



UNIVERSITI PUTRA MALAYSIA

**FEED FATTY ACIDS AND THEIR UTILIZATION IN THE GIANT PANDA
(*Ailurupoda melanoleuca*)**

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FEED FATTY ACIDS AND THEIR UTILIZATION IN THE GIANT PANDA

(Ailurupoda melanoleuca)

NUR AISHAH BINTI ABDULLAH

A project submitted to the
Faculty of Veterinary Medicine, University Putra Malaysia

In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Feed Fatty Acids and their Utilization in Giant Panda (*Ailurupoda melanoleuca*)”, by Nur Aishah binti Abdullah 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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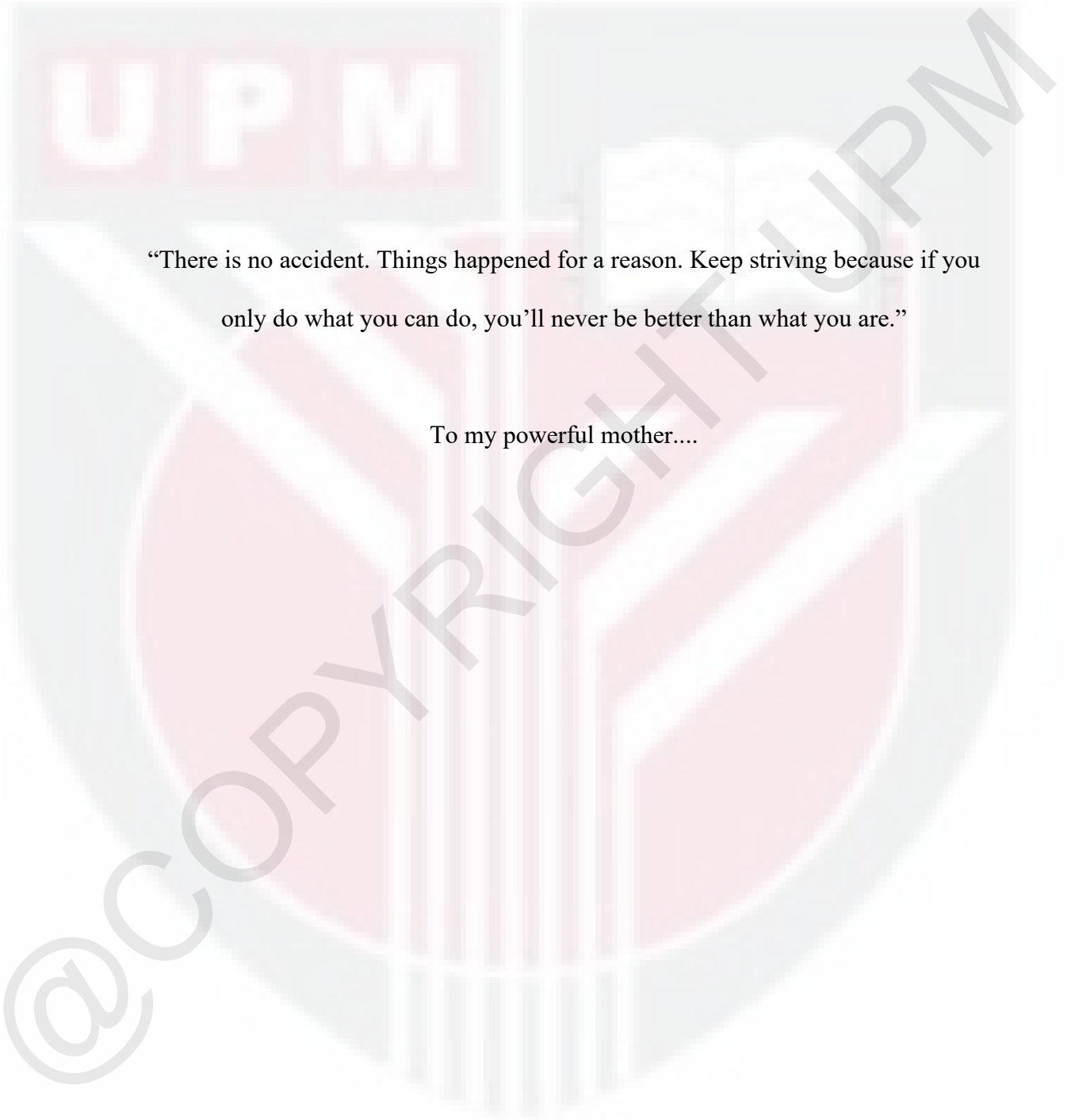
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DEDICATION

“There is no accident. Things happened for a reason. Keep striving because if you only do what you can do, you’ll never be better than what you are.”

To my powerful mother....



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Alhamdulillah, praises to Allah the Almighty for the infinite blessings and His grace. I would like to extend my deepest gratitude to the truly inspiring supervisor, Associate Professor Dr. Goh Yong Meng for his patience, guidance and continuous support throughout completing my final year project. I would also like to thank my co-supervisor, Dr. Hafandi Ahmad for helping me with the application to take samples from Zoo Negara as the research about Giant Panda is restricted and difficult to be approved. In addition, thank you to all lecturers, post graduate students and batch mates that have been directly or indirectly involved in putting up the puzzles of this project into one.

This thesis is dedicated to my beautiful mother, Nur Azah Khadijah Koh binti Abdullah for her concern and understanding during my absence at home as I need to dedicate more time for this project. I would also want to acknowledge Dr. Maehdi Ebrahimi and Puan Rosmawati Mohd Hanipah for their patience in assisting me to perform the total lipid extraction procedures. Without them, I won't be able to get through this adventurous journey starting from sampling, laboratory work up and till the day of completing this project.

Not to forget, sincere thanks to every single soul that involved directly and indirectly in making this happened as well as for being my pillar of strength through the years.

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LIST OF ABBREVIATION

%	percentage
FAME	Fatty Acid Methyl Ester
GLC	Gas Liquid Chromatography
FID	Flame Ionization Detector
μl	microlitre
μm	micrometre
ml	millilitre
°C	degrees celcius
Rpm	revolutions per minute
A.melanoleuca	Ailuropoda melanoleuca
v/v	volume/volume
EFA	essential fatty acid
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

**ASID LEMAK DAN PENGGUNAANNYA PADA *AILURUPODA*
*MELANOLEUCA***

Oleh

Nur Aishah binti Abdullah

2017

Penyelia : Profesor Madya Dr. Goh Yong Meng

Penyelia Bersama : Dr. Hafandi Ahmad

Beruang Panda memperoleh hampir kesemua keperluan nutrien daripada bahan tumbuhan. Bahan makanan ini tidak dapat dimanfaatkan sepenuhnya memandangkan tumbuhan mempunyai kandungan asid lemak yang lebih rendah, serta ciri sistem pencernaan Beruang Panda yang lebih mirip kepada haiwan karnivor. Analisa asid lemak tinja merupakan pendekatan yang boleh digunakan untuk menerangkan fungsi fisiologi salur penghadaman Beruang Panda. Kajian ini dilakukan memandangkan Beruang Panda merupakan haiwan herbivor yang unik. Tambahan pula, dapatan kajian ini berpotensi untuk menyumbang kepada repositori data saintifik Beruang Panda yang terhad pada masa ini. Kajian ini menggunakan sampel tinja dari dua ekor Beruang Panda (*A. melanoleuca*) dewasa serta anak mereka yang berumur 2 tahun 6 bulan. Sampel tinja telah diperolehi untuk analisis daripada 9 Januari sehingga 20 Februari 2017 di Zoo Negara,

Malaysia. Analisis asid lemak juga dilakukan ke atas empat spesies buluh serta bahan makanan harian lain yang dimakan oleh Beruang Panda tersebut. Tahap asid lemak dalam diet telah ditentukan selepas asid-asid lemak tersebut diekstrak dan dianalisis menggunakan kromatografi gas.

Keputusan menunjukkan bahawa asid palmitik (16:0) merupakan asid lemak yang paling banyak dijumpai pada batang buluh dan asid α -linolenik (18:3 n-3) bertumpu pada daun buluh. Peratusan asid lemak tepu adalah yang tertinggi pada kesemua empat spesies buluh diikuti oleh asid lemak politaktepu n-3, asid lemak politaktepu n-6 dan akhir sekali asid lemak monotaktepu. Keputusan juga menunjukkan bahawa Beruang Panda mampu mensintesis asid lemak berantai panjang menggunakan asid lemak daripada makanan mereka. Ini bermakna Beruang Panda mempunyai enzim desaturase dan elongase yang membolehkan proses desaturasi dan pemanjangan rantai asid lemak dijalankan dalam badan mereka.

Kata kunci: *A. melanoleuca*, analisa asid lemak, analisa nutrisi, buluh tempatan, n-3, n-6, penggunaan

ABSTRACT

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

FEED FATTY ACIDS AND THEIR UTILIZATION IN THE GIANT PANDA

(Ailurupoda melanoleuca)

By

Nur Aishah binti Abdullah

2017

Supervisor: Associate Prof. Dr. Goh Yong Meng

Co-supervisor: Dr. Hafandi Ahmad

The Giant Panda derived most of its nutritional needs from plant materials. Dietary utilization of these plant materials is complicated by the comparatively lower plant fatty acid contents, and fact that the digestive system of the Giant Panda has characteristics that are consistent with carnivores. In the absence of other body fluids and tissues, faecal fatty acid profiles of the Giant Panda provide an important hint to explain the functions of its digestive system. These are crucial to understand how Giant Panda's acquire energy and other nutritional needs from its herbivorous diet. The aims of this research were to study the fatty acid profiles of bamboo plants in the Giant Panda's diet, and to determine the faecal fatty acid profiles of Giant Pandas. This information would provide insights into how Giant Pandas are able to fulfil their fatty acid requirements, especially the long chained fatty acids that are crucial for the various

physiological functions of the body. Four local species of bamboo plants, and faecal samples of two adult Giant Pandas (*A.melanoleuca*) and their cub were obtained for analysis. The study was conducted from 9th January until 20th of February 2017 at Zoo Negara Malaysia. Identification and quantification of fatty acid content was done by using gas liquid chromatography (GLC) following the procedure of total lipid extraction and fatty acids methyl esters (FAME).

Results showed that palmitic acid (16:0) was the dominant fatty acids in the shoots of bamboo plants, while α -linolenic acid (18:3 n-3) is the major fatty acid found in leaves. Total saturated fatty acids (TSFA) were consistently high followed by the unsaturated fatty acids comprising of the PUFA n-3, n-6 and MUFA. Faecal fatty acid profiles showed the presence of very long chain n-3 and n-6 fatty acids which are important for cellular functions. In conclusion, the current study showed that while bamboo lacked the longer chain essential fatty acids, the Giant Pandas were able to satisfy their physiological requirements through *de novo* synthesis. This indicates that the Giant Panda do have the ability, as well as the specific enzymes for the elongation and desaturation of fatty acids, a feature found in most herbivorous and omnivorous animals.

Keywords: *A.melanoleuca*, fatty acid analysis, local bamboos, n-3, n-6, nutritional preferences, utilization

1.0 INTRODUCTION

The Giant Panda (*A. melanoleuca*) also known as Panda Bear or simply Panda is a native animal to south central China. Even though it belongs to the order Carnivora, the Giant Panda's diet is over 99% bamboo (Schaller et al., 1989). According to World Wide Fund of Nature (WWF) update on 2016, they stated that there are only 1,864 Giant Pandas left in the wild. As a result of farming, deforestation, and other development, the Giant Panda has been driven out of the lowland areas where it once lived and listed as an endangered species in International Union of Conservation of Nature (IUCN) red list.

Anatomically, this animal exhibits characteristics which are consistent with their dietary preference. Giant Pandas in the wild will occasionally eat other grasses, wild tubers, or even meat in the form of birds, rodents or carrion. In captivity, they may receive honey, eggs, fish, yams, shrub leaves, oranges, or bananas along with specially prepared food. Their diets consist mainly of bamboo leaves despite its carnivorous-like digestive tract and its ability to digest cellulose and acquire energy from its diet has received attention from the scientific community (Dierenfeld et al., 1982 as cited by Zhu et al, 2011). The aims of this research were to study the fatty acid profiles of bamboo plants in the Giant Panda's diet, and to determine the faecal fatty acid profiles of Giant Pandas. This information would provide insights into how Giant Pandas are able to fulfil their fatty acid requirements, especially the long chained fatty acids that are crucial for the various physiological functions of the body.

2.0 LITERATURE REVIEW

2.1 Definitions

Giant Panda is a large, black-and-white carnivore with scientific name of *A.melanoleuca*, originated from China that normally feeds on bamboo (Collins Dictionary, 2010). Bamboo leaves are a subfamily of *Bambusoideae* with a hollow woody-walled stems of ringed joints as well as edible young shoots in the grass family of *Poacea* (Encyclopaedia Britannica, 2016).

2.2 Giant Panda (*Ailurupoda melanoleuca*)

This specific black and white carnivore is the rarest member of the bear family and lives in temperate broadleaf and mixed forests where the habitats are mainly in bamboo forests high in the mountains of southwest China. Giant Panda is in the phylum of Chordata as chordates are all bilaterally symmetrical with distinct heads and is considered as Mammalian of the Carnivora order. It belongs to the same members of the bear family, Ursidae but the Giant Panda is the only member of the genus Ailuropoda because it is not very closely related to any other bears. One of the most similar characteristic that can be related with Giant Panda, is *Ailurus fulgens* known as the Red Panda. The Giant Panda consumes around 12 to 38 kilogram of bamboo leaves as part of their main diet (WWF, 2016). The adults can grow up to 100 to 150 kilogram, however a newborn Giant Panda is only a 100gram of weight and about 1/900th to the size of the dam (Kelly, 2015).

The Giant Panda has a similar typical body shape of a bear with black fur on its ears, eye patches, muzzle, legs, arms as well as the shoulders. The rest of the animal's coat is white. Following weirdness on how they appeared to be black and white, there is speculation suggests that the bold colouring provides effective camouflage of their thick, wooly coat in the cold forest and rocky habitat (Dudley, 1997). It is a terrestrial animal and generally solitary. Each adult has a defined territory, and a female is not tolerable by other females in her range. Besides, Giant Pandas communicate through vocalization as well as scent marking by clawing trees or spraying urine. Social interactions do occur primarily during the breeding season. After copulation, the male adult will abandoned the female and the female raise the cub alone (Loucks et al, 2003).

2.3 Diets and digestive system

The Giant Panda is classified as a carnivore but its diet is surprisingly herbivours in nature, consist primarily and exclusively of bamboo. However, they still have the digestive system of a carnivore, with the carnivore-specific genes (Li, 2010) that enable them to obtain both energy and protein in small amounts from the consumption of bamboos. The Panda's digestive system is partially adapted for processing bamboo as they have a tough throat and oesophageal lining with thick stomach mucosa and enlarged colon surface. However, their short intestines are ill-suited to digest cellulose effectively, thus digestion of cellulose is done by microbes in its gut. Pandas born with sterile intestines and thus require the tranfection of bacteria from their mother's faeces to facilitate digestion (Zhu et al, 2011). Thus, to compensate

for the bamboo's poor digestibility. Giant Panda needs to ingest huge quantities of bamboos to fulfil their nutrient requirements.

In general, their diet consisted of 99% bamboo roots, shoots and leaves. Only a few bamboo species are widespread at the high altitudes that they inhabit in the wild. These include *Bashania fangiana* in Wolong (sub alpine conifer forest region) (Wei, 1999), *Fargesia spathacea*, *F. robusta*, *Sinarundinaria chungii*, *S. nitida*, and *S. fangiana* found in lower altitudes. Occasionally they also consume eggs, small or infant animals, as well as foraging in farmlands for pumpkin, kidney beans, wheat and domestic pig food (Hu and Wei 2004).

Their diet was also reported to vary with seasons. Between April to June, they eat bamboo stems while on July to October, leaves was preferred. Between November to March, old shoots, stems as well the leaves are consumed. Regular time of feeding is 14 hours per day with a sitting position and forelegs free while eating. They used an accessory lobe "thumb" on the pad of each forepaw which can be flexed to controls the position of the bamboo stalk before grasping the stalk with their strong teeth. Consumption of vast quantities of feed is possible and non detrimental because of the rapid passage of large amounts of indigestible plant material through their short and straight digestive tract (Ciochon, 2007).

On the other hand, the rapid passage of ingesta limited the potential of microbial digestion thus limiting any alternative forms of digestion causing them to defecate up to 40 times a day (Dierenfeld, 1982). To further conserve energy, the low body surface area to body volume of the Giant Panda indicated a lower metabolic rate compare to other herbivore (Ciochon, 2007). It is thus clear that the sedentary lifestyle allows Giant Pandas to survive on nutrient poor resources such as bamboo. Similarly,

several features includes flattened molars and extensive jaw muscles which attach from the top of the head to the mouth enables the Giant Pandas to crush and grind the fibrous plant material (Ishak et al, 2016).

2.4 Characteristics of the bamboo leaves

Bamboo is in the grass family Poaceae with a large distribution throughout the tropics and subtropics region. In Peninsular Malaysia, 59 species of native bamboo are currently known (Wong, 2011). Some local species includes *Fargesia dracocephala*, *Fargesia rufa*, *Dendrocalamus asper*, *Bambusa vulgaris* and *Thyrsostachys seamensis*. The growth pattern of the bamboos are solely depends on the local soil and climatic condition with some species contribution as other factor. The typical growth ranges from 3–10 cm per day during the favourable growth period, (Li et al, 2002) but however, the size range for mature bamboo is species-dependent, with the smallest bamboos reaching only several inches high at maturity.

As the shoots and its rhizome system mature, taller and larger culms are produced each year until the plant approaches its particular species limits of height and diameter. Soft bamboo shoots, stems, and leaves are the major food source of the Giant Panda of China, the Red Panda of Nepal as well as the bamboo lemurs of Madagascar. Bamboos were also the primary diets of mountain gorillas of Africa where they has been and documented consuming bamboo sap (Soderstrom and Calderon, 1979).

Bamboo shoots are low in calories, high in dietary fibre and rich in various nutrients including protein, carbohydrates, amino acids, minerals, fat, sugar, fibre and inorganic salts. Fresh shoots are also a good source of thiamine, niacin, vitamin A,

vitamin B6, and vitamin E (Nirmala et al, 2007). They are rich in protein containing between 1.49 and 4.04 (average 2.65 g) per 100 g of fresh bamboo shoots with 17 amino acids and 67% of the total amino acid content is constitute of tyrosine (Kozukue et al, 1999). The fatty acid is comparatively low (0.26% to 0.94%), however the shoots contained some important essential fatty acids.

Good sources of phytosterols that are the precursors of many pharmaceutically active steroids are present and act as a nutraceuticals or “natural medicine” that helps in cholesterol-lowering activity (Brufau, 2008). It is also indicated that there is an overall decrease in all the nutrient components in 10 days old shoots compared to the freshly collected shoots (Nirmala et al, 2007). Protein is also in leaves, branches and stems. Leaves contain the highest levels of protein and rich in calcium around summer (Sastry, 2008).

2.5 Mystery of Giant Panda's nutrition

The panda has gut structure, gut function and gut enzymes like a carnivore with the absence of multiple stomachs, nor an enlarged cecum, nor the gut microbes that usually present in herbivorous animals such as cattle and sheep. A study published in the Proceedings of the National Academies of Science states that “The Giant Panda genome codes for all necessary enzymes associated with a carnivorous digestive system but lacks genes for enzymes needed to digest cellulose, the principal component of their bamboo diet (Zhu et al, 2011). The gut microbes of pandas can digest small amount of fibre where 92 percent of cellulose will ends up eliminated in its faeces. Thus, if only 8 percent of ingested cellulose into short chain fatty acids, this probably means that the panda gets most of its energy from the digestible carbohydrate and protein provided by bamboo leaves and stalks, not from the fat itself.

3.0 Methodology

3.1 Study subjects and faecal sampling

The subject in this study project involves two adult animals, Fu Wa (Male, currently known as Xing Xing) and Feng Yi (Female, currently known as Liang Liang) Giant Pandas and their cub, Nuan (also known as Nuan Nuan) from the National Zoo of Malaysia, Kuala Lumpur. The adult Pandas were born in 2006 in China and being housed in Malaysia on loan for 10 years as a part of international agreement and kept in separate exhibits. Nuan, the Panda cub was born on September 2015. Their faecal samples are processed fresh and taken only once. Next, the samples were subjected to fatty acids determination.

All the Pandas were fed with 4 types of bamboos that were cultivated locally which are Buluh Galah (*Bambusa heterostachya*), Buluh Betung (*Dendrocalamus asper*), Buluh Botol (*Bambusa ventricosa*) and Buluh Siam (*Thyrsostachys siamensis*). Besides, they also are fed with carrots, apples, pears and special cake baked by the zookeeper. All bamboos are also subjected for fatty acid profile determination. This is crucial to compare the fatty acid changes in the diets and in the faeces, thus enable us to identify the utilization of the feed in conjunction with the carnivorous digestive system of Giant Panda. The bamboos were sampled on the day of harvest (Day 1), (Day 3) post harvest and then again (Day 7) post harvest. This is to visualize possible deterioration of fatty acid profiles with increasing storage time.

3.2 Fatty Acids Profile Determination

3.2.1 Total Lipid Extraction

Briefly, 9 gram of faeces is crushed and homogenised in 40 ml of chloroform:methanol (2:1 v/v) based on the method of Folch et al., (1957), modified by Rajion (1985) in a tube. The tubes were shaken vigorously before 6ml of fresh chloroform-methanol (2:1, v/v) and 3 ml of normal saline were added to facilitate phase separation. The aliquot was then vortexed at 2000 rpm for about 30 seconds using IKA MSI Shaker and centrifuged at 3000 rpm for 5 minutes to obtain 2 phases of liquid. The lower phase contained 86 parts chloroform: 14 parts methanol: 1 part water (Shahidi and Wanasundra, 1998) while the upper phase contained 3: 48: 47 parts of chloroform, methanol and water respectively. Next, the upper phase will be discarded while the lower phase liquid was collected in a round bottom flask and evaporated using rotary evaporation at 70°C for 5 minutes after transferring into the methylation tube.

3.2.2 Fatty Acid Methyl Esters (FAME) Preparation

Lastly, FAME test was conducted where the previously extracted free fatty acids will then be subjected to transmethylation to obtain their FAME using 20% methanolic boron trifluoride (BF₃). 100 µl of the internal standard, heneicosanoic (21: 0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to transmethylation. 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. Saponification process is done by adding 2ml of 0.66N of methanolic potassium hydroxide (KOH) to saponify the lipid sample.

The sample is then heated in a boiling water bath for 10 minutes with occasional shaking for every 5 minutes and after the mixture has cooled down, 2 ml of 20% methanolic boron trifluoride (BF₃) were added to initiate trans-esterification before it was heated again for 20 minutes in a boiling water bath (Rajion, 1985). Next, 4ml of petroleum ether and 4 ml of distilled water were added after cooling. The mixture is then vortexed at 200rpm for 30 seconds and centrifuged at 300 rpm for another 5 minutes to increase separation. Lastly, the upper petroleum phase was transferred to a 4ml screw-capped vial (Kimble Glass Inc., USA) using pasteur capillary pipettes, closed tightly and stored at 4°C. The final product of FAME will then be subjected to GLC.

3.2.3 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). 1 µl was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a FID detector. High purity hydrogen (Malaysian Oxygen Bhd., Malaysia) was the carrier gas at 40 ml/minute. 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 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/minute, and then held for 20 minute to facilitate optimum 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 % 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 detected fatty acids was obtained with a programmed PC under Microsoft Excel 2013 (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 related to nutritional intake.

All steps are then repeated using different sample which are 3 gram for Buluh Botol (*Bambusa ventricosa*) and Buluh Siam (*Thyrsostachys siamensis*), 10 gram for Buluh Galah (*Bambusa heterostachya*) and Buluh Betung (*Dendrocalamus asper*) while only 2 grams for the cake. It is important to ensure all apparatus are free from any form of lipids contamination. Before and after fatty acids extraction, all apparatus will be soaked in Decon 90 for 2 hours, scrubbed, and washed with tap water. These apparatus will then be soaked with distilled water overnight, rinsed, and oven dried at 60°C.

3.3 Data analysis

The fatty acid values were expressed both in absolute amount of each fatty acid and as a total fatty acid in unit of percentage (%). Datasets were analysed using IBM SPSS Version 22 (SPSS Inc, Chicago, IL, USA). Prior to that initial data tabulation was performed using Microsoft Excel 2013 (Microsoft Corp., Redmond, USA). All statistical procedures were conducted at 95 % confidence level.

4.0 RESULTS

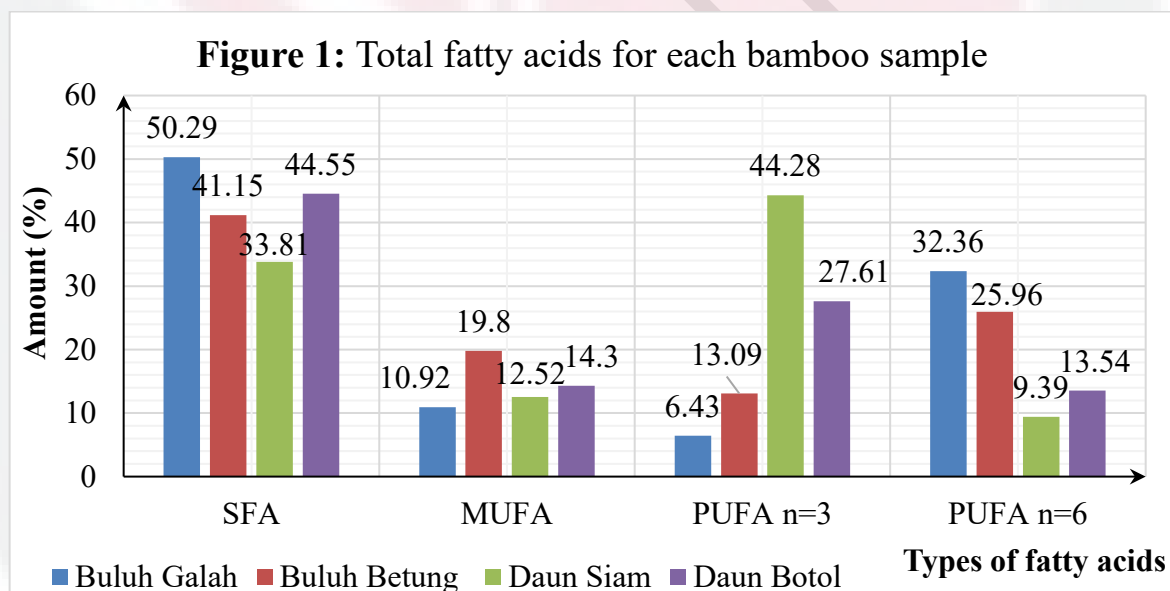
4.1 Fatty Acids from Different Types and Parts of Bamboos (Day 1)

Table 1: Fatty Acid Profiles from different types and parts of fresh bamboo sample

*All values were expressed in % of total fatty acids

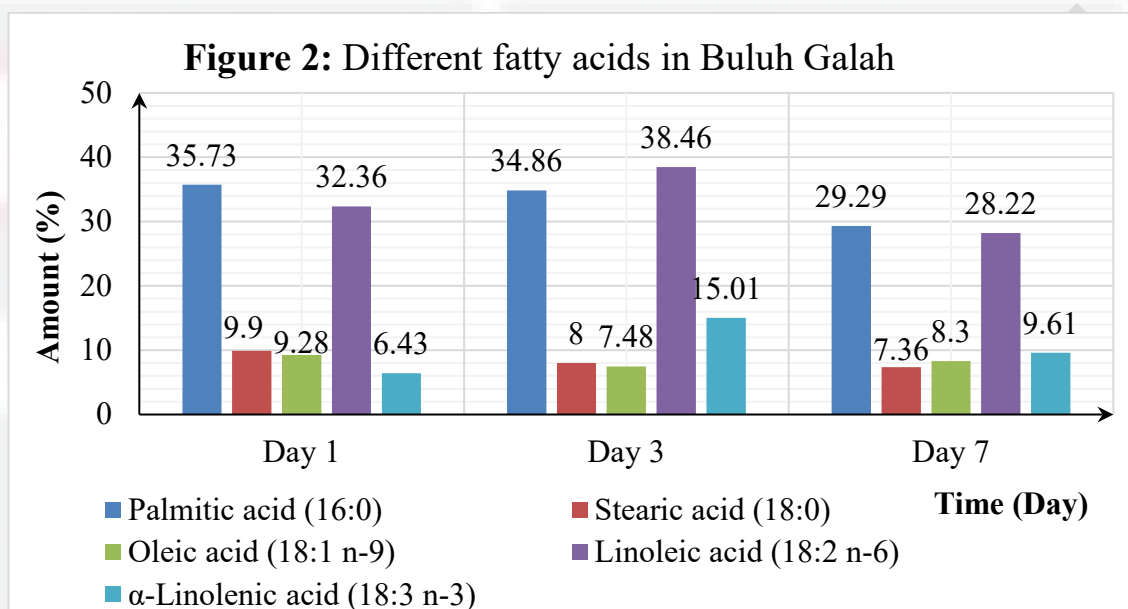
Fatty acids	Different types and parts of fresh bamboo sample			
	Buluh Galah (<i>B.heterostachya</i>)	Buluh Betung (<i>D. asper</i>)	Daun Siam (<i>T.siamensis</i>)	Daun Botol (<i>B. ventricosa</i>)
	MEAN ± STANDARD DEVIATION			
Myristic acid (14:0)	1.96 ± 0.13	1.88 ± 0.81	1.27 ± 0.17	1.83 ± 0.21
Palmitic acid (16:0)	29.29 ± 2.06	29.63 ± 0.36	18.93 ± 1.27	32.90 ± 0.50
Palmitoleic acid (16:1 n-7)	3.48 ± 0.87 ^a	1.04 ± 0.10 ^b	3.11 ± 0.17	3.41 ± 0.10
Stearic acid (18:0)	7.36 ± 0.37	6.23 ± 0.87	4.74 ± 0.81	8.10 ± 0.62
Oleic acid (18:1 n-9)	8.30 ± 2.36	12.82 ± 4.74	8.77 ± 1.60	10.27 ± 0.26
Linoleic acid (18:2 n-6)	38.46 ± 1.18	34.33 ± 2.40	8.11 ± 2.05	15.97 ± 0.27
α-Linolenic acid (18:3 n-3)	9.61 ± 0.55	12.73 ± 1.91	54.43 ± 3.94	23.87 ± 0.94

The fatty acids profiles from different part of bamboos are depicted in Table 1. The dominant fatty acids were palmitic acid (16:0), oleic acid (18:1 n-9) and linoleic acid (18:2 n-6) for both Buluh Galah and Buluh Betung while for Daun Siam and Daun Botol, the fatty acids which are in abundance were palmitic acid (16:0), α -linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6). When the fatty acids were evaluated individually, the result showed that the palmitic acid (16:0) was the highest in shoots while α -linolenic acid (18:3 n-3) was present in large amount in the leaves.

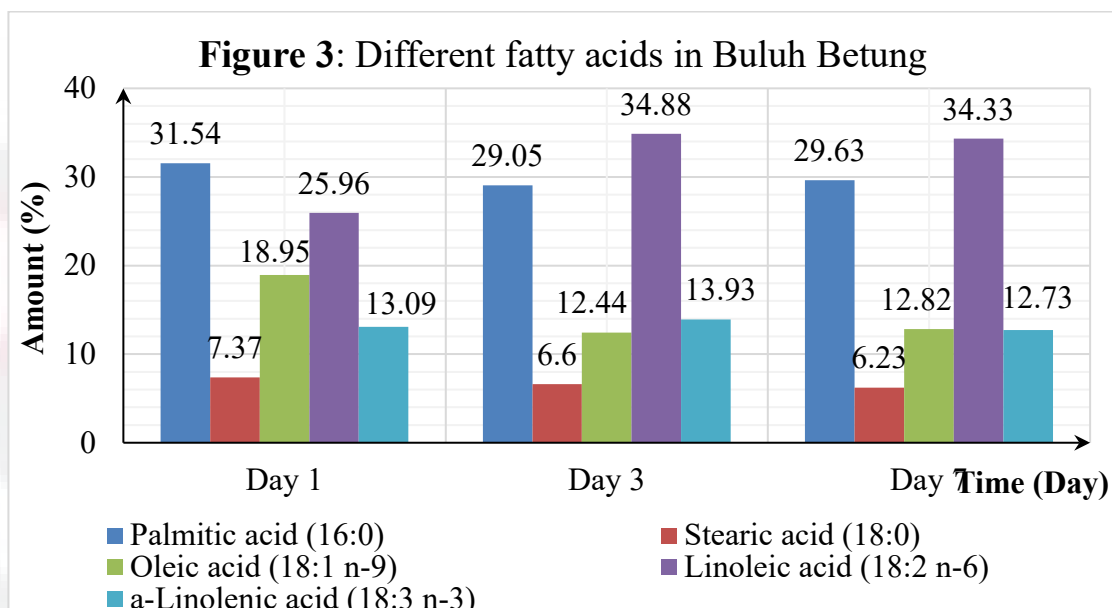


The percentage of TSFA was the highest in all four samples. High percentage of PUFA was due to high total amount of n-3 and n-6 that present in the diet consumed. Daun Siam has lowest amount of TSFA as it has lowest level of palmitic acid (16:0) compared to the other sample. The high percentage of MUFA in Buluh Betung was due to increase of oleic acid (18:1 n-7) in the diet. The information can be observed as illustrated in graph above.

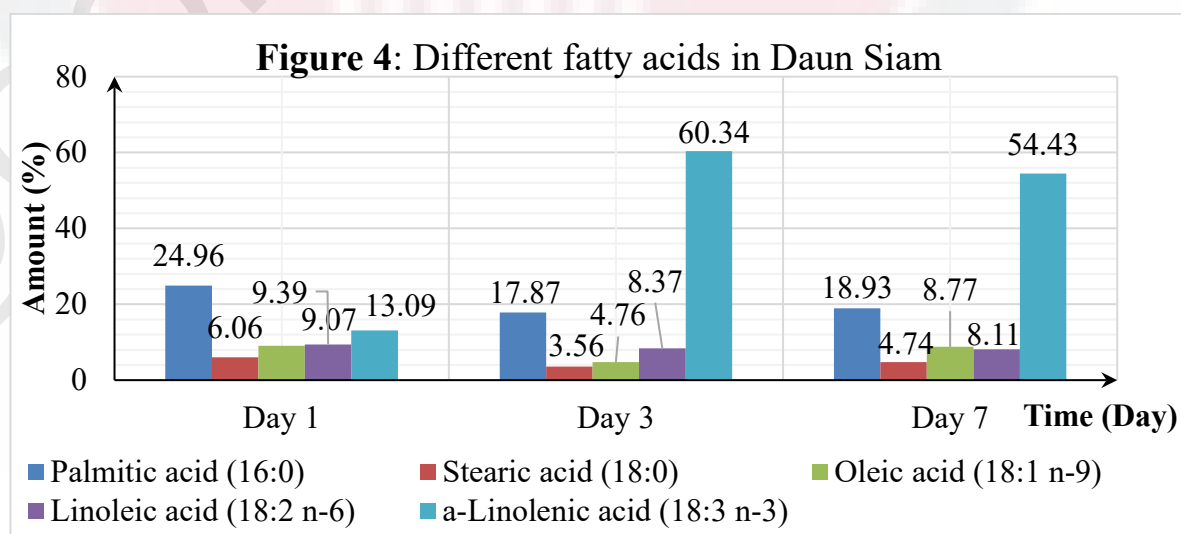
4.2 Trend of Changes of Fatty Acid Following Storage Time



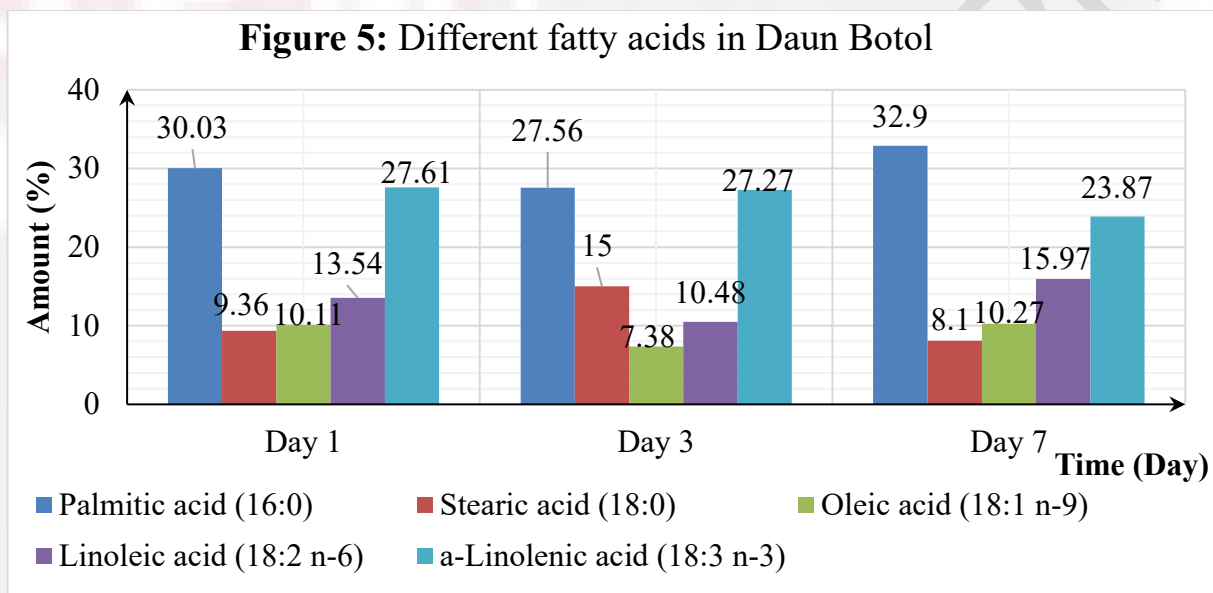
From the first graph that has been plotted, the highest amount of fatty acid recorded was palmitic acid (16:0) followed by linoleic acid (18:2 n-6), stearic acid (18:0), oleic acid (18:1 n-9) and α -linolenic acid (18:3 n-3) on day 1 for Buluh Galah before the amount declined accordingly on day 3 and 7, only for palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1 n-9). However, there is increase in linoleic acid (18:2 n-6) and α -linolenic acid (18:3 n-3) on day 3 before they started to decrease again on day 7.



On the 2nd graph, the highest amount of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1 n-9) is on day 1 for Buluh Betung before the amount declined on day 3 and day 7 but there is slight increase of oleic acid (18:1 n-9) on day 7. However, there is increase in linoleic acid (18:2 n-6) and α -linolenic acid (18:3 n-3) on day 3 after day 1.



Next for Daun Siam, the highest amount of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1 n-9) and linoleic acid (18:2 n-6) are on day 1 before the amount declined on day 3 but there is slight increase for all of them on day 7.



The highest amount of α -linolenic acid (18:3 n-3) is on day 1 before the amount decreased on day 3 and 7. While for palmitic acid (16:0), oleic acid (18:1 n-9) and linoleic acid (18:2 n-6), the highest amount are on day 7 with an increase in stearic acid (18:0) on day 3.

Table 2: Total amount of linoleic acid (18:2 n-6) and α -Linolenic acid (18:3 n-3)

Fatty acids	Buluh Galah	Buluh Betung	Daun Siam	Daun Botol
Linoleic acid (18:2 n-6) Day 1	32.36 \pm 3.35	25.96 \pm 8.66	9.39 \pm 1.02	13.54 \pm 1.72
α-Linolenic acid (18:3 n-3) Day 1	6.43 \pm 0.53	13.09 \pm 6.16	44.28 \pm 4.37	27.61 \pm 4.66
Linoleic acid (18:2 n-6) Day 3	34.88 \pm 1.64	28.22 \pm 7.86	8.37 \pm 0.15	10.48 \pm 0.70
α-Linolenic acid (18:3 n-3) Day 3	15.01 \pm 5.42	13.93 \pm 0.56	60.34 \pm 0.81a	27.27 \pm 2.45b
Linoleic acid (18:2 n-6) Day 7	38.46 \pm 1.18	34.33 \pm 2.40	8.11 \pm 2.05	15.97 \pm 0.27
α-Linolenic acid (18:3 n-3) Day 7	9.61 \pm 0.55	12.73 \pm 1.91	54.43 \pm 3.94	23.87 \pm 0.94

*All values were expressed in % of total fatty acids

Daun Siam has the highest amount of α -Linolenic acid (18:3 n-3) which is PUFA n-3 for all 3 days (D1:44.28 \pm 4.37, D3: 60.34 \pm 0.81, D7: 54.43 \pm 3.94) while Buluh Galah has the highest amount of Linoleic acid (32.36 \pm 3.35) which is PUFA n-6 for all 3 days (D1: 32.36 \pm 3.35, D3: 34.88 \pm 1.64, D7: 38.46 \pm 1.18) compared to other samples. However, there is significant difference in the amount of α -Linolenic acid (18:3 n-3) on day 3 between the leaves and it is higher in Daun Siam.

4.3 Fatty Acids of Faecal Sample from Different Giant Pandas

Table 3: Fatty Acids of Faecal Sample from Different Giant Pandas

Fatty Acids	Faeces sample		
	Fu Wa	Feng Yi	Nuan
	MEAN ± STANDARD DEVIATION		
Myristic acid(14:0)	3.32 ± 0.18a	0.79 ± 0.10b	1.02 ± 0.19b
Palmitic acid (16:0)	17.06 ± 1.20b	24.30 ± 0.01a	23.95 ± 0.82ab
Palmitoleic acid (16:1 n-7)	0.29 ± 0.00b	1.82 ± 0.00ab	1.99 ± 0.62a
Stearic acid (18:0)	5.84 ± 0.15	6.76 ± 1.20	9.34 ± 1.58
Oleic acid (18:1 n-9)	6.80 ± 4.54	5.49 ± 0.53	10.17 ± 0.38
Linoleic acid (18:2 n-6)	17.80 ± 0.38	17.06 ± 1.18	30.55 ± 7.82
α-Linolenic acid (18:3 n-3)	29.12 ± 5.36ab	37.10 ± 3.38a	13.72 ± 1.96b
Arachidonic acid (20:4 n-6)	5.31 ± 0.24a	2.44 ± 0.82b	1.60 ± 0.11 b
Eicosopentaenoic acid (20:5 n-3)	0.50 ± 0.21	0.99 ± 0.24	0.99 ± 0.04
Docosapentaenoic acid (22:5 n-3)	5.26 ± 0.37a	1.58 ± 0.62b	1.20 ± 0.59b
Docosahexanoic acid (22:6 n-3)	6.73 ± 0.62a	1.45 ± 0.24b	3.10 ± 0.96b

*All values were expressed in % of total fatty acids

From the table 3 below, Feng Yi utilize shorter fatty acid chain such as myristic acid (14:0), oleic acid (18:1 n-9) and linoleic acid (18:2 n-6) while Fu Wa is able to absorb more palmitic acid (16:0), palmitoleic acid (16:1 n-7), stearic acid (18:0) and α -Linoleic acid (18:3 n-3). However, only myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1 n-7) and α -Linoleic acid (18:3 n-3) showed significant difference between the two adult panda. Besides that, there was significant effect on amount of longer chain fatty acid which are α -Linoleic acid (18:3 n-3), arachidonic acid (20:4 n-6), docosahexanoic acid (22:6 n-3) and decosapentanoic/clupanodonic acid (22:5 n-3) between the individual panda.

5.0 DISCUSSION

5.1 Feed Fatty Acid Profile

In general, among the three main fatty acids in bamboos were palmitic, linoleic and α -linolenic acids, but the composition was remarkably different among these fractions (Kozukue and Kozukue, 1981). However, in this study showed that the dominant fatty acids were palmitic acids (16:0), oleic acid (18:1 n-9), stearic acid (20:0), linoleic acid (18:2 n-6) and α -linolenic acid (18:3 n-3) where palmitic acid (16:0) and α -linolenic acid (18:3 n-3) were the highest in both shoots as well as leaves, respectively. Shoots had higher amount of linoleic acid than α -linolenic acid compared to leaves.

This is because the bulky part contains higher concentration of linoleic acid, but limited amount of α -linolenic acid. Since the linoleic acid is the predominant PUFA n-6 in this case for both shoots but lowest among the adult panda, thus this can be explained by the dietary preference in which they prefer to eat stems during October to March. For shoots of 3-5 days, the level of fat will usually increase significantly compared to younger or juvenile one before it started to deteriorates for example that can be seen in *Dendrocalamus* family such as *D. asper* (Nirmala et al 2007).

5.2 Importance of n-3 and n-6 Fatty Acids Chain

EFA includes both α -linolenic acid, linoleic acid and other longer n-3 to n-6 fatty acids were vital for the optimal functioning of the mammalian body including inflammation and immune response regulation which is crucial for development of a good skin/fur health in this case. EFA is required in mammals as they lacked the $\Delta 15$ desaturase and $\Delta 12$ desaturase which is important for necessary n-3 and n-6 fatty acids synthesis (Innis, 1996). The elongation of α -linolenic acid and linoleic acid into extensive series of n-3 and n-6 fatty acids could be beneficial, however mammals are reported to have little ability to transform α -linolenic acid and linoleic acid to their longer chain derivatives (Roche, 1999). Therefore, supplementation of preformed decosahexanoic acid present in different parts of bamboos such as stem and shoots is required for the optimum growth of the Giant Panda.

All the bamboo species surveyed in this study, Buluh Betung, Daun Siam and Daun Botol have similar ratio n-6: n-3 for all 3 days while Buluh Galah had the highest recorded ratio. As for the ratio UFA: SFA, only Buluh Galah and Buluh Betung are consistent in value with Daun Siam as the highest ratio. High n-3 fatty acids could increase the bioavailability of n-3 PUFAs, thus changed the ratio of n-6/n-3 PUFAs and altered fatty acid distribution. If there is high ratio of n-6:n-3, the value of ratio UFA:SFA would be decreased as the n-3 PUFAs can significantly modify the distribution and bioavailability of fatty acids, and particularly, may block the absorption of SFAs in the gastrointestinal tract (Yang et al, 2017).

5.3 Changes of Fatty Acid Profiles with Time of Storage

Storage time was shown to have an effect on the bamboo's fatty acid profiles. There is a noted increase in fatty acid amount after day 3 (Figures 2 to 5) indicating the cell wall damage in the samples that could be responsible for the release of FA into the surroundings, thus increasing fatty acid concentrations. This is because in tissues, enzyme responsible for conversion and synthesis of particular fatty acid is being kept separately to its substrate.

However when the bamboos has been chopped off, the large surface area cause mixing up of the enzymes and substrate leading to break down of the cellular wall which increase the made fatty acids available thus explained why some of the fatty acids parameter in the results increase on day 3 of storage. Within 7 days, progressive degradation of the fatty acids will take place and the amount of the fatty acids profiles will declined as well. It is best to conclude that the best time to feed the Giant Panda for optimum fatty acid requirement is between day 1 to 3 after the bamboos has been chopped off.

5.4 Ability of Giant Panda to Fulfil their Physiological Needs for Long Fatty Acids Chain

It is interesting to note that the faecal samples showed evident presence of longer chain n-3 and n-6 fatty acids. There was significant amount of longer chain fatty acid that had its origin in the linoleic acid (18:2 n-6), such as arachidonic acid (20:4 n-6). Even the amount of linoleic acid (18:2 n-6) of both animals does not differ much. Since decosapentanoic/clupanodonic acid (22:5 n-3) was present as well, it indicated possible elongation from alpha-linolenic acid, an important precursor fatty acid for the n-3 family. Desaturation utilizes desaturases to synthesize unsaturated fatty acids from full-length saturated fatty acid substrates (Mansilla and Mendoza, 2005).

The process involved where all desaturases require oxygen to consume nicotinamide adenine dinucleotide (NADH) which is specific for the double bond that has been induced into the substrate as illustrated in figure 6 below. However, arachidonic acid (20:4 n-6) can also present from the sloughing off of the cell from gastrointestinal tract as it was a typical fatty acids of the cell membrane. Since the longer fatty acid chain was present in the faeces, even the Giant Panda do not have any intake of them in the diets and this proved that the Giant Panda do have the ability as well as specific enzymes to make the conversion to some extent.

Omega-3 fatty acids

Omega-6 fatty acids

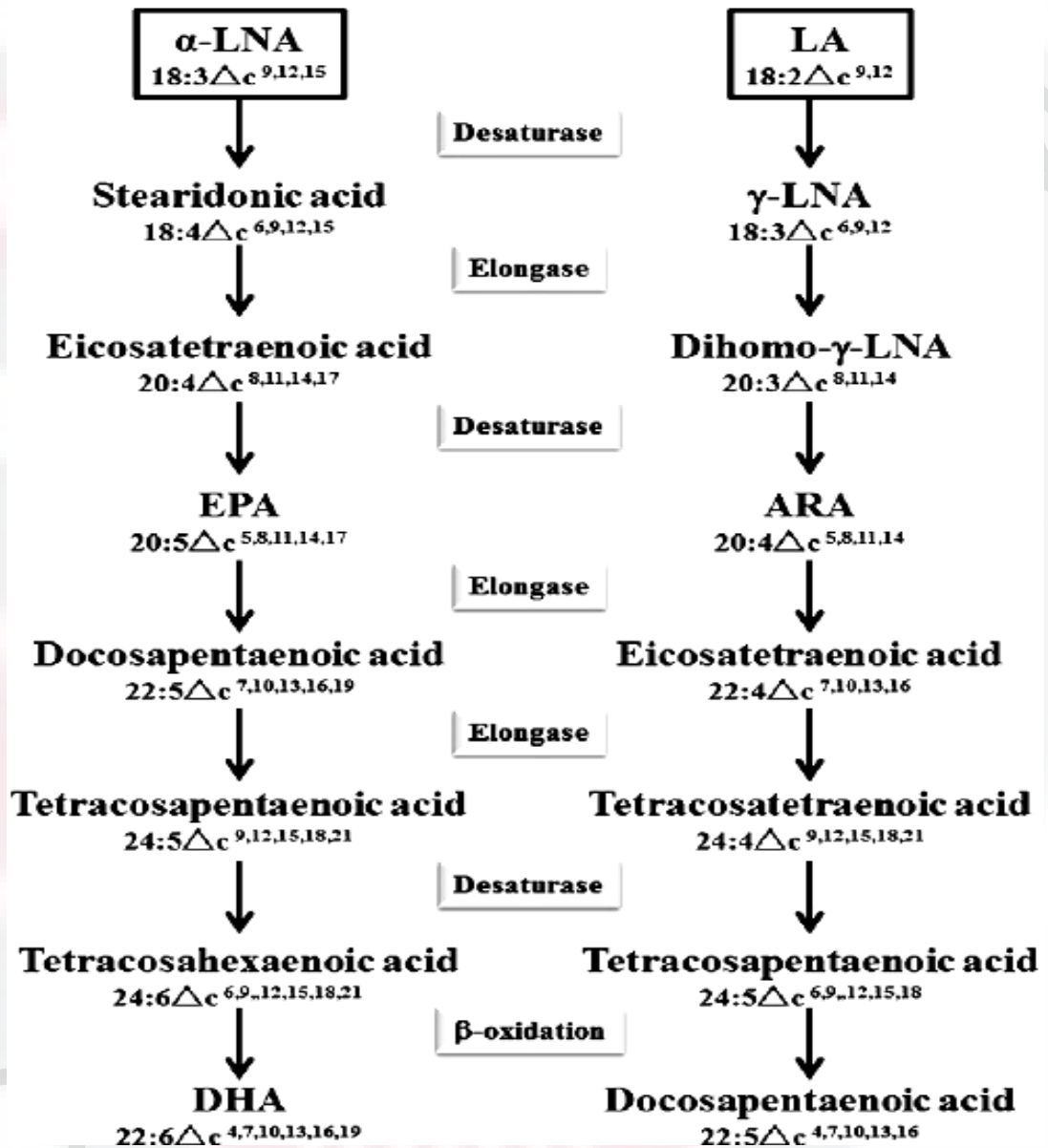


Figure 6: Enzymatic process of desaturation and elongation (Rustan and Dreven, 2005)

The difference in amount of fatty acids presence in each individual might be influenced by several factors such as gender, physiological state and age factor. For Nuan case, there are presences of longer chain fatty acid however it is lesser than Fu Wa and Feng Yi because she is still suckling and does not consume as much bamboos as her parents did. In comparable with other animals with carnivorous digestive system such as the feline family, it shown that cats fed with diets which is rich in linoleic acid appeared to be able to convert the linoleic acid into arachidonic acid. However, feline are not able to convert to a very long chain fatty acid (Sprecher, 2000).

Besides that, another study established that eicosopentaenoic acid (20:5 n-3) can be further converted into docosapentaenoic acid, clupanodonic acid (22:5 n-3) by canine after feeding with EFA although the amount of conversion appeared small (National Research Council, 2006). In short, additional questions may remain for case involving Giant Panda, such as whether the derived EPA might help modify the inflammatory response or benefits the skin and hair. Lastly, as for the total SFA and USFA with their ratios, there is no significant difference between the faecal sample of Fu Wa and Feng Yi and this may suggested that the utilization of fatty acid might be almost similar with the adult panda.

6.0 CONCLUSIONS

In conclusion, the most dominant fatty acid of bamboos plants are palmitic acid (16:0) in the shoots and α -linolenic acid (18:3 n-3) in the leaves. Since deterioration take place approaching Day 7 of storage, it is concluded that the maximum storage period for the bamboos is between Day 1 to Day 3 post harvest. Since bamboo lacked the longer chain essential fatty acids it is believed that the Giant Pandas were able to satisfy their physiological requirements through *de novo* synthesis. In short, this indicates that they have the ability to do the conversion at least to some extent, as well as the specific enzymes for the elongation and desaturation of fatty acids which is a feature found in most herbivorous and omnivorous animals.

7.0 RECOMMENDATIONS

Profiling of the fatty acid in this experiment might not been sufficient to establish a true fatty acid profile of *A. melanoleuca* because the sample size was small. However, in the future improvement in terms of larger sample size can be done to conclude the normal fatty acid composition. Since the study might have come to a conclusion that Giant Panda do have the ability to convert short fatty acid chain to a longer one, it is best to obtain blood and tissue samples from the animals to determine the actual n-3 and n-6 conversion ability among Giant Panda.

However, one year or more advance application for access to the animal should be done as Giant Panda in Zoo Negara was in high restricted security. Next, Giant Panda that are fed with different diets or supplementation can also be studied in order to study the relationship of diet consumed with the fatty acid profiles and in the same time enable us to determine the digestibility ability of the animals after being fed with different kind of feed. In short, further study of the diversity of lipid metabolism in Giant Panda is also crucial to develop a better understanding on the fluctuation of the fatty acid profiles.

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APPENDICES



Plate 1: Animal subject 1 (Fu Wa)



Plate 2: Animal subject 2 (Feng Yi)



Plate 3: Animal subject 3 (Nuan)



Plate 4: Buluh Galah (*Bambusa heterostachya*)



Plate 6: Buluh Botol (*Bambusa ventricosa*)



Plate 5: Buluh Betung (*Dendrocalamus asper*)



Plate 7: Buluh Siam (*Thyrsostachys siamensis*)