertindakbalas terhadap punca lain yang terdapat dalam persekitaran.

**Kata Kunci:** komposisi asid lemak, buaya jenuh, asid lemak perlu, diet

**ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999-Project.

**BLOOD FATTY ACIDS ANALYSIS OF CAPTIVE FALSE GHARIAL**

*(Tomistoma schlegelii)*

By

**Nur Nabila binti Sarkawi**

**2016**

**Supervisor: Dr. Tengku Rinalfi Putra Tengku Azizan**

**Co-supervisor: Dr. Hafandi Ahmad**

Fatty acid is an important physiological tool to further understand the animal's body mechanism. Any data obtained from the analysis might help in captive wildlife management and thus improving their quality of life especially for those species that are on the brink of extinction. Research on the fatty acid composition in false gharial is limited compared to other crocodylian species. Therefore, fatty acid study was done on five captive false gharials consisting of two males and three females. The fatty acid composition is then further investigated by taking into account their essential fatty acid composition in the diet. Blood sample was collected from lateral tail vein using 18G spinal needle for plasma fatty acid analysis and feed sample comprised of chicken and fish meat was also analysed. Then, identification and quantification of fatty acid

composition was done by using gas chromatography following the procedure of total lipid extraction and fatty acids methyl esters (FAME) preparation. The plasma fatty acid result showed all of the false gharials have predominant increase in unsaturated fatty acids. Specifically, polyunsaturated fatty acids was the highest, followed by saturated fatty acids and monounsaturated fatty acids with 47.30%, 29.24% and 23.47% respectively. Based on feed fatty acid analysis, chicken and fish was known to be the dietary source of omega-6 polyunsaturated fatty acid. As there was lack of dietary source in omega-3 polyunsaturated fatty acid, eicosapentaenoic acid (EPA, C20:5 n-3) value in plasma was also found to be low. On the contrary, docosahexaenoic acid (DHA, C22:6 n-3) was significantly high compared to other species of crocodylian and in relation to the fatty acid values obtained from feed (chicken and fish). Thus, several possible contributing factors were pointed out regarding the high DHA content in the plasma. In conclusion, the fatty acid composition in false gharial is not solely affected by the diet, but it can also depend on how its body mechanism react to other sources from the environment.

**Keywords:** fatty acid composition, false gharial, essential fatty acid, diet

## 1.0 INTRODUCTION

Crocodile is a reptile in the Order of Crocodylia. It consists of family Alligatoridae, Crocodylidae and Gavialidae. There are about 23 species of crocodiles recognized. The False Gharial or *Tomistoma schlegelii* is one of the species listed as 'vulnerable' by the IUCN Red List due to insufficient data. This species is also known as Tomistoma, Sunda Gharial, or Malayan Gharial and is categorised as freshwater crocodile. The ecology including its nesting, size and age of sexual maturity, diet and population demography remains poorly known. According to the study done by Stuebing *et al.* (2006), the current distribution of *T. schlegelii* in South East Asia is restricted to peat swamps of Indonesia and Malaysia. In Malaysia itself, it was reported to be found in Sarawak, Perak, Pahang and Selangor states.

This species is known to have a broad diet. Pigs, monkeys, and snakes were the most commonly reported animals eaten by *T. schlegelii*. Others include fish, shrimp, birds, otters, turtles and monitor lizards. The stomach content revealed prey items including vertebrates, invertebrates and plant materials. Usually captive *T. schlegelii* are fed with chicken, beef, pigs, rats and fish and the frequency of feeding might vary according to management preferences.

The diet given to the crocodiles is believed to have direct relation to the fatty acid composition in their body. Fatty acid profile is an important physiological tool to further understand the animal's body mechanism. Any data obtained from the study might help in the improvement of the management and thus improving their quality of life especially in those species that are on the brink of extinction. Since the study of

this species is limited, no data of fatty acid profile for this species has been reported to date.

Fatty acids consist of saturated and unsaturated fatty acids. Saturated fatty acids are considered as bad cholesterol, unlike the unsaturated. Unsaturated fatty acids consist of monounsaturated and polyunsaturated fatty acids. The polyunsaturated fatty acids, namely omega-3 and omega-6 PUFA are essential fatty acids as they can only be acquired from the diet and cannot be synthesized by the body. They provide fluidity and stability in terms of cell membrane structure. In addition to the previously mentioned role, omega-3 and omega-6 PUFA also contribute in maintaining the animal's health condition. Omega-3 plays a crucial role in brain structure and function. Along with omega-3, omega-6 fatty acids is essential in brain function and normal growth and development. Information obtained from the fatty acid profile of this species in some ways reflect the major concern in its health status and especially the suitability of the diet.

Realizing the benefits of fatty acids in animals, this study was undertaken with the objective to establish the fatty acid profile in plasma from captive false gharial with regular diet of raw chicken and catfish.

## 2.0 LITERATURE REVIEW

### 2.1 False Gharial (*Tomistoma schlegelii*)

Crocodiles are classified under reptiles and under the Order of Crocodylia. The ancestral crocodylian branched out into three families known as Crocodylidae (true crocodiles and tomistoma), Alligatoridae (alligator and caimans) and Gavialidae (gharials).

*Tomistoma* was originally described by Müller as *Crocodylus (Gavialis) schlegelii* in 1838, but soon after was assigned with his own genus *Tomistoma*. The most appropriate taxonomic affiliation of the species has attracted considerable attention. Molecular studies have suggested that *Tomistoma* is closely related to *Gavialis*. However, on the basis of morphology, *Tomistoma* is in its own subfamily (Tomistominae; long slender snout like Gharials but true crocodylian-like skull bones) with the “true” crocodiles, and this is the currently recognised taxonomic relationship. The snout of this species is similar to the slender snout of the Indian Gharials (*Gavialis gangeticus*) but slightly wider and thus known as the false gharial. *T. schlegelii* also known as Tomistoma, Sunda gharial, and Malayan gharial.

According to The IUCN Red List of Threatened Species, this species is listed as Vulnerable and listed in Appendix I as the most endangered species for CITES. In Wildlife Act 2010, *T. schlegelii* regarded as totally protected wildlife as specified in the second schedule. It is one of the least-known among 23 crocodile's species (Stuebing *et al.*, 2006).

*T. schlegelii* is native to Southeast Asia with an estimation of 2500-3000 specimens remaining (Rodder *et al.*, 2010). It is a large freshwater crocodylian whose extant distribution is currently limited to Malaysia (Peninsular Malaysia and Sarawak), Indonesia (East Sumatra, West Java and Kalimantan) and Brunei (Stuebing *et al.*, 2006). This species prefers the peat and freshwater swamp forests along rivers (Bezuijen *et al.*, 2001).

## **2.2 Diet and Feeding of *Tomistoma Schlegelii***

In the wild, crocodile's hatchling start feeding on small aquatic invertebrates such as crustaceans, insects, snails and later include tadpoles, small frogs and fish in their diet. As they grow, the proportion of fish increases and later birds and mammals are also consumed (Huchzermeyer, 2003). *T. schlegelii* has a broad diet and are not only specific to fish. It has always been incorrectly classified as fish eater like the Gavialis (Vitt and Caldwell, 2009). As being reported by the fishermen and former crocodile hunters, monkeys, pigs and snakes were the most commonly reported items eaten by *T. schlegelii*. Other reported prey were fish, shrimp, birds, otters, turtles and monitor lizards. Some farm-raised *T. schlegelii* in Sumatra, Indonesia are fed with adult chickens, late-age chicken embryos, snake meat and monitor lizard meat (Bezuijen *et al.*, 1997). According to Staniewicz (2011), the stomach contents of false gharial showed a varying prey items, such as vertebrates, invertebrates and plant material.

### 2.3 Association of diet with fatty acid composition

According to Staton (1990), the essential fatty acid requirements of vertebrate species are being influenced by a various kind of diets and environmental factors. Depending on the sources of fat in the food, the fatty acids composition also varies according to the crocodile species as different species may have different nutritional requirements of saturated and unsaturated fatty acids (Huchzermeyer, 2003). Dietary changes that alter the fatty acid composition of tissues in wildlife may affect their patterns of migration and hibernation (Geiser *et al.*, 1994; Harlow and Frank, 2001; Pierce and Williams, 2005). In alligators, the diet appears to be directly related to the fatty acid composition of their fat, thus farmers should be able to change the diet of their animals to produce lipid which exhibit a particular fatty acid spectrum (Peplow *et al.*, 1990)

### 2.4 Lipid and Fatty acid

Lipid are organic compounds that are insoluble in water but soluble in non-polar organic solvent such as chloroform and ether (Contreras *et al.*, 2012). According to Fahy *et al.* (2005), lipids are classified into simple and complex group. The simple lipid comprised of fatty acids, sterols and acylglycerols while complex lipids such as glycerophospholipids and glycosphingolipids. There are various biological functions of lipids, such that fats and oils that are the main forms of energy storage in many organisms. Phospholipids and sterols are the major structural elements of biological membranes.

Fatty acids are carboxylic acid which comes in either saturated or unsaturated form (Baeza-Jimenez *et al.*, 2014). Fatty acids are stored in the form of triglycerides that, when provided by hydrolysis of triglycerides contain ample amount of energy sources for the body (Denniston *et al.*, 2005). In nature, unsaturated fatty acids are the most abundant followed by saturated fatty acids, with double bonds and a *cis* configuration. For higher animals, both saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) can be synthesized internally from non-lipid sources. However they are incapable of 'de novo' synthesis of the essential polyunsaturated fatty acids (PUFA). Therefore, neither SFA nor MUFA are essential in animal's diet (Hulbert and Abbott, 2011). 'De novo' synthesis refers to the synthesis of complex molecules from simple molecules.

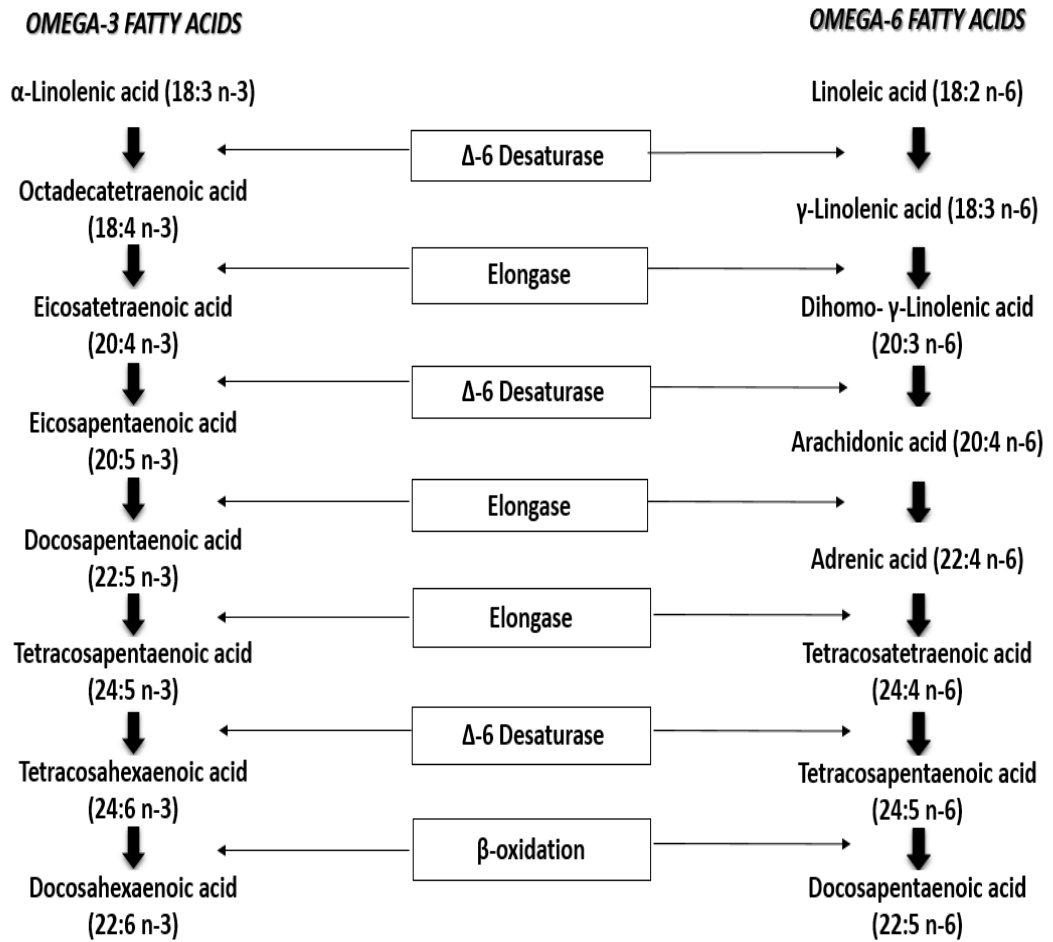
Fatty acid that has no carbon-carbon double bond is known as saturated fatty acids (SFA) (Woods *et al.*, 2005). The most common saturated fatty acid in animals, plants and microorganisms is palmitic acid (16:0). Stearic acid (18:0) is a major fatty acid in animals and some fungi, and a minor component in most plants. Myristic acid (14:0) has a widespread occurrence, occasionally as a major component (Rustan and Drevon, 2005). Consumption of SFA increases level of low-density lipoprotein (LDL) which is known to increase risk for cardiovascular disease (Hoenselaar, 2011).

Unsaturated fatty acids, with double bonds comprised of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). MUFA contain only one double bond in their chemical composition Oleic acid (18:1) is the most common MUFA in animals. It is also found in microorganisms. Palmitoleic acid (16:1 n-7) also

occurs widely in animals, plants and microorganisms, and is a major component in some seed oils (Rustan and Drevon, 2005).

While MUFA containing of only one carbon-carbon double bond, PUFA containing two or more carbon-carbon double bond. PUFA are the major components of cell membrane phospholipids (Pereira *et al.*, 2012) and consisting of essential fatty acids, of which they cannot be synthesized by the body and must be acquired from the diet (Bruno, 2005). These essential fatty acids are  $\alpha$ -linolenic acid (ALA) and linoleic acid (LA). These precursors will undergo an enzymatic process of desaturation and elongation (Baeza-Jimenez *et al.*, 2014) to produce long chain PUFA as illustrated in Figure 1. ALA, an Omega-3 PUFA will synthesized eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Whilst the omega-6 PUFA, LA will be synthesized into arachidonic acid (ARA, 20:4 n-6). Omega-3 and omega-6 fatty acids cannot be interconverted, and both are essential nutrients (Rustan and Drevon, 2005).

However, long chain PUFA can only be synthesized to a limited extend, thus they must be also acquired from diet or supplement (Pereira *et al.*, 2012). ALA is found in higher plants and algae, whereas EPA and DHA are major fatty acids of marine algae, fatty fish and fish oils (Rustan and Drevon, 2005). The occurrence of LA is in a major component in plant lipids and in mammals it is derived only from dietary vegetables, plant and marine oils. Meanwhile, ARA is a major component of animal phospholipids. It is also a major component of marine algae and some terrestrial species such as mosses (Gurr *et al.*, 2002).



**Figure 1:** Enzymatic process of desaturation and elongation (Rustan and Drevon, 2005)

## 2.5 Health Implications

The fatty acid composition plays an important role in animal health status. Dietary fatty acids can have effects on metabolic, immunological and cardiovascular events in numerous ways (Rustan and Drevon, 2005). Usually the fatty acids in ectothermic animals and plants contain higher proportions of unsaturated fatty acids as compared to those of birds and mammals (Barboza *et al.*, 2009). A high level of saturated fat in the diet of crocodile might disturb the metabolism in the liver and a deficiency of unsaturated fatty acid in the diet might cause dermatosis (Staton and Edwards, 1987). There is an equilibrium that exists between these fatty acids and disturbance of this balance may manifest to dermatitis, fatty liver, fertility problems and lower growth rates (Isberg, 2007). In many species, DHA is an important component of sperm tail lipids and thus may contribute to reproductive failure in captive crocodylians (Lance *et al.*, 2000). In the study made by Osthoff *et al.* (2010), there is slight difference in the fatty acid composition of adipose tissue between healthy wild Nile crocodiles (*Crocodylus niloticus*) and pansteatitis affected. Dietary deficiency of omega-3 PUFA during development lead to reduced EPA in the retina of animals and resulting in visual function impairment.

However, not all PUFA can be associated to be beneficial for the health, as omega-6 PUFA are considered as pro-inflammatory and omega-3 PUFA as anti-inflammatory. Therefore, it is depending on the omega-6: omega-3 ratio since the biosynthetic pathways for both relies on the same enzymes (Pereira *et al.*, 2012). Woods *et al.*, (2005) suggested that a balanced ratio of omega-6 and omega-3 PUFA is needed for normal growth and development in addition to the possibility of reducing

cardiovascular disease and other chronic diseases along with improving mental health status.

From the aspect of behavioural alteration, the information of how changes in dietary omega-3 fatty acids can alter behaviour is less known (Innis, 2005). However, there was a study done by feeding monkey with low omega-3 diet and it was observed that the monkey has increased in stereotypic activity (Innis, 2005). Thus, from the establishment of a preliminary study in fatty acid profile in relation to the diet will help to extract the information regarding its diet suitability, physiological mechanism and health status.

### 3.0 MATERIALS AND METHODS

#### 3.1 Crocodiles and Husbandry

This study was carried out at Pusat Konservasi Hidupan Liar Sungai Dusun, Kuala Kubu Baru, Selangor. A total of five crocodiles (n=5) were selected with two males and three females. There was only one juvenile and the other four were adults. All animals were given with the same diet. They were fed with raw commercial chicken once a week and raw catfish once a month. These false gharials were placed inside an earthen pond filled with fresh water and having a stocking density of eight with semi-wild environment.

**TABLE 1:** Details of the *T. schlegelii* involved

<i>Animal ID</i>	<i>AGE</i>	<i>SEX</i>	<i>DIET</i>	<i>HOUSING</i>
<i>FG1</i>	Adult	Male	Raw chicken and raw catfish	Kept in earthen freshwater pond, having stocking density of eight with semi-wild environment
<i>FG2</i>	Adult	Female		
<i>FG3</i>	Adult	Female		
<i>FG4</i>	Adult	Female		
<i>FG5</i>	Juvenile	Male		

#### 3.2 Crocodile's Restraining and Blood Collection

The crocodiles involved were handled according to animal ethic requirement. Prior to restraining and blood collection, water was drained out of the pond. Firstly, the crocodile's body was lassoed using rope and a wet towel was used to cover the eyes. This procedure helps to limit visual stimulation to the crocodile. Then, adhesive tape was wrapped around the snout to secure the jaw, taking care not to block the

nostrils. The body was also pinned down by handlers sitting from its cranial to caudal part of the body, and all the limbs were tied using rope to immobilize the crocodiles. Blood samples were collected from the lateral tail vein using 5 inches 18G spinal needle fitted to a 10 ml plastic syringe. The area of skin where the blood collection was to be made was first swabbed with 70% alcohol. Approximately 10 ml of blood was collected which was then transferred into 6 mL ethylenediaminetetraacetic (EDTA) tubes (Becton-Dickinson Company Vacutainer® K<sub>2</sub> EDTA), stored in 4°C and process within 24 hours. Plasma was then collected after centrifuging the EDTA tubes at 1000 x g for 10 minutes. Aliquots of blood were then pipetted into 1.5 mL Eppendorf tubes and stored at -20°C until analysis.

### **3.3 Plasma Fatty Acids Profile Determination**

#### **3.3.1 Total Lipid Extraction**

The total fatty acids profile were extracted from feeds, and plasma using chloroform: methanol 2:1 (v/v) based on the method of Folch *et al.* (1957) modified by Rajion (1985). About 1 mL of plasma and 1 g of feed samples were transferred to the glass tubes using micropipette. Then, 6 mL of fresh chloroform-methanol (2:1, v/v) and 3 mL of normal saline solution were added to facilitate phase separation. The aliquot was then vortexed at 2000 rpm for about 30 seconds using IKA MS1 Shaker and centrifuged at 3000 rpm for 5 minutes. The lower phase contained 86 parts chloroform: 14 parts methanol: 1 part water (Shahidi and Wanasundara, 1998). The upper phase contained 3: 48: 47 parts of chloroform, methanol and water respectively. The non-lipid contaminants would be retained in the

aqueous upper phase (Christie, 1982). After complete separation, the upper phase was discarded and lower phase was transferred to another glass tubes.

### 3.3.2 Fatty Acid Methyl Esters (FAME) Preparation

Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) were carried out using 20% methanolic boron trifluoride ( $\text{BF}_3$ ) according to methods in AOAC (2007). 100  $\mu\text{l}$  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 within the samples.

The sample was heated in the water bath at 70°C for 5 minutes and then dried on a heating block (40 °C) under a constant and mild flow of pure nitrogen gas. After drying the chloroform: methanol, 2ml of 0.66N methanolic potassium hydroxide (R & M Chemicals, Essex, U.K.) was added to saponify the lipid sample. The samples were heated in a boiling water bath for 10 minutes with occasional shaking for every five minutes. After the mixture had cooled down, 2 mL of 20%  $\text{BF}_3$  (Sigma Chemical Co., St. Louis, Missouri, USA) were added to initiate trans-esterification and the mixture was reheated for 20 minutes in a boiling water bath (Rajion, 1985).

After cooling, 4mL of petroleum ether (boiling point 40-60 °C) and 4 mL of distilled water were added and the mixture was vortexed at 200 rpm for 30 seconds. The mixture was then centrifuged at 3000 rpm for 5 minutes to increase phase separation. The upper petroleum phase was transferred to a 4 mL screw-capped vial

(Kimble Glass Inc., USA) using pasteur capillary pipettes, closed tightly and stored at 4 °C until analysis by gas-liquid chromatography.

### 3.3.3 Gas-Liquid Chromatography

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) (Figure 4). 1 µl was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a FID detector. High purity nitrogen (Malaysian Oxygen Bhd., Malaysia) was the carrier gas at 40 ml/min. High purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used for the flame ionization detector in the gas-liquid chromatography. The injector temperature was programmed at 250°C and the detector temperature was 300°C. The column temperature program initiated runs at 100°C, for 2 minutes, warmed to 170 °C at 10 °C /min, held for 2 minutes, warmed to 220°C at 7.5 °C /min, and then held for 20 min to facilitate optimal separation.

Identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to standards obtained from Sigma (St. Louis, MO, USA). Both gravimetric calculations and normalised percentage (%) of total fatty acids were used to determine the differences in fatty acids composition. Peak areas were 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 acid values were expressed both as the absolute amount of each fatty acids and as a total fatty acids in unit of percentage (%). Data from all five *T. schlegelii* were analysed using descriptive analysis in Microsoft Excel 2013 (Microsoft Corp., Redmond, USA) by establishing the mean  $\pm$  standard deviation, minimum value and maximum value.

## 4.0 RESULTS

### 4.1 Plasma Fatty Acid Profile of *T. schlegelii*

The plasma fatty acids profile from each of five *Tomistoma Schlegelii* is depicted in Table 2. Based on table 3, the dominant fatty acids were oleic acid, palmitic acid, docosahexanoic acid, linoleic acid and stearic acid. When the fatty acids evaluated individually, the results showed that the oleic acid (18:1 n-9) was the highest in FG1 and FG2. Oleic acid is the omega-9 MUFA. Whilst, for FG3, FG4, and FG5, the dominant fatty acid was docosahexanoic acid (22:6 n-3) which is an omega-3 PUFA. The lowest percentage of fatty acid accounted for FG1, FG2, FG3, and FG4 was eicosopentaenoic acid (20:5 n-3), an omega-3 PUFA and the lowest for FG5 was the myristic acid (14:0) which is a type of SFA.

Looking it as a whole from the mean value regardless of the age and sex, the highest percentage of fatty acid belongs to docosahexanoic acid (DHA) and the lowest was eicosopentaenoic acid (EPA).

**Table 2:** Plasma Fatty Acid Profile of *T. schlegelii* (n=5)

All values were expressed in percentage (%)

FATTY ACIDS	TOTAL FATTY ACIDS IN PLASMA (%)				
	FG1	FG2	FG3	FG4	FG5
Myristic acid (14:0)	0.30	0.37	0.38	0.54	0.19
Palmitic acid (16:0)	23.29	18.77	22.66	25.44	23.93
Palmitoleic acid (16:1 n-7)	2.84	2.93	1.74	1.59	1.83
Stearic acid (18:0)	5.93	4.55	7.42	5.32	6.15
Oleic acid (18:1 n-9)	26.54	28.52	22.01	11.90	17.41
Linoleic acid (18:2 n-6)	15.25	18.67	16.23	14.65	15.99
$\alpha$ -Linolenic acid (18:3 n-3)	1.30	1.29	1.37	1.05	1.92
Arachidonic acid (20:4 n-6)	1.67	1.27	2.20	2.39	2.35
Eicosopentaenoic acid (20:5 n-3)	0.22	0.10	0.31	0.24	0.30
Docosapentaenoic acid, clupanodonic acid (22:5 n-3)	1.34	0.28	1.26	0.63	1.19
Docosapentaenoic acid, osbond acid (22:5 n-6)	0.56	0.31	0.79	1.48	1.02
Docosahexanoic acid (22:6 n-3)	20.57	22.67	23.56	34.55	27.49
Total Saturated Fatty Acid	29.71	23.96	30.51	31.52	30.50
Total Unsaturated Fatty Acid	70.29	76.04	69.49	68.48	69.50
Total MUFA	29.38	31.45	23.76	13.49	19.25
Total PUFA n-3	23.44	24.34	26.51	36.47	30.89
Total PUFA n-6	17.48	20.25	19.22	18.52	19.36
Ratio n-6:n-3	0.75	0.83	0.73	0.51	0.63
Ratio UFA : SFA	2.37	3.17	2.28	2.17	2.28
Ratio PUFA : SFA	1.38	1.86	1.50	1.74	1.65

Total SFA = 14:0 + 16:0 + 18:0

Total Unsaturated Fatty Acid = 16:1 n-7 + 18:1 n-9 + 20:5 n-3 + 18:3 n-3 + 22:5 n-3 +  
22:6 n-3 + 20:4 n-6 + 18:2 n-6 + 22:5 n-6

Total MUFA = 16:1 n-7 + 18:1 n-9

Total PUFA n-3 = 20:5 n-3 + 18:3 n-3 + 22:5 n-3 + 22:6 n-3

Total PUFA n-6 = 20:4 n-6 + 18:2 n-6 + 22:5 n-6

**Table 3:** Descriptive Analysis of Plasma Fatty Acid Profile of *T. schlegelii* (n=5)

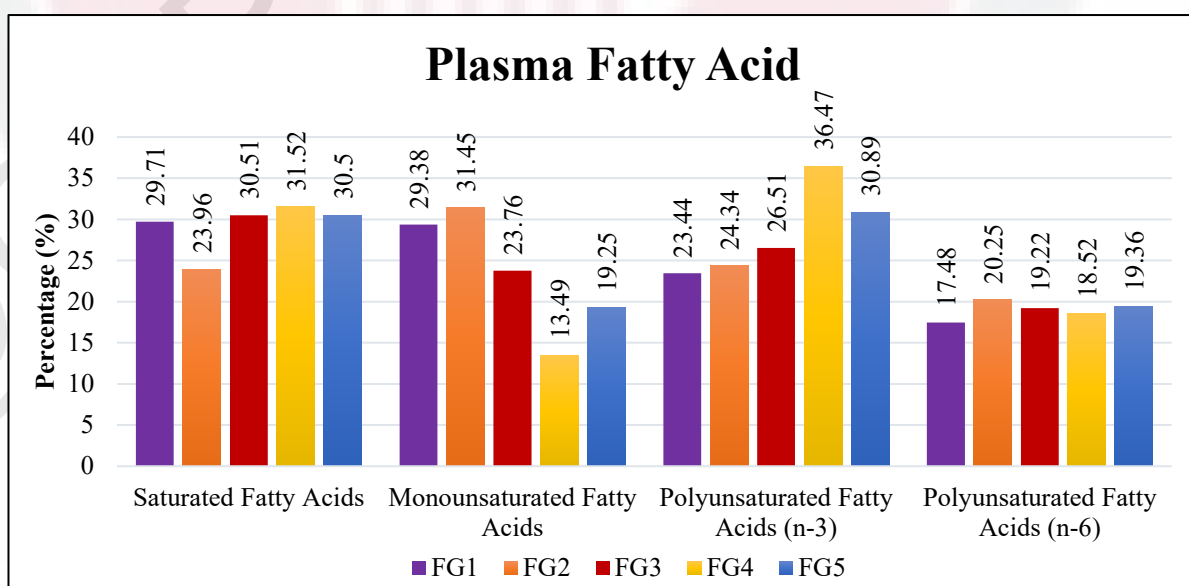
FATTY ACIDS	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	MEAN $\pm$ STANDARD DEVIATION
Myristic acid (14:0)	0.36	0.127789	0.19	0.54	0.36 $\pm$ 0.127789
Palmitic acid (16:0)	22.82	2.487241	18.77	25.44	22.82 $\pm$ 2.487241
Palmitoleic acid (16:1 n-7)	2.19	0.644616	1.59	2.93	2.19 $\pm$ 0.644616
Stearic acid (18:0)	5.87	1.06411	4.55	7.42	5.87 $\pm$ 1.06411
Oleic acid (18:1 n-9)	21.28	6.774572	11.9	28.52	21.28 $\pm$ 6.774572
Linoleic acid (18:2 n-6)	16.16	1.536398	14.65	18.67	16.16 $\pm$ 1.536398
$\alpha$ -Linolenic acid (18:3 n-3)	1.39	0.322071	1.05	1.92	1.39 $\pm$ 0.322071
Arachidonic acid (20:4 n-6)	1.98	0.488242	1.27	2.39	1.98 $\pm$ 0.488242
Eicosapentaenoic acid (20:5 n-3)	0.23	0.084143	0.1	0.31	0.23 $\pm$ 0.084143
Docosapentaenoic acid, clupanodonic acid (22:5 n-3)	0.94	0.462763	0.28	1.34	0.94 $\pm$ 0.462763
Docosapentaenoic acid, osbond acid (22:5 n-6)	0.83	0.448185	0.31	1.48	0.83 $\pm$ 0.448185
Docosahexanoic acid (22:6 n-3)	25.77	5.513186	20.57	34.55	25.77 $\pm$ 5.513186
Total Saturated Fatty Acid	29.24	3.020687	23.96	31.52	29.24 $\pm$ 3.020687
Total Unsaturated Fatty Acid	70.76	3.020687	68.48	76.04	70.76 $\pm$ 3.020687
Total MUFA	23.47	7.350193	13.49	31.45	23.47 $\pm$ 7.350193

Total PUFA n-3	28.33	5.384185	23.44	36.47	28.33 ± 5.384185
Total PUFA n-6	18.97	1.033867	17.48	20.25	18.97 ± 1.033867
Ratio n-6:n-3	0.69	0.123288	0.51	0.83	0.69 ± 0.123288
Ratio UFA:SFA	2.45	0.406485	2.17	3.17	2.45 ± 0.406485
Ratio PUFA:SFA	1.62	0.19021	1.38	1.86	1.62 ± 0.19021

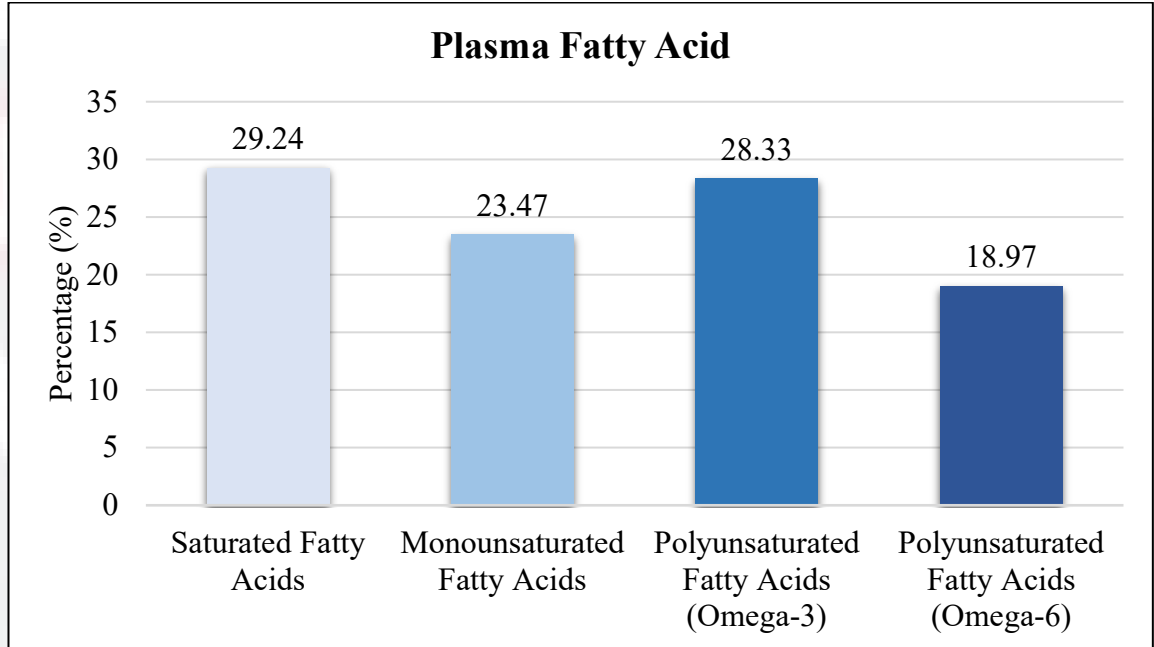
All values were expressed in percentage (%)

The percentage of total PUFA was the highest in all five *T. schlegelii* followed by SFA and MUFA. On the contrary, in FG2 the MUFA was higher compared to SFA. Higher percentage of PUFA was due to high total amount of omega-3 and omega-6 in the plasma. These information can be observed as illustrated in Figure 3. Then, the percentage of total SFA was high in all samples except for FG2 as there was a higher levels of palmitic acid (16:0) in FG1, FG3, FG4 and FG5. The high percentage of MUFA in FG2 was due to an increase of oleic acid (18:1 n-9) in the plasma (Figure 2).

**Figure 2:** Plasma Fatty Acid for each *T. schlegelii* (n=5)



**Figure 3:** Plasma Fatty Acid of *T.schlegelii* expressed in total mean value (%)



#### 4.1 Feed Fatty Acid Profile

The total fatty acids of feed from raw chicken and raw catfish is depicted in Table 4. Chicken is known to be the dietary source of omega-6, thus the results showed that linoleic acid (18:2 n-6) is among the dominant fatty acids with 17.16%. This can be correlate to the 16.16% of linoleic acid composition in plasma. However, there was only 0.73% of arachidonic acid in the chicken which does not really comply with those value in plasma. As for the catfish, it does not contain high omega-3 especially there was 0.00% for both EPA and DHA. It contain of higher omega-6 as it was due to fact that it lives in the freshwater.

**Table 4:** Feed Fatty Acid Profile of Raw Chicken and Raw Catfish

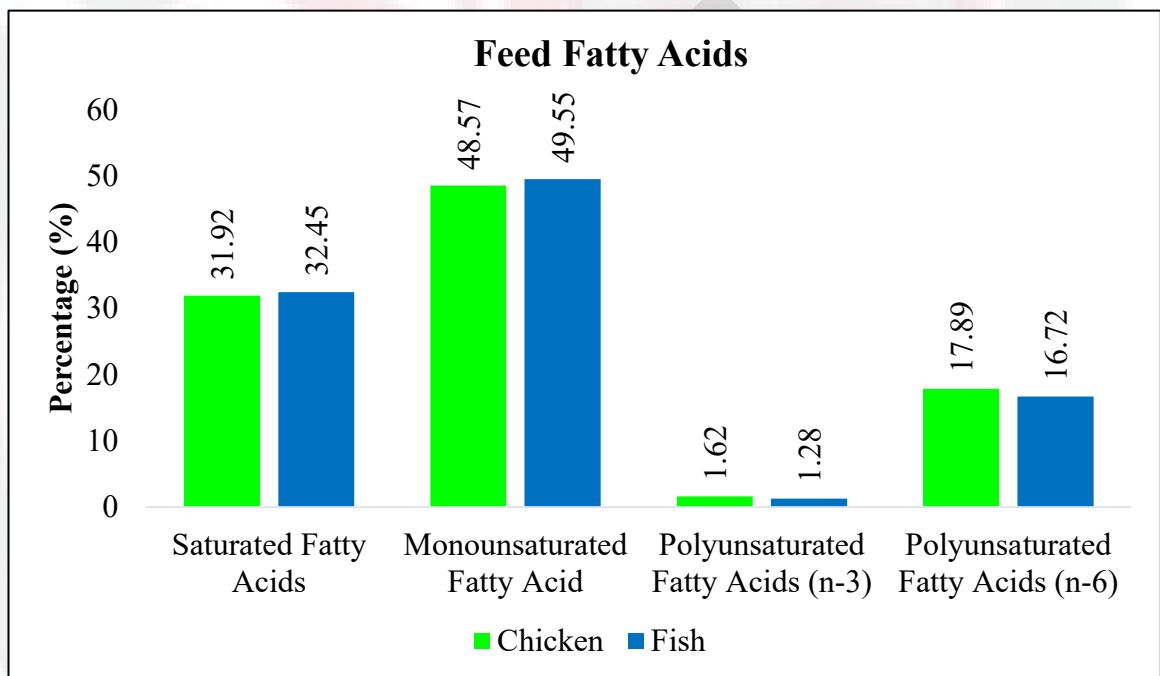
FATTY ACIDS	TOTAL FATTY ACIDS IN FEED (%)	
	Chicken	Fish
Myristic acid (14:0)	0.91	1.87
Palmitic acid (16:0)	27.59	25.04
Palmitoleic acid (16:1 n-7)	5.45	7.66
Stearic acid (18:0)	3.43	5.54
Oleic acid (18:1 n-9)	43.12	41.89
Linoleic acid (18:2 n-6)	17.16	15.64
$\alpha$ -Linolenic acid (18:3 n-3)	1.17	1.28
Arachidonic acid (20:4 n-6)	0.73	1.08
Eicosapentaenoic acid (20:5 n-3)	0.09	0.00
Docosapentaenoic acid, clupanodonic acid (22:5 n-3)	0.15	0.00
Docosapentaenoic acid, osbond acid (22:5 n-6)	0.00	0.00
Docosahexanoic acid (22:6 n-3)	0.21	0.00
Total Saturated Fatty Acid	31.92	32.45
Total Unsaturated Fatty Acid	68.08	67.55
Total Monoenes	48.57	49.55
Total PUFA n-3	1.62	1.28
Total PUFA n-6	17.89	16.72
Ratio n-6:n-3	11.01	13.06
Ratio UFA : SFA	2.13	2.08
Ratio PUFA : SFA	0.61	0.55

All values were expressed in percentage (%)

The total feed fatty acids results showed an increase of MUFA, SFA and PUFA in descending order for both chicken and fish which was different from the total plasma fatty acids profile that has much higher level of PUFA. The high total percentage of MUFA in chicken and fish was due to a significant increase of the individual fatty acids of oleic acid which is 43.12% and 41.89% respectively. The palmitic acid was high for both feeds which explains the level of SFA that comes in second. For PUFA, the omega-3 is much lower than omega-6 in both feeds.

The omega-3 PUFA is the lowest in chicken as there is low levels of those four types of omega-3 PUFA namely; ALA, EPA, DPA (clupanodonic acid) and DHA. Similar to raw catfish, which also has the lowest omega-3 PUFA as there is low amount of ALA and absence of EPA, DPA (clupanodonic acid) and DHA.

**Figure 4:** Feed Fatty Acids in Raw Chicken and Raw Catfish



## 5.0 DISCUSSION

### 5.1 Plasma Fatty Acid Profile

In general, among the dominant plasma fatty acids in captive *T. schlegelii* were docosahexanoic acid, palmitic acid, oleic acid, and linoleic acid. Similarly, the meat fat composition of captive *Crocodylus niloticus*, *Crocodylus porosus*, *Crocodylus johnstoni*, *Caiman latirostris* and *Caiman yacare* showed a significant increase of those individual fatty acids. (Mitchell *et al.*, 1995; Hoffman *et al.*, 2000; Elena *et al.*, 2007). However, unlike *T. schlegelii*, there was no increase in docosahexanoic acid (DHA) can be found from these species. Lance *et al.* (2000) found that the EPA and DHA were higher in the wild than captive-reared alligators while LA was higher in the captive compared to those in wild. The summary of inter-species comparison from different studies illustrated on Table 5.

**Table 5:** Inter-species comparison for dominant individual fatty acids from different studies

<b>Fatty Acids</b>	<i>Tomistoma schlegelii</i>	<b>Hoffman et al. (2000)</b> <i>Crocodylus niloticus</i>	<b>Mitchell et al. (1995)</b> <i>Crocodylus porosus</i> and <i>Crocodylus johnstoni</i>	<b>Elena et al.(2007)</b> <i>Caimanlatirostris</i> and <i>Caiman yacare</i>
<b>Palmitic acid (16:0)</b>	22.82	25.38	22.5	21.85
<b>Stearic acid (18:0)</b>	5.87	9.89	7.4	15.36
<b>Oleic acid (18:1 n-9)</b>	21.28	43.05	33.1	34.92
<b>Linoleic acid (18:2 n-6)</b>	16.16	9.05	15.2	8.40
<b>Docosahexanoic acid (22:6 n-3)</b>	25.77	0.90	1.3	0.57

All values were expressed in percentage of total identified fatty acids (%)

From the results, the total plasma fatty acids showed that there is higher PUFA, followed by SFA and MUFA. Different to those results, the total SFA of ten captive caimans (*C. latirostris*, and *C. yacare*) were high followed by MUFA and PUFA with 41.4%, 39.1 % and 10.7% respectively (Elena *et al.*, 2007). The total plasma fatty acids of captive and wild yacare caiman (*C. yacare*) and captive Nile crocodiles (*C. niloticus*) showed higher MUFA, followed by SFA and PUFA (Hoffman *et al.*, 2000; Vicente-Neto, 2010).

The percentage of omega-3 fatty acid from all *T. schlegelii* showed a predominant increase as compared to omega-6 fatty acid with 28.33% and 18.97%

respectively. Increase of omega-3 fatty acid in plasma was also reported by Morpurgo *et al.* (1993) but in wild Nile crocodiles (*Crocodylus niloticus*) as compared to captive ones. Peplow *et al.* (1990) stated that lipids in American alligators (*Alligator mississippiensis*) fed on fish diets contained 11.1% DHA and 4.0% EPA, while alligators fed on beef diets contained minimum amount of these fatty acids. From the comparison, the difference exist as being a monogastric animal, the diet strongly influences the fatty acid composition (Osthoff *et al.*, 2009).

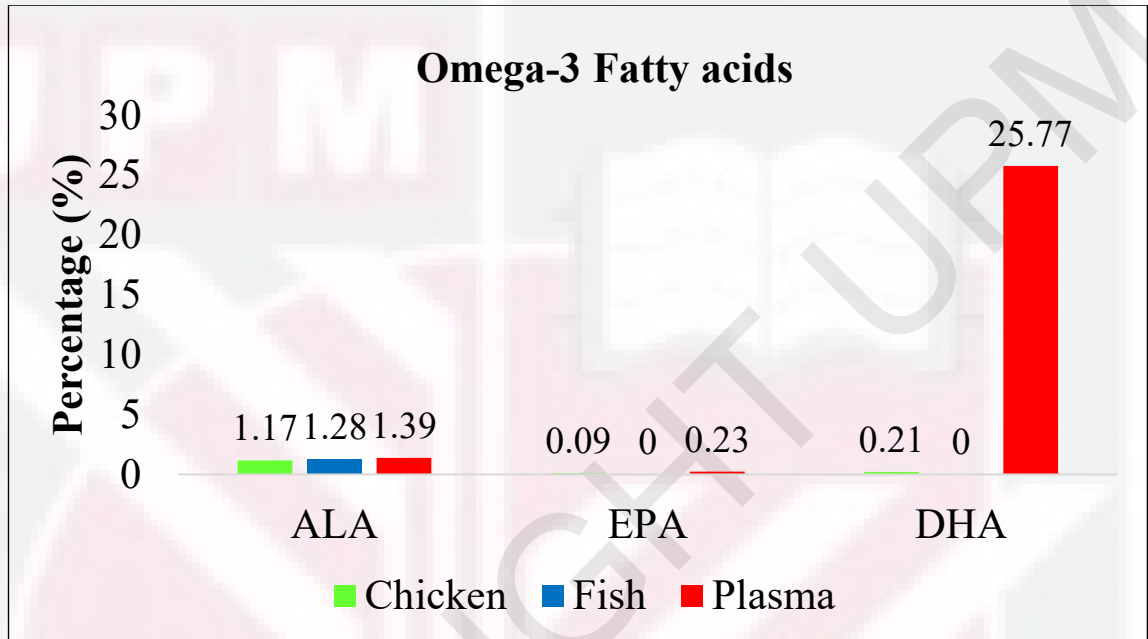
## 5. 2 Feed Fatty Acid Composition

There were two types of essential fatty acids that can be acquired from the diet namely; omega-3 and omega-6 PUFA. The wide changes in fatty acid profiles within a species is mostly due to feeding behaviour and physiology of the gastrointestinal tract (Vicente-Neto, 2010). These Tomistomas were fed with raw commercial chicken and raw catfish.

Based on the results being shown in Table 3, both feed has high omega-6 content as compared to omega-3 PUFA. Contradict to the plasma fatty acids profile that has higher omega-3 than omega-6 PUFA, due to high levels of DHA. However, in the chicken, the DHA only contain about 0.21% while in catfish, DHA was not found. The omega-3 composition in feed and plasma can be summarized as in Figure

5.

**Figure 5:** Comparison of omega-3 essential fatty acid composition in chicken, fish and plasma



Fish is known to provide a good source of omega-3 PUFA (Chedoloh *et al.*, 2011). However, since catfish is a type of freshwater fish, it contains higher levels of omega-6 PUFA than marine species and require less dietary requirement of Omega-3 PUFA. Freshwater fishes showed small range of DHA (0.63% - 1.41%), EPA (0.11% - 0.25%) and AA (1.41% - 4.46%). (Nur Airina and Jamaludin, 2012). Furthermore, in the study conducted by Zzaman *et al.* (2014), fatty acids profile from muscles of three freshwater fish; patin (*Pangasius pangasius*), tilapia (*Oreochromis niloticus*) and catfish (*Clarius batrachus*) showed 0%, 0.05% and 0.16% DHA respectively. This explains the level of omega-3 PUFA in the catfish. Similarly in chicken meat, it is also known to provide the dietary sources of PUFA, unfortunately not the major contributor

for DHA in this study. They might be the sources to other types of fatty acids such as omega-6 PUFA as in both of these feed, the omega-6 content can be considered high.

There are many possibilities to relate with the unexpectedly high levels of DHA in the plasma despite having a minimal value in the feed. Firstly, poikilothermic vertebrates display a diversity of lipid metabolism fluctuations. There is still limited information regarding the control of lipid deposition and synthesis in these animals (Sheridan, 1994; Morpurgo and Gelman 1991). Apart from that, the synthesis of long chain PUFA of omega-3 and omega-6 requires the same enzymes, and generally the increase of one of these essential fatty acids reflects the decrease of the other (Pereira *et al.*, 2012). These could be also attributed by species difference other than the variations in diet (Hoffman *et al.*, 1990). Lastly, as this animal is living in the semi-wild environment, it provided us with information that the false gharial may have access to any prey items inside the pond or from the land which we may overlooked.

In addition, we cannot run from the fact that there might be due to any technical issues such as from the machine itself. Important parameters that can be identified such as column temperature, carrier gas flow-rate, injection temperature, split ratio and sample size were known to affect the analysis by gas and liquid chromatography (Barwick, 1999).

## 6.0 CONCLUSION

In conclusion, the fatty acids profile of captive false gharial in this study showed a predominant increase in unsaturated fatty acids specifically polyunsaturated fatty acids due to an increase in omega-3 and omega-6 polyunsaturated fatty acids. Then, it is followed by saturated fatty acids and monounsaturated fatty acids. In relation to diet provided to the captive false gharial, the chicken and catfish is known to be the dietary source omega-6. Nonetheless, this shows that the diet provided is suitable for the consumption by the false gharial as the diet contain essential fatty acids needed for their well-being.

## **7.0 RECOMMENDATIONS**

Profiling of the fatty acids have not been done on *T. schlegelii*. This is only a preliminary study for this species and the sample size acquired (n=5) might not been sufficient to establish a true fatty acid profiles. However in the future, an improvement in terms of higher sample size can be done to conclude the normal fatty acid composition in this species. Additionally, samples can be obtained from both that kept in wild and captivity to study the comparison between each if there is any. Crocodiles that are fed with different diet or supplementation can also be studied in order to determine the relationship of diet with fatty acids composition which believes to have a major effect in the composition. This will in turn, reflects the nutritional benefits to the animals. Further study on the diversity of lipid metabolism in reptiles is also required to have better understanding on the fluctuations of fatty acids composition.

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## **APPENDICES**



**Plate 1:** The false gharial subjected for this study



**Plate 2:** The restraining procedure



**Plate 3:** Blood sampling via lateral tail vein



**Plate 4:** The fatty acids methyl ester (FAME) was quantified by using gas chromatography.