UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF TOTAL LIPID AND OMEGA 3 CONTENT IN SKIN AND FLESH OF CHICKEN THIGH FROM CHICKEN FED WITH EXTRUDED FLAXSEED

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LIST OF ABBREVIATIONS

Abbreviations	Full form
1. ALA	Alpha linolenic Acid
2. CVD	Cardiovascular Disease
3. DHA	Docosahexaenoic Acid
4. DPA	Docosapentaenoic Acid
5. EPA	Eicosapentaenoic Acid
6. FAME	Fatty Acid Methylation
7. GC	Gas Chromatography
8. n-3 PUFA	Omega-3 Polyunsaturated Fatty Acid
9. SFA	Saturated Fatty Acid
10. SRM 1546a	Standard Reference Material

ABSTRACT

DETERMINATION OF TOTAL LIPID AND OMEGA 3 CONTENT IN SKIN AND FLESH OF CHICKEN THIGH FROM CHICKEN FED WITH EXTRUDED FLAXSEED

Nabila Idzni Kamalrudin

Among the terrestrial animals, chicken has the potential for increasing the n-3 PUFA intake in humans as they can synthesize eicosapentaenoic (EPA) and docosahexaenoic (DHA) from alpha-linolenic acid (ALA). Many researchers found that feeding chicken with food that rich in sources of n-3 PUFA such as flaxseed is effective in producing high n-3 PUFA chicken meat depends on the dietary treatment duration. Thus, this study aimed to determine and compare the total lipid, EPA and DHA content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with extruded flaxseed on day 21st, 28th, 35th and 40th respectively. The total lipid was extracted using cold lipid extraction, while the EPA and DHA content were determined using acid- catalyzed fatty acid methylation method (FAME) and gas chromatography. The result showed that there was a significant difference ($p<0.05$) of total lipid and EPA content in the skin between the control and treatment group on day 28th, 35th and 40th. The EPA content in the skin was the highest and double up on day 40th. However, the total lipid content in the flesh remained the same after the dietary flaxseed treatment. There was also a significant difference ($p<0.05$) on day 28th and 40th for DHA content in the skin between the control and treatment group. However, the DHA content in both skin and flesh were decreasing in both control and treatment groups. In conclusion, the enrichment of chicken with high n-3 diet did not affect the total lipid content in the flesh. The longer the duration of flaxseed feeding, the more the increment of EPA can be seen in both skin and flesh. While for the DHA conversion was a tissue-specific type and this resulted in low DHA content in both skin and flesh.

ABSTRAK

PENENTUAN JUMLAH KANDUNGAN LEMAK DAN OMEGA-3 DALAM KULIT DAN DAGING PEHA AYAM SELEPAS DIBERI MAKAN *FLAXSEED*

Nabila Idzni Kamalrudin

Antara haiwan darat, ayam mempunyai potensi dalam meningkatkan pengambilan omega-3 dalam kalangan konsumer kerana ia dapat mensintesis asid lemak omega-3 seperti EPA dan DHA dari ALA. Ayam juga merupakan haiwan yang berpotensi tinggi untuk menjalani proses pengkayaan dengan omega-3. Hal ini kerana, ramai penyelidik mendapati bahawa pemberian makanan yang kaya dengan kandungan omega-3 seperti *flaxseed* kepada ayam berkesan dalam menghasilkan daging ayam yang tinggi kandungan omega-3 dan ianya bergantung pada tempoh rawatan diet tersebut. Oleh itu, kajian ini bertujuan untuk menentukan dan membandingkan jumlah kandungan lipid, EPA dan DHA dalam kulit dan daging ayam antara ayam yang diberi diet komersial dan ayam yang diberi diet *flaxseed* pada hari ke 21, 28, 35 dan 40. Lipid daripada sampel diekstrak dengan menggunakan kaedah extraksi lipid sejuk. Sementara untuk penentuan kandungan EPA dan DHA, kaedah metilasi asid lemak yang dikatilkan asid (FAME) dan gas kromatografi digunakan. Hasil kajian menunjukkan bahawa terdapat perbezaan yang signifikan ($p<0.05$) untuk jumlah kandungan lipid dan EPA dalam kulit ayam di antara kumpulan control dan rawatan pada hari ke 28, 35 dan 40. Kandungan EPA dalam kulit mempunyai nilai yang tertinggi dan meningkat dua kali ganda pada hari ke 40. Walaubagaimanapun, kandungan jumlah lipid dalam daging kekal sama antara kumpulan control dan rawatan. Untuk kandungan DHA, terdapat perbezaan yang signifikan ($p<0.05$) dalam kulit antara kumpulan control dan rawatan pada hari ke 28 dan 40. Kandungan DHA dalam kulit dan daging, kedua-duanya menurun antara kumpulan control dan rawatan setelah diberi diet *flaxseed*. Kesimpulannya, pengkayaan ayam dengan diet yang tinggi dengan omega-3 tidak mempengaruhi jumlah kandungan lipid dalam daging. Semakin lama tempoh masa pemberian diet *'flaxseed'*, semakin banyak kenaikan EPA boleh dilihat. Penurunan kandungan DHA setelah diberi diet *flaxseed* adalah kerana, proses penukaran DHA merupakan jenis tisu khusus.

CHAPTER 1

INTRODUCTION

1.1 Background

Gallus gallus domesticus or referred to as chickens were originated as junglefowl in Asia and were domesticated over 3000 years ago (Al-Nasser et al., 2007). Chicken consumption has been increasing from years to years due to consumer health awareness all around the world as chickens contain high nutritive values. Malaysia becomes self-sufficient in poultry meat production especially chicken and the overall production can meet the domestic demands in this country. Chicken is one of the staple food which provides a source of protein necessary for Malaysian diet (Samsuddin, Sharaai, & Ismail, 2015).

Another reason why chicken becomes a staple food among Malaysian because, in terms of economy, the price of chicken is lower than beef and pork. Besides, chicken prices have been more consistent compared to other poultry products (Samsuddin et al., 2015). In terms of nutrition, chicken meat contains higher protein, lower saturated fat, and cholesterol compared to other meat. However, commercial chicken meat contains low omega 3 long-chain polyunsaturated fatty acids (n-3 PUFA) (Bhalerao, Hegde, Katyare, & Kadam, 2014).

Omega-3 long-chain polyunsaturated fatty acid (n-3 FA), acids including eicosapentaenoic (EPA) docosahexaenoic (DHA), are the dietary fats that provide a lot of health benefits. They are incorporated in many parts of the body including cell membranes (Swanson, Block, & Mousa, 2012). They play a very significant role in the prevention and management of cardiovascular disease and other non-communicable diseases such as diabetes, hypertension, cancer and other inflammatory diseases (Gómez Candela, Bermejo López, & Loria Kohen, 2011). However, the conversion rate of ALA to EPA and DHA is low in the human as the omega 3 desaturase enzyme that functions as an activator for omega 3 production is the absence in the body (Burdge, 2006).

Adequate intake of n-3 PUFA has shown a lot of positive impact on human health. Increasing the consumption of fatty fish which is known as the main dietary source of n-3 PUFA is one of the approaches to increase n-3 PUFA dietary intake of an individual. Fatty fish such as wild salmon, anchovies and mackerel have a relatively large concentration of n-3 PUFA. (Zivkovic, Telis, & Hammock, 2011).

However, the fish consumption might be restricted due to several reasons such as consumer preferences, affordability and seasonal availability. Among the terrestrial animals, poultry like the chicken has the potential for increasing the n-3 PUFA intake in humans as they can synthesize long-chain n-3 fatty acids such as EPA and DHA from ALA (Mirshekar, Boldaji, Dastar, Yamchi, & Pashaei, 2015). Therefore, enrichment of poultry meat with n-3 sources could partially provide an alternative to increase n-3 consumption.

Chicken is the best candidate to undergo the enrichment process as researchers managed to prove that feeding chicken with food that rich in sources of n-3 PUFA such as flaxseed is effective in producing high n-3 PUFA chicken meat (Bhalerao et al., 2014). According to Anjum et al (2013) flaxseed is one of the most common meal supplemented diets for the enrichment of chicken meat due to its high content of a-linolenic acid (ALA) which is also known as n-3 PUFA. Besides, it also contains all essential amino acid of the protein and acts as an excellent source of fiber, lecithin, vitamins and mineral.

Flaxseed can be enriched to the chicken meat with different inclusion rate and feeding period. Zuidhof et al (2009) stated that the level of the long-chain n-3 PUFA eicosapentaenoic acid can be increased significantly in the chicken meat with increased level and duration of flaxseed feeding. Another study also reported that when the chicken is being fed with flaxseed for a longer duration, it will increase the ALA deposition in the meat itself and increase the conversion efficiency to EPA and DHA (Mirshekar et al., 2015). Thus, the purpose of this study is to determine and compare the n-3 PUFA in the skin and flesh of chicken enriched with 3% inclusion rate of extruded flaxseed on different feeding duration.

1.2 Problem Statement

World Health Organization, stated that cardiovascular disease (CVD) is the main cause of death globally. National Health Morbidity Survey (NHMS, 2015) reported the same findings, where cardiovascular disease is the main contributor to total morbidity and mortality in Malaysia. One of the ways to lower the prevalence of CVD is by encouraging people to consume food that rich in n-3 PUFA. Several studies support this statement, that consuming food that contains high in n-3 PUFA can reduce the risk of getting cardiovascular and other diseases (Zuidhof et al., 2009).

However, in Malaysia the consumption of n-3 PUFA is low. Based on a study conducted by Kock et al (2012), analysis of typical Malaysian diets based on a 7-day rotation menu had shown that the n-3 PUFA to be only 0.3% kcal. Besides, based on RNI 2017 report, the average intake of n-3 PUFA is also reported to remain low among Malaysians. Another study is conducted among Malaysian elderly also found the same finding, where more than 40% did not meet the RNI requirement (Rosney, Haron, Salleh, & Shahar, 2018). Based on all of these findings, it can be concluded that the average intake of n-3 among Malaysian is low.

Thus, one of the alternatives to counter this alarming scenario is by encouraging people to consume marine fish. Marine fish such as mackerel, salmon and cod are a well-known food that rich in n-3 PUFA. Instead of a good source of n-3 PUFA, fish is also one of the main sources of protein-based food. However, fish consumption can be limited to certain consumers. In developing countries such as Malaysia, factors such as increment in income and urbanization among populations do affect the intake of fish in the diet (Ahmad et al., 2016).

Compared to other types of food, fish may be considered expensive by consumers (Pieniak, Verbeke, Perez-Cueto, Brunsø, & De Henauw, 2008). A study conducted in Malaysia reported that those from poverty-low income households would spend less on fish products as it is no considered as basic necessities. They allocate more to other essential household items such as meat, clothing and medical expenses (Tan, Yen, & Hasan, 2015). Thus, an alternative to increase the consumption of omega-3 sources of food other than fish is needed.

Besides fish, chicken is also a main source of protein almost all around the world. Viewing this as a good alternative, many researchers had conducted a study on enrichment of poultry products with n-3 PUFA. A study conducted by Kartikasari et al (2012), stated that the enrichment of chicken with dietary n-3 PUFA increased the levels of EPA and DHA in the meat.

One of the reasons is because the presence of hepatic elongase and desaturase enzyme in the chicken which can produce n-3 (López-Ferrer, Baucells, Barroeta, Galobart, & Grashorn, 2001) and may increase the conversion efficiency from ALA to EPA and DHA (Baeza et al., 2013). Another study also obtained the same result where dietary replacement of sunflower oil with n-3 FA rich oil sources significantly improved the total n-3 PUFA in breast and thigh meat of a chicken (Kalakuntla et al., 2017). Plus, several studies have proved that feeding omega 3 PUFA enriched poultry feed, is an effective way of producing chicken meat with high omega 3 PUFA content. (Bhalerao et al., 2014).

Enrichment of chicken meat with n-3 PUFA is also an opportunity for the chicken production sector to add value to their product. Flaxseed that contains a high level of n-3 PUFA has gained popularity as a nutritional supplement, especially for the enrichment process. It contains a high proportion of PUFA such as ALA (about 55%) and linolenic acid (14%) (Bekhit et al., 2018). However, it was reported that the price of flaxseed is approximate twice the price of wheat and corn (Zuidhof et al., 2009). This may lead to an increase in the cost of production and the price of chicken might be more expensive compared to the commercial chicken in the market (Kanakri, Carragher, Hughes, Muhlhausler, & Gibson, 2017). Thus, this study is being conducted to determine the optimal feeding duration of flaxseed for the enrichment procedure.

1.3 Significance of the study

This study could provide several benefits in many aspects. Firstly, the findings of this study highlight the n-3 PUFA content in the chicken meat after feeding with flaxseed enriched with 3% inclusion rate and different feeding duration. Also, from this study, it will provide the total lipid, EPA and DHA content in the enriched n-3 PUFA chicken meat and a comparison can be made with non-enriched n-3 PUFA chicken. Thus, it is beneficial for the community whom concern about healthy food and essential nutrients especially n-3 PUFA containing in the end-products after food undergoing the enrichment process.

Besides, the findings of this study also can be used as baseline data in order to introduce new products to the consumers. In order to introduce this new product to the Malaysian, it is important to have a clear database on the nutritional composition of the products as this will help the consumer to choose a healthier choice. By having this study, enriched n-3 PUFA chicken can be introduced to the Malaysian population as an alternative food that can substitute fish in terms of increasing n-3 PUFA intake. Consumers will have more options in choosing a healthier product as their cooking ingredient. As a result, the prevalence of cardiovascular disease (CVD) in Malaysia can be reduced and improved their quality of life.

Furthermore, there were limited studies about the enrichment of chicken meat with n-3 PUFA in Malaysia. Also, limited studies were available about the optimal inclusion rate and feeding duration of n-3 PUFA. Thus, through this finding, it can be used by the chicken production sector or company to produce high quality and healthy poultry products in the food industry. Besides, this finding also provides an optimal feeding duration for the enrichment procedure that can help in reducing the cost production of the sector during the enrichment procedure. This may help the company to plan its finances wisely and produce high-quality end-products at a reasonable price. Lastly, enriched n-3 PUFA chicken has been suggested by many researchers as it provides a lot of benefits in terms of their nutritional values.

In a conclusion, this study was beneficial to individuals, governments, agencies, non-governmental agencies, and the food industries. Malaysia becomes self-sufficient in production of poultry meat, which consists of chicken, ducks and quails. Plus, the overall production can meet the domestic demand in Malaysia. Furthermore, chicken meat is vital in Malaysia since protein-based food is needed in a human’s diet and the meat is acceptable by Malaysians socially and religiously. Thus, this study can help many agencies, especially the chicken production sector in order to fulfill their customer needs that demanding for healthy and nutritious poultry products.

1.4 Objectives

1.4.1 General Objective

To study the total lipid content and omega-3 content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with extruded flaxseed on different days.

1.4.2 Specific Objective

i. To determine and compare the total lipid content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with extruded flaxseed on day 21^{st}, 28^{th}, 35^{th} and 40^{th} respectively.
ii. To determine and compare the EPA content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with extruded flaxseed on day 21^{st}, 28^{th}, 35^{th} and 40^{th} respectively.
iii. To determine and compare the DHA content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with extruded flaxseed on day 21^{st}, 28^{th}, 35^{th} and 40^{th} respectively.

1.5 Null hypothesis

There is no significant difference of total lipid, EPA and DHA content in skin and flesh of chicken thigh between chicken fed with common feed and feed enriched with 3% inclusion rate extruded flaxseed on day 21^{st}, 28^{th}, 35^{th} and 40^{th} respectively.

CHAPTER 2

LITERATURE REVIEW

2.1 Chicken

2.1.1 Overview of chicken

The current poultry which includes birds such as chicken, turkey, duck, goose, ostrich, quail, pheasant, guinea fowl and peafowl are a domesticated fowl used for both meat and egg production. Chickens became the most popular poultry worldwide regardless of culture and religion (Al-Nasser et al., 2007). Among western countries, the highest consumption rates were recorded where US ranking first followed by European Union ranking fifth from 2004 to 2006. During 2006, US was the largest consumer of poultry meat per capita (around 54kg/year) in the world (Magdelaine, Spiess, & Valceschini, 2008). In Malaysia, chicken production industries had rapidly increased due to the increase in demand for consumption. Malaysia also has become self- sufficient in chicken meat production as it is able to meet the domestic demand from the consumers (Samsuddin et al., 2015).

2.1.2 Chicken as a functional food

Food that consist of active components which can exert a positive effect on physiological processes in the organism and provide beneficial effects on human health can be referred as functional food.(Singer, 2000; Jimenez Colmenero et al., 2001; Narahari, 2003; Siro et al., 2008; Peric, Rodic, & Milosevic, 2011). In other words, it must have a higher content of certain substances such as vitamins, fatty acids, antioxidants, probiotics and prebiotics which can create a positive impact on consumers' health (Surai et al., 1995 Grashorn, 2005; Peric, Rodic, & Milosevic, 2011).

It should not be in a form of pill or capsule and must be in its original shape and form (Siro et al., 2008; Peric, Rodic, & Milosevic, 2011). Plus, the functional food was not allowed to be consume in a greater quantity or more than usual for beneficial purposes. Poultry meat is one of the animals that can be enriched with useful and beneficial substances. This is because the nutrients that consumed by the chicken daily can easily transfer into their products (Pritchard, 2003; Grashorn 2005, 2007; Peric, Rodic, & Milosevic, 2011).

2.1.3 Enriched chicken with n-3 PUFA

Enrichment of chicken with n-3 PUFA can improve its functional values towards human health. To be more specific, enhancing the functional value of chicken meat through dietary intervention appear to be more safe, efficient and does not create any considerable side effects. Chicken is widely consumed and its fat composition can be successfully enriched with PUFA n-3 by feeding the chicken special diet

Among the studies that had been conducted, several studies managed to show a significant increase in the level of n-3 PUFA in enriched chicken meat. A study from Zhuidof et al (2004) stated that levels of the long-chain n-3 fatty acid eicosapentaenoic (EPA) increased significantly in breast meat with increased level and duration of flaxseed feeding. Anjum et al (2013) also found the same findings where the level of ALA in thigh meat increased significantly when the chicken fed with extruded flaxseed.

Another study also showed that it is possible to increase the n-3 fatty acid content in chicken meat around 81mg of fatty acids per 100 g of meat by feeding the chicken with a diet that high in ALA (Kartikasari et al., 2012). Based on The Canadian Food Inspection Agency, food can be labeled as a source of n-3 PUFA, if it meets the threshold of 0.3g or more per 100g serving in a prepackaged meal (Zuidhof et al., 2009).

Each part of the chicken has different lipid and fatty acid composition, especially in thigh and breast. It was reported that in thigh meat, the predominant lipids are triglycerides compared to breast meat that contains more phospholipids (González-Esquerra & Leeson, 2001). Thus, the enrichment of breast meat with n-3 PUFA might be more difficult to achieve because the potential for the EPA and DHA to deposit is smaller (Betti, Perez, Zuidhof, & Renema, 2009). This was supported by a study from Mirshekar et al., (2015) which found that breast meat needs 17th days to reach the threshold level to be labeled as n-3 food sources. Compared to thigh meat which needs only 7th days of flaxseed oil consumption prior to slaughter to reach the standard of a threshold.

2.2 Omega 3-PUFA

2.2.1 Overview of Omega-3 PUFA

Omega-3 polyunsaturated fatty acid (n-3 PUFA) is an essential fatty acid that consists of EPA and DHA. They are not synthesized by the body and must be obtained through diet or supplementation (Leaf & Kang, 2001). EPA and DHA are abundance in marine animals and can be produced by water plants such as algae. However, it is quite challenging to obtain an adequate intake of EPA and DHA through diet alone. Alpha-linolenic acid (ALA) is a shorter chain n-3 and also the precursor for EPA and DHA is an important component of the diet as it is found in many land plants that are commonly eaten. However, it does not provide the same health benefits as EPA and DHA (Swanson et al., 2012).

Although it is possible for the body to use elongase and desaturase enzymes to convert ALA to EPA and DHA, several research suggests that only a small amount can be synthesized in the body from this process (Swanson et al., 2012). For example, a study suggested that only 2 to 10% of ALA is converted to EPA or DHA (Chiu et al., 2008), and other studies found even less (Goyens, Spilker, Zock, Katan, & Mensink, 2005) found an ALA conversion of 7% for EPA, but only 0.013% for DHA. Another study from Hussein et al (2005) found an ALA conversion of only 0.3% for EPA and <0.01% for DHA (Hussein et al., 2005)

2.2.2 Omega-3 and Cardiovascular disease

Omega 3 (n-3 PUFA) plays a significant role in the prevention and management of cardiovascular. The ingestion of food that rich in EPA helps to inhibit very-low-density lipoprotein (VLDL) formation. Plus, it has been shown to reduce total cholesterol and triglyceride concentration (De Caterina, Liao, & Libby, 2000).

Besides, n-3 PUFA has resulted in a favorable change in high-density lipoprotein (HDL) (Leaf & Kang, 2001). HDL has been found to increase in most patients using DHA supplementation (Jacobson, Glickstein, Rowe, & Soni, 2012). Some studies have reported that n-3 supplements help in improving the flow-mediated arterial dilation and the mechanical function of the heart.

2.2.3 Omega-3 and Inflammatory disease

Omega-3 also plays a role during the anti-inflammatory process in the human body for the prevention of other non-communicable diseases such as diabetes, hypertension and cancer. It exerts anti-inflammatory characteristics through different mechanisms such as suppressing the production of interleukin-2 and inhibit lipopolysaccharide-induced inflammation (Mozaffarian & Wu, 2011). They also bind to specific nuclear receptors and transcription factors that regulate gene expression such as PPAR-α, HNF4α and SREBP-1c (Mozaffarian & Wu, 2011). The rapid process of transcription can directly impact the inflammatory pathways. Besides, n-3 also modifies the production of eicosanoids which can help in reducing the inflammation. For instance, it helps in reducing the levels of thromboxane A2 and leukotriene B4, lead to reduced inflammation (Mozaffarian & Wu, 2011).

2.2.4 Recommended intake

Based on Recommended Nutrient Intake (2017), Malaysian should consume 3% to 7% kcal of omega-6 PUFA (LA), while omega-3 PUFA at 0.3 to 1.2% kcal. This range of omega-3 FA intakes is recommended with due consideration to the present omega-3 content of habitual Malaysian diets and the practicability of increasing the intake which requires substantial changes in dietary habits. Three ways have been recommended to increase the n-3 intake, firstly eat more pulses, tofu and fish. Secondly, use cooking oil that combined palm olein with an n-3 rich vegetable oil such as canola and soybean. Last but not least, include the n-3 rich source of food in the household food basket.

2.3 Meal supplemented diet

2.3.1 Flaxseed

The production of flaxseed or linseed production was approximately 2.9 million metric tons during 2016 and this number is expected to increase annually based on FAO (2017). This increment is expected due to the whole world has become more aware about the health benefits of flaxseed and flaxseed oil consumption which also lead to the increase of global production of this oil-seed (Avazkhanloo, Shahir, Khalaji, & Jafari Anarkooli, 2019).

Flaxseed is one of the most common supplemented meal diets for enriching chicken meat because of its high content of ALA (Anjum et al., 2013). Another study by Apperson and Cherian (2017) also reported that flaxseed is a rich source of ALA, which has beneficial effects in increasing n-3 PUFA in chicken body tissues. Besides, other studies also reported that enrichment of flaxseed in daily diets of chicken had shown positive effects on controlling human cardiovascular diseases (Lewinska, Zebrowski, Duda, Gorka, & Wnuk, 2015).

Contradictory, flaxseed may contain anti-nutrients such as non-starch polysaccharides, mucilage, linattine, cyanogenic glycosides, trypsin inhibitors and phytic acid. All of these nutrients can adversely affect the health of gastrointestinal health and nutrient utilization in broiler chickens (Madhusudhan et al., 1986; Bhatty, 1993; Alzueta et al., 2003; Apperson & Cherian, 2017). Another study also reported that the inclusion of flaxseed in broiler diets could increase the viscosity of ileal digesta, decrease the digestibility of nutrients and increase the growth of broiler chickens at the early stage of life (Alzueta et al., 2003).

Rodríguez et al (2001) also mentioned that water-soluble non-starch polysaccharides are the main component responsible for flaxseed antinutritional effects in broiler chickens. Heat processing, such as autoclaving, have a major effect on the structural arrangement of lipid, carbohydrate and protein in flaxseed (Yu & Damiran, 2011). Therefore, the application of appropriate, innovative and value-capable processing technology plays a significant role in decreasing the anti-nutritional compounds and changing the molecular structure for broilers in flaxseed-based diets.

Extruded flaxseed is the term used for flaxseed after it undergoes an extrusion and expansion process. These processes are hydro-thermal processing technology using a combination of moist heat and shear treatment concurrently (Eastman et al., 2001; Avazkhanloo, Shahir, Khalaji, & Jafari Anarkooli, 2019). A study reported that these two processes (Singh, Gamlath, & Wakeling, 2007) could cause structural change when the raw ingredients or complete feed were exposed to shear and temperatures between 100-180 (Guy, 2001; Aslaksen et al., 2006; Day and Swanson, 2013; Beck, Knoerzer, & Arcot, 2017).

Thus, this may lead to reduction in digestive viscosity Beck et al (2017) and an increase in nutrient solubility and digestibility (Alonso, Orúe, Zabalza, Grant, & Marzo, 2000). Besides, the secondary structure of protein, lipid and carbohydrate can also be changed through these processes, especially in complete rations containing a variety of non-starch polysaccharides. (Guerrero, Kerry, & De La Caba, 2014).

2.3.2 Fish oil

Supplementation of chicken diet with fish oil is also one of the possible ways to increase the level of n-3 PUFA in the meat (Kartikasari et al., 2012). The level of n-3 PUFA increases significantly in chicken meat fed with fish oil (Schreiner, Hulan, Razzazi-Fazeli, Böhm, & Moreira, 2005). Besides, a study from Chekani-Azar, Shahriar, Maheri-Sis, Ahmadzadeh, & Vahdatpoor, (2008) also found that the level of the PUFA content increased with increasing level of fish oil in the diet. High PUFA content indicated the increased levels of n-3 and n-6 precursors in the chicken tissues.

In addition, the level of saturated fatty acid (SFA) also reported slightly lower in chicken meat after fish oil is added to their diet (López-Ferrer et al., 2001). However, the supplementation of fish oil in the chicken diet may result in negative effects on the sensory properties of the meat. It has been reported that chicken fed with a longer period of fish oil has the poorest sensory quality scores (López-Ferrer, Baucells, Barroeta, & Grashorn, 1999). Another study also reported that the use of fish oil resulted in a negative impact on the sensory quality of the meat (Schreiner et al., 2005).

2.4 Total lipid and fatty acid content analysis

2.4.1 Lipid

Lipids are consisting of biomolecules of carbon and hydrogen which are nonpolar solvents that soluble in organic but water-insoluble form. The increasing interest in the extraction and analysis is due to the established relationship between many lipid components and various health effects, as well as being a significant source of energy and supplying vital nutrients for the body. There are several type of lipid extraction and fatty acid analysis procedure (Señoráns & Luna, 2012).

2.4.2 Soxhlet method

Soxhlet method is a conventional lipid extraction technique. A sample is dried in this process, ground into tiny particles and put in a porous thimble. The thimble is put in an extraction chamber designed to monitor the flow of solvents using a siphon. As the solvent moves through the sample, the lipids are extracted and transferred into the flask. By the end of the extraction phase, which would take around 6-24 hours, the solvent- and lipid-containing flask is extracted, the solvent is evaporated and the residual lipid mass is calculated and analyzed,

Due to its simple and unattended use, this method is one of the most commonly used techniques. However, this method also has disadvantages such as the use of flammable organic liquid solvents, the possibility of emitting potential toxicity during extraction, the high cost and purity of solvents required, the laborious procedure and the time-consuming procedure. (Señoráns & Luna, 2012).

2.4.3 Folch method

This method is considered the most reliable and traditional means for quantitatively extracting lipids from various animal tissues. The method consists of homogenizing the tissue with 2:1 chloroform: methanol mixture and washing the extract by adding 0.2 times its volume of either water or an appropriate salt solution. The resulting mixture is divide into two phases and the lower phase is the total pure lipid extract (Folch, Lees, & Sloane Stanley, 1987).

2.4.4 Bligh and Dyer Method

This method can be considered as a alteration of the Folch method. The method used by Bilgh and Dyer's is a mixture of chloroform–methanol–water mixture for total extraction of lipids from the fish muscle. A reduction in solvent/

sample ratio (1part sample to 3 parts 1:2 chloroform/methanol followed by 1 or 2 parts chloroform) is one of the advantages of this method. In contrast, the Folch method employs a ratio of 1-part sample to 20 parts 2:1 chloroform/methanol, followed by several steps of the crude extract washing. Despite this reduction in solvents, the Bligh and Dyer method is still thought to yield >95% of total lipids recovery.

Señoráns & Luna (2012) states that, although the procedure was developed using cod muscle, it can be applied to any tissue containing 80% water. In recent years, these traditional methods have been replaced with new procedures which use modern extraction techniques such as microwave-assisted extraction, accelerated solvent extraction or supercritical fluid extraction with general advantages such as less extraction times, less use of solvents and greater reliability (Señoráns & Luna, 2012).

2.4.5 Acid-catalyzed fatty acid methylation method (FAME)

In order to analyze fatty acid, it needs to be in fatty acid methyl esters form before injected into gas chromatography (GC). This is because if in their free, underivatized from, fatty acids may be difficult to analyze due to these highly polar compounds tend to form hydrogen bonds and may lead to adsorption issues.

Thus, reduce the fatty acid polarity will make the analysis process more amenable. In addition, the polar carboxyl functional groups must be first neutralized to distinguish between the very slight differences exhibited by unsaturated fatty acids. This then will allow the column in GC to perform separation by the degree of unsaturation, position of unsaturation and the cis and trans configuration of unsaturation (Buchanan, Stenerson, & Sidisky, 2011).

2.4.6 Gas chromatography

Fatty acid methyl esters are then isolated and identified using an Agilent Technologies 7890A GC System (Agilent Technologies; Santa Clara, CA, USA) equipped with an Omegawax 250 capillary column (60 m × 0.25 mm internal diameter, 0.25 μm film thickness, Supelco, Bellefonte, PA, USA), a flame ionization detector (FID), an Agilent Technologies 7693 autosampler, and a split injection system (split ratio 50: 1). The injection volume is 1μl, the injector and detector temperature are 300 °C and 270 °C, respectively. The temperature program is 50 to 190 at 20 °C min-1, then from 190 to 250 at 4 °C min-1, and held at 250 °C for 8 min. The carrier gas is helium at 1.18 mL min-1, at a constant flow.

CHAPTER 3

METHODOLOGY

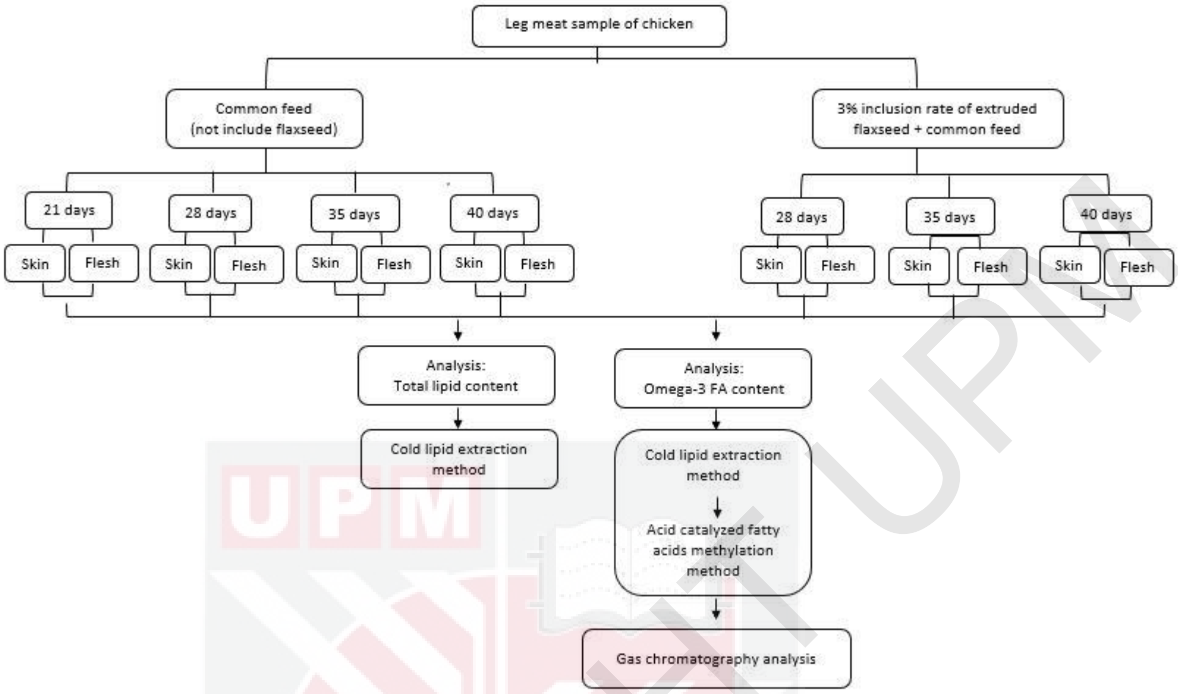
3.1 Sample selection and sampling method

Samples used for this study were obtained using purposive sampling. The company provided five pairs of thigh parts from five Cobb breed chickens for each day that consist of a control and treatment groups.

3.2 Reagents and chemicals

The determination of total lipid content, EPA and DHA in all samples used similar reagents. For the cold lipid extraction method, the reagents used throughout the experiment were dichloromethane-methanol 2:1 (v/v), butylated hydroxytoluene, potassium chloride (0.44% & 0.88%) and water- methanol. Next, for acid-catalyzed fatty acids methylation (FAME) method the reagents used were acetyl chloride, methanol, tricosanoic acid, potassium carbonate and hexane. Meanwhile, for gas chromatography, helium was used as the gas carrier.

3.3 Experimental design



The control group was fed with a commercial diet in the market while for the treatment group was fed with a commercial diet added with a 3% inclusion rate of extruded flaxseed. All of the chickens were slaughtered at a different time points (day 21st, 28th, 35th and 45th). For the control group, they provided the samples for day 21st, 28th, 35th and 40th. As for the treatment group they only provided the samples for day 28th, 35th and 40th.

As for the sample preparation, the chicken thigh was separated to the skin and flesh part. Each sample for each day was pulled from a total of five pairs of thigh parts from five chickens. All the samples were labeled by day and prepared as duplicate. Then, all the samples were kept in -20° freezer prior to the analysis day.

3.4 Determination of total lipid, EPA and DHA content

The total lipid content was extracted out using cold lipid extraction (Folch et al,1957) with a modification from Bligh and Dyer method. As for the determination of EPA and DHA content, acid-catalyzed fatty acids methylation (FAME) method (Christie, 2003) and gas chromatography (GC) were used. The cold lipid extraction method was used to extract the lipid from the skin and flesh sample.

First, approximately 1-2 grams of chicken sample is homogenized for 1 minute with an aliquot of dichloromethane-methanol (2:1 v/v) added with butylated hydroxytoluene (CH2CL2/MeOH (2:1) + BHT). Next, the mixture is filtered onto No 1 filter paper and the solid residue resuspended and homogenized with CH2CL2/MeOH (2:1) added with BHT. After 1 minute, this step was repeated twice and the combined filtrates were transferred to a separation funnel. 30ml of 0.44% potassium chloride (KCl) was added in water-methanol (3:1 v/v). The mixture is shaken thoroughly and allowed to settle for 4 hours or overnight.

After that, the upper layer was removed by aspiration and the bottom layer contains the purified lipid which can be recovered was transferred to a round bottom flask. Then, the solvent was evaporated using rota-evaporator and the purified lipid was make up until 10ml. From this 10ml extracted lipid, it will be divided into two parts. The first 6ml was used for the total lipid content quantification, while the remainder, 3ml was used for FAME to analyze EPA and DHA content

For total lipid quantification calculation:

Total lipid (g) = Weight of dried vial with extracted lipid -weight of dried vial

$$\text{Percentage of total lipid (\%)} = \frac{\text{Weight of extracted lipid (g)}}{\text{weight of sample}} \times 100\%$$

Next, before the samples were analyzed by gas chromatography, fatty acid needs to be transesterified to methyl ester. This can be achieved through acid-catalyzed fatty acids methylation method (FAME). The portion of lipid (3ml) for fatty acid analysis is transferred into a Pyrex Vial and Nitrogen was used to dry the lipid. 200ml of tricosanoic acid (C23:0) was added in toluene. The addition of tricosanoic C23:0 in the sample during this FAME method was intended to spike the EPA and DHA for gas chromatography analysis**.**

After that, 2ml of acetyl chloride/methanol (C2H3CIO/MeOH (1:10) and the mixture is shaken thoroughly. Left in the oven at 100C for 1 hour. Next, let the sample cooled down and 2 ml of K2CO3 was added. 3ml of n-Hexane and the mixture is shaken thoroughly using a vortex. The sample was being centrifuged for 3 minutes at 1000 rcf. The top layer was taken and inject in the GC for further analysis.

Fatty acid methyl esters are then isolated and identified using an Agilent Technologies 7890A GC System (Agilent Technologies; Santa Clara, CA, USA) equipped with an Omegawax 250 capillary column (60 m × 0.25 mm internal diameter, 0.25 μm film thickness, Supelco, Bellefonte, PA, USA), a flame ionization detector (FID), an Agilent Technologies 7693 autosampler, and a split injection system (split ratio 50: 1). The injection volume is 1µl, the injector and detector temperature are 300 °C and 270 °C, respectively. The temperature program is 50 to 190 at 20 °C min-1, then from 190 to 250 at 4 °C min-1, and held at 250 °C for 8 min. The carrier gas is helium at 1.18 mL min-1, at a constant flow.

Instead of the samples, standard reference material (SRM) SRM 1546a was also used in this analysis. This SRM is intended primarily for validation of methods for determining fatty acids, cholesterol, proximate, calories, elements, vitamins, and amino acids in canned meat products and similar materials. It also used for quality assurance when assigning values to in-house reference materials.

The meat homogenate (SRM 1546a) is a mixture of pork and chicken products blended together in a commercial process. Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids and Reference Mass Fraction Values for Fatty Acids were used as a reference value for total lipid, EPA and DHA content in the SRM homogenate meat. This homogenate meat was treated as the sample and undergo the same procedure that consists of cold lipid extraction method, FAME method and gas chromatography.

3.1 Statistical analysis

All data were analyzed by using IBM SPSS Statistical Software (Version 22). The results were presented in descriptive and analytical statistics. Descriptive statistic was used to analyze mean and standard deviation for total lipid, EPA and DHA content in both skin and flesh of chicken thigh fed with common feed and feed enriched with a 3% inclusion rate on day 21st, 28th, 35th and 40th. While for analytical statistics, ANOVA was used to compare the mean of total lipid, EPA and DHA content among all the samples. Tukey's post hoc test was used to know the significant different between all the samples. A p-value <0.05 and a 95% confidence interval were considered to indicate statistical significance.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Total lipid content analysis

Total lipid was determined by measuring the weight of the extracted lipid (6ml) from the sample before and after placed in the oven. Raw skin and flesh samples were used in this experiment. The total lipid content in the samples was expressed as g/100g and was converted to a percentage. For total lipid SRM 1546a, the value obtained is 12.25g/100g. It did not achieve the certified value, 18.96g/100g. This might due to the different methods used to extract out the lipid from the sample.

4.1.1 Total lipid in skin

In Table 1, the control group, there is a significant difference ($p<0.05$) in total lipid content on day 21st, 28th, 35th and 40th. Day 35th has the highest total lipid content (14.25%) while day 28th has the lowest (9.25%) compared with the other days. As for the treatment group, there is a significant difference ($p<0.05$) in total lipid content on day 28th. As day 28th (11.75%) has the lowest total lipid content while both day 35th and 40th (15.75%) have the highest total lipid content. As for the comparison between the control and treatment group, there is a significant difference ($p<0.05$) in total lipid content on day 28th, 35th and 40th.

Table 1: Total lipid content in the skin (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	12.25±0.35[a]	-
28	9.25±0.35[b]	11.75±0.35[a]*
35	14.25±0.35[c]	15.75±0.35[b]*
40	11.25±0.35[d]	15.75±0.35[b]*

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, $p<0.05$
(*) - comparison between control and treatment group, indicate sig. value

4.1.2 Total lipid in flesh

For the control group, there is no significant difference ($p>0.05$) in total lipid content on day 21st, 28th, 35th and 40th in Table 2. Day 28th and 35th, both have the highest total lipid content (2.75%) compared with the other days. As for treatment group, there is no significant difference in total lipid content on day 28th, 35th and 40th Day 40th has the lowest total lipid content (2.25%) compared with day 28th and 35th (2.75%). There is also no significant difference in total lipid content between the control and treatment group on day 28th, 35th and 40th.

The increment of total lipid content in the skin between the control group and the treatment group was due to the increasing age of the chicken. Older chicken (day 40th) has much higher body weight compared to young chicken (day 21st). Hence, chicken with a higher weight have higher percentage of skin and visible fat (Fereidoun et al., 2007). Enrichment of extruded flaxseed does not cause the increment of the total lipid (Kartikasari et al., 2012).

On the contrary, there are no changes or increments of total lipid content in flesh in all three days as the percentage of lipid remains the same. The findings from the current study are concur with the studies from Zelenka, Schneiderova, Mrkvicova, & Dolezal (2008), Kartikasari et al., (2012), Kanakri et al., (2017) which also found that there was no effect of increasing dietary ALA levels on the total lipid content in the thigh meat. This is because the effect of feeding chicken with flaxseed diet did not change the crude fat percentage in the meat, but only altered the fatty acid composition of the lipid fraction.

Table 2: Total lipid content in flesh (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	2.25±0.35[a]	-
28	2.75±0.35[a]	2.75±0.35[a]
35	2.75±0.35[a]	2.75±0.35[a]
40	2.25±0.35[a]	2.25±0.35[a]

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, $p<0.05$
(*) - comparison between control and treatment group, indicate sig. value $p<0.05$

4.2 EPA content analysis

The remainder of 3 ml lipid was used for EPA content determination. EPA content was determined by using the standard curve of 37 component FAME mix with the concentration ranging from 12.5ug/ml – 200ug/ml. From that, a linear calibration curve was plotted with the equation of y = 0.3855x - 0.485 (**R^2**= 0.998) as shown in Appendix I. The retention time for EPA is at the 25.5th minutes while for the internal standard (C23:0), it was detected at the 29.50th minutes. Due to the unavailable data on EPA certified value, eicosanoic acid (C20) value was used as the reference value for the EPA content of SRM 1546a. The value obtained is 0.1594g/100g and achieved the certified value, 0.0329g/100g.The EPA content in the samples was expressed as g/100g and was converted to a percentage.

4.2.1 EPA content in skin

Based on Table 3, there is a significant difference ($p<0.05$) in EPA content on day 21st, 28th, 35th and 40th in the control group. Day 35th has the highest EPA content with 0.67% while day 21st has the lowest (0.39%) compared with the other days. As for the treatment group, there is a significant different ($p<0.05$) in EPA content on day 28th and 40th. Day 40th has the highest EPA content compared with other days. There is a significant difference ($p<0.05$) in EPA content on day 28th, 35th and 40th between the control and treatment groups. The EPA content is the highest and double up on day 40th.

Table 3: EPA content in the skin (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	0.39 ± 0.07^{a}	-
28	$0.54\pm0.02^{b*}$	0.44 ± 0.07^{a}
35	$0.67\pm0.07^{c*}$	0.37 ± 0.07^{b}
40	0.45 ± 0.14^{d}	$0.86\pm0.14^{c*}$

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, $p<0.05$
(*) - comparison between control and treatment group, indicate sig. value $p<0.05$

4.2.2 EPA content in flesh

In Table 4, as for the control group, there is a significant difference ($p<0.05$) in EPA content on day 40th. Day 40th has the highest while day 28th has the lowest EPA content. On day 40th for the treatment group, there is also a significant difference ($p<0.05$) in EPA content. As day 40th has the highest EPA content. For comparison between treatment and control group, there is no significant difference ($p>0.05$) in EPA content on day 28th, 35th and 40th. However, EPA content slightly increases on day 28th and 40th.

From the findings, it can be summarized that the increments of EPA content in both skin and flesh were inconsistent among days 21st, 28th, 35th and 40th. In the skin, the EPA content between control and treatment group was increasing on day 40th. As for the flesh, the EPA content between control and treatment group increasing on day 28th and 40th.

The inconsistent EPA increment in the present study might be due to insufficient conversion of ALA into long-chain n-3 in broilers resulted in a slight increase of EPA (Mirshekar et al., 2015). In the current study, a 3% inclusion rate of extruded flaxseed is used to enrich the chicken. This rate is the same amount used by a study from (Kartikasari et al., 2012), however, instead of extruded flaxseed, they used flaxseed oil. From this study 3% flaxseed oil had been identified as the minimal sufficient level for the enrichment process and the optimal n-6: n-3 ratio can be achieved.

However, this finding contradicted with the present finding where little differences and increment in EPA content were found even after the dietary treatment. While in a previous study, they used a much higher inclusion rate of extruded flaxseed (5%, 10% and 15%) and managed to increase the level of ALA in the thigh meat (Anjum et al., 2013). Thus, it can be concluded that the higher the inclusion rate of extruded flaxseed fed to the chicken the more significant increment of ALA can be seen.

Besides, the competition that exists between n-6 and n-3 fatty acids for a delta-6-desaturase enzyme which is required for elongation of ALA to EPA might be one of the possible reasons to explain the decreasing trend of EPA content on day 28th and 35th in the skin while on day 35th in flesh respectively (Mirshekar et al., 2015). In contrast with the decreasing trend on day 28th and 35th, on day 40th the EPA content increases in the skin while minimally increase in the flesh. This is because the longer the duration of flaxseed consumption, the ALA content in the flaxseed increased the content of n-3 fatty acid in the thigh muscles (Mirshekar et al., 2015).

Table 4: EPA content in the flesh (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	2.16 ± 0.31^a	-
28	1.63 ± 0.23^a	2.97 ± 0.35^a
35	1.95 ± 0.23^a	1.88 ± 0.25^a
40	6.05 ± 0.88^b	6.81 ± 1.00^b

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, $p<0.05$
(*) - comparison between control and treatment group, indicate sig. value $p<0.05$

4.3 DHA content analysis

The remainder of 3 ml lipid was used for DHA content determination. DHA content was determined by using the standard curve of 37 component FAME mix with the concentration ranging from 12.5ug/ml – 200ug/ml. From that, a linear calibration curve was plotted with the equation of y = 0.3742x – 0.6358 ($\mathbf{R^2}$= 0.6358) as shown in Appendix I. The retention time for DHA is at the 29.4th minutes while for the internal standard (C23:0), it was detected at the 29.50th minutes. Due to the unavailable data on DHA certified value, ALA (C18:3) value was used as the reference for the DHA content of SRM 1546a. The value obtained is 0.012g/100g. However, it did not achieve the certified value, 0.133g/100g. This might due to the different gas chromatography settings and conditions used in this analysis. The EPA content in the samples was expressed as g/100g and was converted to a percentage.

4.3.1 DHA content in skin

For the control group, there is a significant difference ($p<0.05$) in DHA content on day 40th as shown in Table 5. Day 40th has the highest DHA content compared with other days. As for the treatment group, there is a significant difference ($p<0.05$) in DHA content on day 28th. For comparison between the control and treatment groups, there is a significant difference on day 28th and 40th. DHA content in all three days is decreasing between control and treatment group.

Table 5: DHA content in the skin (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	$0.16{\pm}0.07^{a}$	-
28	$0.17{\pm}0.07^{a}$*	$0.11{\pm}0.00^{a}$
35	$0.17{\pm}0.07^{a}$	$0.15{\pm}0.07^{b}$
40	$0.22{\pm}0.07^{b}$*	$0.16{\pm}0.07^{b}$

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, p<0.05
(*) - comparison between control and treatment group, indicate sig. value p<0.05

4.2.2 DHA content in the flesh

As shown in Table 6, for the control group, there is a significant difference (p<0.05) in DHA content on day 40th. Day 40th has the highest DHA content (0.98%) compared with other days. As for the treatment group, there is no significant difference (p>0.05) on day 28th, 35th and 40th. However, the DHA content is increasing in all three days. As for the comparison between control and treatment group, there was no significant difference on day 28th, 35th and 40th. The DHA content is decreasing between control and treatment group on all three days.

There is a decreasing trend of DHA content in both skin and flesh even after receiving dietary flaxseed treatment. Previous study from Zelenka et al., (2008) also found the same finding where the level of DHA content shows no significant different in the treatment group. This might due to the changes in n-3 PUFA as a result of dietary treatments that were somewhat tissue specific. Previous study reported that, the uptake of n-3 was selective according to the tissue. For instance, brain and liver have high affinity for DHA (Bézard, Blond, Bernard, & Clouet, 1994).

In thigh meat the greatest responder to dietary ALA was DPA (Kartikasari et al., 2012). Docosapentaenoic acid (DPA) is another long-chain omega-3 fatty acid and a metabolite of DHA can be formed through the internal metabolic pathways (Mohebi-Nejad & Bikdeli, 2014). Due to the same level of EPA and DHA, this might reflect the limited capacity for DPA to be converted to DHA as they need to compete for the same enzyme (Kartikasari et al., 2012). Thus, this resulted in less concentration of DHA in thigh meat even after enriched with 3% inclusion rate of extruded flaxseed.

Besides, it also depends on the capabilities of the chicken to synthesize DHA (Elongases, Gregory, Geier, Gibson, & James, 2013) Compared to EPA, the conversion rate for DHA is much lower due to the difference in the number of carbons they had (Cholewski, Tomczykowa, & Tomczyk, 2018). Lopez-Ferrer et al., (2001) stated that the ALA in flaxseed requires the process of elongation and desaturation in order to generate EPA and DHA. However, this process is limited in animals.

Instead of the plant-based oil, animal-based oil such as fish oil also can be good source of DHA (Lee, Whenham, & Bedford, 2019). Further conversion process is not needed, as EPA and DHA are naturally presence in the fish oil. This is because, fish usually consumed water plants such as microalgae that provide direct source of these essential fatty acid (Swanson et al., 2012). However, the enrichment of chicken with fish oil might affect the sensory taste and odour of the animal products due to the low oxidative stability (Schreiner et al., 2005).

Table 6: DHA content in the flesh (n=2)

	Mean ± SD (%)	
Day	Control	Treatment
21	0.63 ± 0.09^{ab}	-
28	0.54 ± 0.07^{a}	0.40 ± 0.05^{a}
35	0.69 ± 0.11^{ab}	0.59 ± 0.07^{a}
40	0.98 ± 0.18^{b}	0.60 ± 0.06^{a}

(a b c d) - comparison between days in a similar group
Means with different subscript indicate sig. value, $p<0.05$
(*) - comparison between control and treatment group, indicate sig. value $p<0.05$

CHAPTER 5

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Conclusion

It can be concluded that the enrichment of extruded flaxseed to the chicken does not affect the total lipid content in both skin and flesh. However, the increment of total lipid in the skin is due to the age and body weight factors. Older chicken has a higher body weight that resulted in a higher percentage of skin and fat. As for the EPA content, the increment trend was inconsistent among all four days. Low inclusion rate and competition between n-3 and n-6 for desaturase enzyme might be the possible reasons that lead to the decreasing trend. However, the EPA content increases significantly in the skin and slightly increase in the flesh on day 40th after the dietary treatment. This is because the longer the consumption of extruded flaxseed, the more increment of EPA can be seen. As for DHA content, in both skin and flesh, a decreasing trend can be seen. One of the possible reasons that resulted in this finding is the limited capacity for the DPA to be converted to DHA in the thigh meat. This is because in the conversion of DHA is somewhat tissue-specific and this led to a low concentration of DHA even after dietary treatment.

5.2 Limitations and recommendations

One of the limitations of this study is only two types of fatty acids were analyzed. Other types of fatty acids should be included in the analysis as the enrichment of n-3 PUFA might affect the composition of other fatty acids as well. Plus, the changes that occur in the whole lipid fraction can also be analyzed. Next, the present study only uses one part which is thigh for the analysis. In the future, other parts such as the breast part should be analyzed together so that a comparison can be made as both thigh and breast might have different types and structures of lipid composition. Last but not least, for future recommendation, should include a varied amount of inclusion rates such as 1%, 5% and 10% so that a comparison can be made and the optimal inclusion rate can be determined. As these different inclusions may affect the increment of EPA and DHA in the chicken.

REFERENCES

Ahmad, N. I., Mahiyuddin, W. R. W., Mohamad, T. R. T., Ling, C. Y., Daud, S. F., Hussein, N. C., … Sulaiman, L. H. (2016). Fish consumption pattern among adults of different ethnics in Peninsular Malaysia. *Food and Nutrition Research*, *60*(August). https://doi.org/10.3402/fnr.v60.32697

Al-Nasser, A., Al-Khalaifa, H., Al-Saffar, A., Khalil, F., Al-Bahouh, M., Ragheb, G., … Mashaly, M. (2007). Overview of chicken taxonomy and domestication. *World's Poultry Science Journal*, *63*(2), 285–300. https://doi.org/10.1017/S004393390700147X

Alonso, R., Orúe, E., Zabalza, M. J., Grant, G., & Marzo, F. (2000). Effect of extrusion cooking on structure and functional properties of pea and kidney bean proteins. *Journal of the Science of Food and Agriculture*, *80*(3), 397–403. https://doi.org/10.1002/1097-0010(200002)80:3<397::AID-JSFA542>3.0.CO;2-3

Alzueta, C., Rodríguez, M. L., Cutuli, M. T., Rebolé, A., Ortiz, L. T., Centeno, C., & Treviño, J. (2003). Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilisation and intestinal microflora. *British Poultry Science*, *44*(1), 67–74. https://doi.org/10.1080/0007166031000085337

Anjum, F. M., Haider, M. F., Khan, M. I., Sohaib, M., & Arshad, M. S. (2013). Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. *Lipids in Health and Disease*, *12*(1), 1. https://doi.org/10.1186/1476-511X-12-13

Apperson, K. D., & Cherian, G. (2017). Effect of whole flax seed and carbohydrase enzymes on gastrointestinal morphology, muscle fatty acids, and production performance in broiler chickens. *Poultry Science*, *96*(5), 1228–1234.

https://doi.org/10.3382/ps/pew371

Avazkhanloo, M., Shahir, M. H., Khalaji, S., & Jafari Anarkooli, I. (2019). Flaxseed extrusion and expansion coupled with enzyme and pelleting changed protein and lipid molecular structure of flaxseed and improved digestive enzymes activity, intestinal morphology, breast muscle fatty acids and performance of broiler chickens. *Animal Feed Science and Technology*, (October), 114341. https://doi.org/10.1016/j.anifeedsci.2019.114341

Baeza, E., Chartrin, P., Gigaud, V., Tauty, S., Meteau, K., Lessire, M., & Berri, C. (2013). Effects of dietary enrichment with n-3 fatty acids on the quality of raw and processed breast meat of high and low growth rate chickens. *British Poultry Science*, *54*(2), 190–198. https://doi.org/10.1080/00071668.2013.775695

Beck, S. M., Knoerzer, K., & Arcot, J. (2017). Effect of low moisture extrusion on a pea protein isolate's expansion, solubility, molecular weight distribution and secondary structure as determined by Fourier Transform Infrared Spectroscopy (FTIR). *Journal of Food Engineering*, *214*, 166–174. https://doi.org/10.1016/j.jfoodeng.2017.06.037

Bekhit, A. E. D. A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Mohamed Ahmed, I. A., … Bekhit, A. A. (2018). Flaxseed: Composition, detoxification, utilization, and opportunities. *Biocatalysis and Agricultural Biotechnology*, *13*(December 2017), 129–152. https://doi.org/10.1016/j.bcab.2017.11.017

Betti, M., Perez, T. I., Zuidhof, M. J., & Renema, R. A. (2009). Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes. *Poultry Science*, *88*(8), 1740–1754. https://doi.org/10.3382/ps.2008-00449

Bézard, J., Blond, J. P., Bernard, A., & Clouet, P. (1994). The metabolism and availability of essential fatty acids in animal and human tissues. *Reproduction, Nutrition, Development*, *34*(6), 539–568. https://doi.org/10.1051/rnd:19940603

Bhalerao, S., Hegde, M., Katyare, S., & Kadam, S. (2014). Promotion of omega-3 chicken meat production: An Indian perspective. *World's Poultry Science Journal*, *70*(2), 365–374. https://doi.org/10.1017/S0043933914000373

Buchanan, M. D., Stenerson, K. K., & Sidisky, L. M. (2011). *SLB-IL111 for Fatty Acid Methyl Ester (FAME) Applications*. *6628*(800), 1–8. Retrieved from https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/General_Information/1/t411139.pdf

Burdge, G. C. (2006). Metabolism of α-linolenic acid in humans. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *75*(3), 161–168. https://doi.org/10.1016/j.plefa.2006.05.013

Chekani-Azar, S., Shahriar, H. A., Maheri-Sis, N., Ahmadzadeh, A. R., & Vahdatpoor, T. (2008). Omega-3 fatty acids enrichment and organoleptic characteristics of broiler meat. *Asian Journal of Animal and Veterinary Advances*, *3*(2), 62–69. https://doi.org/10.3923/ajava.2008.62.69

Chiu, C. C., Su, K. P., Cheng, T. C., Liu, H. C., Chang, C. J., Dewey, M. E., … Huang, S. Y. (2008). The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(6), 1538–1544. https://doi.org/10.1016/j.pnpbp.2008.05.015

Cholewski, M., Tomczykowa, M., & Tomczyk, M. (2018). A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids. In *Nutrients* (Vol. 10). https://doi.org/10.3390/nu10111662

De Caterina, R., Liao, J. K., & Libby, P. (2000). Fatty acid modulation of endothelial activation. *American Journal of Clinical Nutrition*, *71*(1 SUPPL.). https://doi.org/10.1093/ajcn/71.1.213s

Elongases, F. A., Gregory, M. K., Geier, M. S., Gibson, R. a, & James, M. J. (2013). *Functional Characterization of the Chicken*. 12–16. https://doi.org/10.3945/jn.112.170290.Materials

Fereidoun, H., Bahram, A., Soltanieh, K., Sadraddin, Abbass, A., & Pouria, H. (2007). Mean percentage of skin and visible fat in 10 chicken carcass weight. *International Journal of Poultry Science*, *6*(1), 43–47. https://doi.org/10.3923/ijps.2007.43.47

Folch, J., Lees, M., & Sloane Stanley, G. . (1987). *a Simple*. *55*(5), 999–1033.

Gómez Candela, C., Bermejo López, L. M., & Loria Kohen, V. (2011). Importancia del equilibrio del índice omega-6/omega-3 en el mantenimiento de un buen estado de salud. recomendaciones nutricionales. *Nutricion Hospitalaria*, *26*(2), 323–329. https://doi.org/10.3305/nh.2011.26.2.5117

González-Esquerra, R., & Leeson, S. (2001). Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. *Canadian Journal of Animal Science*, *81*(3), 295–305. https://doi.org/10.4141/A00-092

Goyens, P. L. L., Spilker, M. E., Zock, P. L., Katan, M. B., & Mensink, R. P. (2005). Compartmental modeling to quantify α-linolenic acid conversion after longer term intake of multiple tracer boluses. *Journal of Lipid Research*, *46*(7), 1474–1483. https://doi.org/10.1194/jlr.M400514-JLR200

Guerrero, P., Kerry, J. P., & De La Caba, K. (2014). FTIR characterization of protein-polysaccharide interactions in extruded blends. *Carbohydrate Polymers*, *111*, 598–605. https://doi.org/10.1016/j.carbpol.2014.05.005

Hussein, N., Ah-Sing, E., Wilkinson, P., Leach, C., Griffin, B. A., & Millward, D. J.

(2005). Long-chain conversion of [13C]linoleic acid and α-linolenic acid in response to marked changes in their dietary intake in men. *Journal of Lipid Research*, *46*(2), 269–280. https://doi.org/10.1194/jlr.M400225-JLR200

Jacobson, T. A., Glickstein, S. B., Rowe, J. D., & Soni, P. N. (2012). Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: A review. *Journal of Clinical Lipidology*, *6*(1), 5–18. https://doi.org/10.1016/j.jacl.2011.10.018

Kalakuntla, S., Nagireddy, N. K., Panda, A. K., Jatoth, N., Thirunahari, R., & Vangoor, R. R. (2017). Effect of dietary incorporation of n-3 polyunsaturated fatty acids rich oil sources on fatty acid profile, keeping quality and sensory attributes of broiler chicken meat. *Animal Nutrition*, *3*(4), 386–391. https://doi.org/10.1016/j.aninu.2017.08.001

Kanakri, K., Carragher, J., Hughes, R., Muhlhausler, B., & Gibson, R. (2017). A reduced cost strategy for enriching chicken meat with omega-3 long chain polyunsaturated fatty acids using dietary flaxseed oil. *British Poultry Science*, *58*(3), 283–289. https://doi.org/10.1080/00071668.2017.1293798

Kartikasari, L. R., Hughes, R. J., Geier, M. S., Makrides, M., & Gibson, R. A. (2012). Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *87*(4–5), 103–109. https://doi.org/10.1016/j.plefa.2012.07.005

Leaf, A., & Kang, J. X. (2001). Omega-3 fatty acids and cardiovascular disease. *World Review of Nutrition and Dietetics*, *89*, 161–172. https://doi.org/10.1159/000059783

Lee, S. A., Whenham, N., & Bedford, M. R. (2019). Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics. *Animal Nutrition*, *5*(1), 11–21. https://doi.org/10.1016/j.aninu.2018.09.001

Lewinska, A., Zebrowski, J., Duda, M., Gorka, A., & Wnuk, M. (2015). Fatty acid profile and biological activities of linseed and rapeseed oils. *Molecules*, *20*(12), 22872–22880. https://doi.org/10.3390/molecules201219887

López-Ferrer, S., Baucells, M. D., Barroeta, A. C., Galobart, J., & Grashorn, M. A. (2001). n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: Linseed oil. *Poultry Science*, *80*(6), 753–761. https://doi.org/10.1093/ps/80.6.753

López-Ferrer, S., Baucells, M. D., Barroeta, A. C., & Grashorn, M. A. (1999). n-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. *Poultry Science*, *78*(3), 356–365. https://doi.org/10.1093/ps/78.3.356

Magdelaine, P., Spiess, M. P., & Valceschini, E. (2008). Poultry meat consumption trends in Europe. *World's Poultry Science Journal*, *64*(1), 53–63. https://doi.org/10.1017/S0043933907001717

Mirshekar, R., Boldaji, F., Dastar, B., Yamchi, A., & Pashaei, S. (2015). Longer consumption of flaxseed oil enhances n-3 fatty acid content of chicken meat and expression of FADS2 gene. *European Journal of Lipid Science and Technology*, *117*(6), 810–819. https://doi.org/10.1002/ejlt.201300500

Mohebi-Nejad, A., & Bikdeli, B. (2014). Omega-3 supplements and cardiovascular diseases. *Tanaffos*, *13*(1), 6–14.

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, *58*(20), 2047–2067. https://doi.org/10.1016/j.jacc.2011.06.063

Peric, L., Rodic, V., & Milosevic, N. (2011). Production of poultry meat and eggs as functional food: Challenges and opportunities. *Biotechnology in Animal Husbandry*, *27*(3), 511–520. https://doi.org/10.2298/bah1103511p

Pieniak, Z., Verbeke, W., Perez-Cueto, F., Brunsø, K., & De Henauw, S. (2008). Fish consumption and its motives in households with versus without self-reported medical history of CVD: A consumer survey from five European countries. *BMC Public Health*, *8*, 1–14. https://doi.org/10.1186/1471-2458-8-306

Rodríguez, M. L., Alzueta, C., Rebolé, A., Ortiz, L. T., Centeno, C., & Trevino, J. (2001). Effect of inclusion level of linseed on the nutrient utilisation of diets for growing broiler chickens. *British Poultry Science*, *42*(3), 368–375. https://doi.org/10.1080/00071660120055359

Rosney, N., Haron, H., Salleh, M., & Shahar, S. (2018). Comparison of fatty acid profiles between successful and usual aging elderly. *Sains Malaysiana*, *47*(8), 1819–1826. https://doi.org/10.17576/jsm-2018-4708-21

Samsuddin, N. S., Sharaai, A. H., & Ismail, M. M. (2015). Sustainability of chicken meat production in achieving food security in Malaysia. *Advances in Environmental Biology*, *9*(23), 1–6.

Schreiner, M., Hulan, H. W., Razzazi-Fazeli, E., Böhm, J., & Moreira, R. G. (2005). Effect of different sources of dietary omega-3 fatty acids on general performance and fatty acid profiles of thigh, breast, liver and portal blood of broilers. *Journal of the Science of Food and Agriculture*, *85*(2), 219–226. https://doi.org/10.1002/jsfa.1948

Señoráns, F. J., & Luna, P. (2012). Sample preparation techniques for the determination of fats in food. *Comprehensive Sampling and Sample Preparation*, *4*, 203–211. https://doi.org/10.1016/B978-0-12-381373-2.00134-4

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: A review. *International Journal of Food Science and Technology*, *42*(8), 916–929. https://doi.org/10.1111/j.1365-2621.2006.01309.x

Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 Fatty Acids EPA and DHA : Health. *Journal of Advanced Nutrition*, *3*, 1–7. https://doi.org/10.3945/an.111.000893.Omega-3

Tan, A. K. G., Yen, S. T., & Hasan, A. R. (2015). At-Home Consumption of Fish Products in Malaysia: An Analysis of Household Expenditure Survey Data. *Marine Resource Economics*, *30*(4), 417–433. https://doi.org/10.1086/682214

Yu, P., & Damiran, D. (2011). Heat-induced changes to lipid molecular structure in Vimy flaxseed: Spectral intensity and molecular clustering. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *79*(1), 51–59. https://doi.org/10.1016/j.saa.2011.01.051

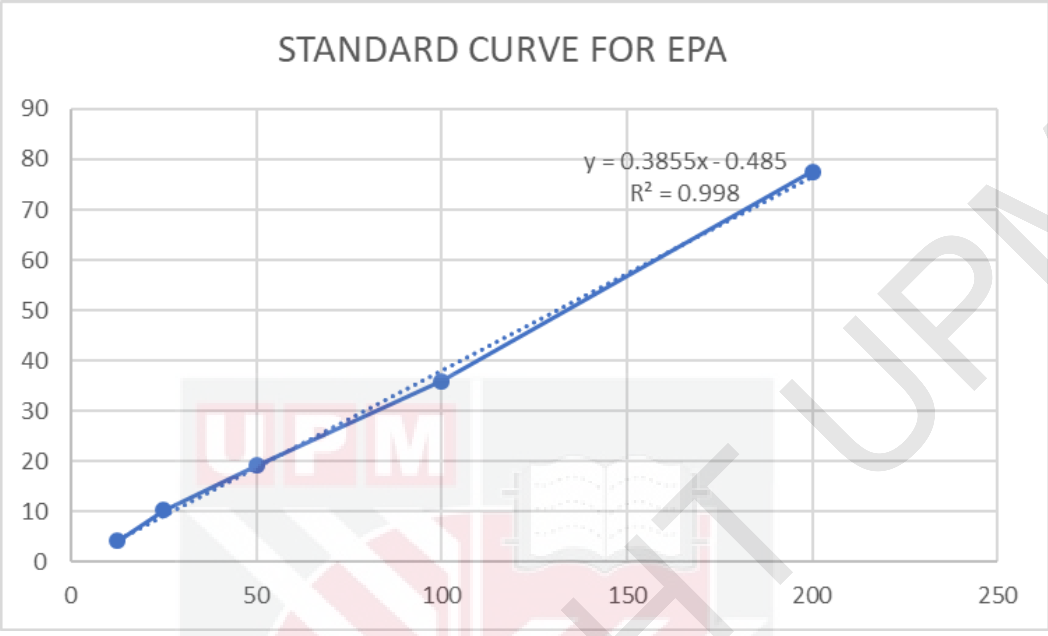
Zelenka, J., Schneiderova, D., Mrkvicova, E., & Dolezal, P. (2008). The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat. *Veterinarni Medicina*, *53*(2), 77–85. https://doi.org/10.17221/1985-VETMED

Zivkovic, A. M., Telis, N., & Hammock, B. D. (2011). Inflammation and Metabolic Health. *Calif Agric (Berkely)*, *65*(3), 106–111. https://doi.org/10.3733/ca.v065n03p106.Dietary

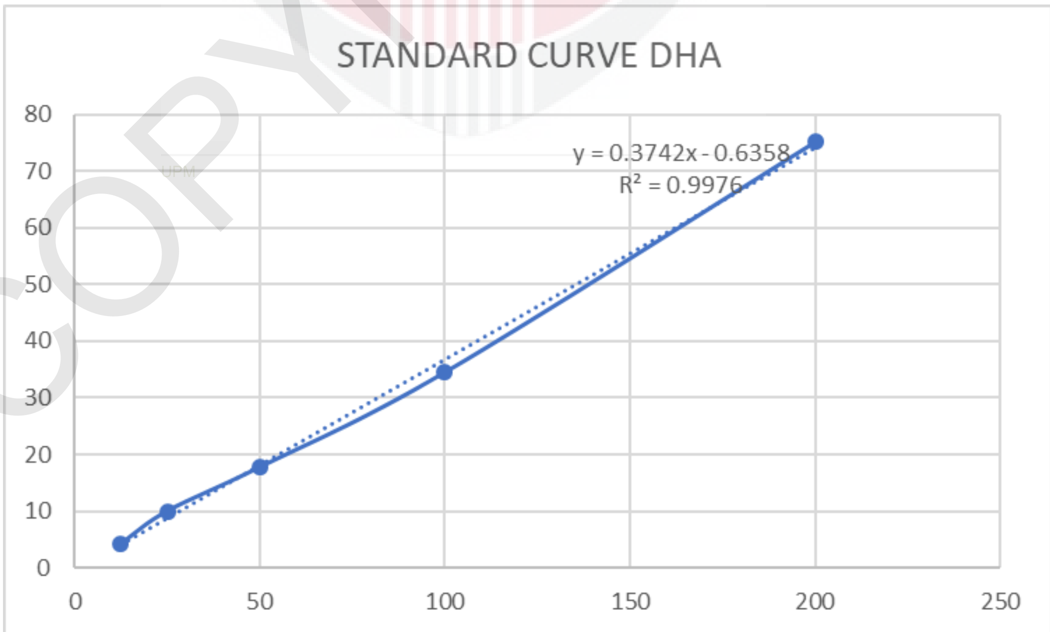
Zuidhof, M. J., Betti, M., Korver, D. R., Hernandez, F. I. L., Schneider, B. L., Carney, V. L., & Renema, R. A. (2009). Omega-3-enriched broiler meat: 1. Optimization of a production system. *Poultry Science*, *88*(5), 1108–1120. https://doi.org/10.3382/ps.2008-00171

APPENDICES

Standard curve of 37 Component FAME Mix for EPA



Standard curve of 37 Component FAME Mix for DHA



Standard reference material and internal standard

Total lipid of Standard Reference Material

	Fat (g/100g)
Reference Values for Proximate and Calories	18.96 ± 0.40
Current analysis values	12.25 ± 0.00

EPA and DHA Standard Reference Material

	Eicosanoic Acid (C20:0) (g/100g)	α-Linolenic Acid (C18:3 n-3) (g/100g)
Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids	0.0329 ± 0.0009	0.133 ± 0.020
Current analysis values	0.1594 ± 0.0000	0.012 ± 0.000