The influence of lipid types and lipid levels on the performance parameters, apparent metabolisable energy and ileal nutrient digestibility in day 1-21 broilers fed maize-soybean based starter diets

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Abstract

The current study investigated the influence of different lipid sources and lipid inclusion level, and their interaction on ileal nutrient digestibility (N, DM, fat, starch, GE), apparent metabolizable energy (AME), and performance parameters (BWG, FI, FCR, and mortality) of broilers fed maize-soybean diets for 21 days post-hatch. A completely randomized design was used with 2 x 3 factorial arrangement of 6 treatments with 8 replicates, and 8 birds per replicate to study the effect of two lipid levels: High (H) and Low (L) and three lipid types: Crude Palm oil (CPO), Soy Oil (SO) and Poultry Fat (Sirona, PF). Six experimental diets (POH, POL, SOH, SOL, PFH and PFL) were formulated using maize and soybean as the main feed ingredient. The inclusion levels of the main feed ingredients and lipid vary between the diets achieving different levels of energy and nutrient density. Other ingredients that were included in the diets show only a slight variation. The lipid sources provided the birds with similar levels of AME within the same lipid inclusion level. High diets were formulated with more lipids than the Low diets, which provided an extra 100Kcal AME (H = L + 100Kcal/kg). Low diets were formulated to contain 6% of crude fat with lower AME. All the lipid type x lipid level interactions are not significant for all the performance parameters, AME, and ileal nutrient digestibility. However, both lipid types and lipid levels significantly ($P < 0.05$) affected body weight gain of broilers. Birds consumed poultry fat source diets had the highest BWG, with similar level in soy oil diets and lowest in diets consist crude palm oil. Diets with low lipid inclusion level resulted significantly better ($P < 0.05$) BWG. Feed intake was influenced ($P < 0.05$) by lipid inclusion level in diets that low lipid inclusion resulted improved feed intake. Feed conversion ratio is significantly impacted ($P < 0.05$) by lipid type, where the soy oil had the best efficiency followed by poultry fat, and diets that included crude palm oil had the highest FCR. AME was significantly higher ($P < 0.05$) in diets containing plant-based oils and increase lipid level contributed to the significant difference ($P < 0.05$) between AME in diets. Ileal digestibility of DM, fat, and GE was significantly higher ($P < 0.05$) in soy and crude palm oils than poultry fat. In general, the result showed that the performance parameters, AME, and ileal nutrient digestibility of broilers are influenced by the various fatty acid profile and the amount of unsaturated and saturated fatty acids in the different sources. Lipid inclusion level affected the AME and performance parameters of broilers.

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List of Abbreviations

AA	Amino Acid
AME	Apparent Metabolisable Energy
ANF	Anti-nutrient Factor
ANOVA	Analysis of variance
BWG	Body Weight Gain
℃	Degree Celsius
Ca	Calcium
CAID	Coefficient of Apparent Ileal Digestibility
DM	Dry Matter
FA	Fatty Acid
FCR	Feed Conversion Ratio
FI	Feed Intake
g	gram
GE	Gross Energy
Kcal	kilocalories
kg	kilogram
kJ	kilojoule
ME	Metabolisable Energy
MJ	Mega Joules
N	Nitrogen
NSP	Non-Starch Polysaccharide
P	Phosphorus
S	Saturated
Ti	Titanium

Chapter One

General Introduction

With the continuous human population growth around the world, demand for animal protein increased (FAO,2012). The cost of feed ingredients for poultry such as corn, maize and soybean rise significantly as the result of shortage, which caused by high demand with limited production amount, and competition for resources (Farrell, 2013). Broiler chickens produce large amount of good quality protein with high efficiency and can be on market in around 30 to 42 days depending on different requirement of the retailer. The fast growth rate of broiler birds requires large amount of energy, which energy dense feed ingredients can be used to meet the production efficiency (Shoaib et al., 2023). Because of the importance of feed to poultry, better methods are investigated to provide the required nutrients to support poultry growth and performances. The rising cost, shortage of conventional feed stuff, and improving production efficiency has led to growing interest in adding energy dense oil ingredients like soybean oil, poultry fat, canola oil and many other lipids (Ravindran et al., 2016; Shoaib et al., 2023).

Adding lipids to the poultry feed ingredients can lower the production cost, decrease the dependence on the traditional feed ingredients and reduce competition for resources. However, adding lipids to the poultry diet also poses nutritional challenges, as the type of lipid and the amount of lipid added to the diet if not formulated correctly, can caused adverse effect such as growth reduction, poor feed conversion ratio, reduce production amount and efficiency, and negatively impact the performance of the bird (Ravindran et al., 2016).

Therefore, this research aims to evaluate different fat sources and fat levels for the intention of maximising the use of fats in poultry diet to increase dietary energy density for the high-performing poultry birds to meet their requirement more efficiently. Also, to investigate the effects of different fat sources and levels on the AME, ileal nutrient digestibility of nitrogen, dry matter, fat, starch and performance parameters of weight gain, feed intake, feed conversion ratio, and mortality in starter broilers (day 1-21 post-hatch).

Chapter Two

2.1 Introduction

Energy is the most expensive nutrient component in poultry feed. Energy is not a nutrient, but the function of carbohydrates, proteins, and lipids (Ravindran & Abdollahi, 2021). The primary source of energy in broiler feed are carbohydrates and lipids. However, if an excess amount of protein is fed to the birds it can also become a source of energy (NRC, 1994). But using protein as an energy is uneconomical. So, the balance between carbohydrates, lipids, and protein in the diet must be carefully constructed (Bell & Weaver Jr., 2001; Murugesan, 2013). The available energy is measured as apparent metabolisable energy (AME) in poultry feeds with MJ/kg or kcal/kg as the unit of measurement. Approximately 70% of the production cost is feed cost, and energy takes up around 70% of the feed cost, so reducing the cost of production, reducing the cost of energy in feed is fundamental (AL-Jaf et al., 2018; Thirumalaisamy et al., 2016). Lipids can provide much larger amount of energy compared to the other feedstuffs, which can be used in broiler diets to suffice broiler birds' high energy demand for maintenance and fast growth (AL-Jaf et al., 2018; NRC, 1994). Due to the increasing price of conventional ingredients, the interest of using fats and oils to improve the energy level of diets have risen. Therefore, there is greater need for exploring and understanding different types of lipid sources and their digestibility in poultry (Ravindran et al., 2016).

Fat is a synonym for lipid which is defined as substances soluble in non-polar organic solvents (chloroform and ether) and insoluble in water. Lipids are categorised into simple, compound, and derived (Gordon, 2003). Simple lipids include triglycerides which also known as triacylglycerols, steryl esters and waxes (Baiao & Lara, 2005). Triglycerides are glycerol esterified with three fatty acids (FAs) and is commonly used in animal feed. Waxes are esters of FAs with long-chain alcohols and can be found on the natural coverage of leaves, stems, insects, skin, feathers, hairs, and the structural material of beehives. They have no nutritional value since they are hydrophobic and cannot be degraded by the digestive enzymes of animals (Ferreira, 1999). Triglycerides are the most important simple lipids as they are the major components of edible oils and fats. The most important compound lipid in food are phospholipids which are esters of glycerol that contain two FAs plus another chemical group, such as, choline and serine (Tancharoenrat, 2012). Lipoproteins are also a compound lipid which make up most of the lipids that are transported in the blood. Derived lipids include a range of compounds which include substances that varies structurally. This group include FA, fat soluble vitamins and other alcohols that derive from simple and compound lipids after hydrolysis (Baiao & Lara, 2005; Gordon, 2003). In terms of nutrition, the important lipids are triglycerides, phospholipids, sterols, and fat-soluble vitamins (Brindley, 1984).

Fats and oils are consisted of triglycerides which are a mixture of glycerol esters of FAs. Fats are solid at room temperature and oils are liquid at room temperature (Gordon, 2003). Fats and oils are lipids which are energy dense that can provide approximately 39 MJ/kg of energy which is equivalent to around 9204 kcal/kg (Bell & Weaver Jr., 2001). It is much higher than the energy value of carbohydrates and proteins. This is due to the carbon atoms in FAs are chemically more reduced than carbon atoms in sugar which the oxidation of triglycerides will release much more energy. So, lipid is usually included in broilers' diet as they require large amount of energy for rapid growth. Around 3-5% lipids are added to poultry diets to increase the energy concentration of the birds' diet. A diverse range of lipids can be used, for example, blended fats, animal fats, vegetable oils, restaurant greases, acid oils and soap stocks (Padley, 1997).

Adding lipids in poultry diet can also provide the birds with essential FAs that maintain the birds' health, and growth. Birds are not able to synthesize all FAs and thus, some are considered essential FAs. Linoleic (18:2, n-6) and linolenic (18:3, n-3) fatty acids are recognized as metabolically essential. Growing chicks and adult birds require 1% linoleic acid, as deficiency of the linoleic acid can cause reduced growth in chicks and poor feathering in broiler birds (Baiao & Lara, 2005; Bell & Weaver Jr., 2001). The essential FA include linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic acid (C20:4) and need to be supplied in the diet. The deficiency of these essential FA may result in impairments in growth and immune system function. Symptoms of linoleic acid deficiency in poultry include retarded growth, increased water consumption and reduced resistance to diseases (Balnave, 1970; Ravindran et al., 2016). In male birds, deficiency symptoms also include lower testes weight and delayed development of secondary sexual characteristics. Decreased egg size is the major outcome of deficiency in laying hens (Watkins, 1991). To ensure adequate supply of these essential FA, a minimum inclusion level of 10 g/kg lipids in poultry diets has been suggested by Leeson and Summers (2009). 20 to 50 g/kg lipids is usually added in commercial poultry diets depending on the relative prices of lipid and cereal grains. The addition of lipid above 40 g/kg is generally avoided in pelleted diets because of the negative effects on pellet quality (Abdollahi et al., 2013a). With new technologies, however, it may be possible to add more than 40 g/kg lipid in these diets.

Using fats and oils also have other advantages such as increasing the palatability of the diet, improve the number of FAs and fat-soluble vitamins in the diet, help binding the particles meanwhile prevent particle separating, control the amount of dust produced while producing pellets which can reduce the amount of wastage, and decrease the rate of passage through digestive tract allowing more time for utilization and absorption of nutrients (Ravindran et al., 2016). Lipids are also carrier of pigments and fat-soluble vitamins such as vitamin A, D, E and K. There are a diverse range of fats and oils which vary in composition that can be used in poultry feed namely, soybean oil, palm oil, canola oil, coconut, oil, fish oil, krill oil, animal fat, tallow, and poultry fat (Sirona) etc (Baiao & Lara, 2005; Ravindran et al., 2016).

The different sources of fats and oils also vary in the amount of energy yield due to different FA content. For example, tallow can provide AME ranging from 6000-10600 kcal/kg (Baiao & Lara, 2005; Ravindran et al., 2016), poultry fat 8000-10200 kcal/kg (Baiao & Lara, 2005; Ravindran et al., 2016), palm oil 5200-8260 kcal/kg (however, other article showed that palm oil has contain around 8260 kcal/kg AME) (Baiao & Lara, 2005; Ravindran et al., 2016), grease 5300-11700 kcal/kg (Ravindran, 2016), and soybean oil 8400-11000 kcal/kg (Baiao & Lara, 2005; Ravindran et al., 2016).

Table 2.1. Lipid sources commonly used in poultry feeds with the approximate energy contribution.

Vegetable Oils	ME (kcals/kg)	Animal Fats	ME (kcal/kg)
Corn Oil	9220	Tallow	8500
Linseed Oil	8690	Lard	8400
Palm Oil	8400	Poultry Fat	9000
Safflower Oil	9220	Fish Oil	9000
Sunflower Oil	8690	Mixed Animal Fat	8100
Soybean Oil	9220	Animal Vegetable Blend	8000
Rapeseed Oil	8800	Mixed Vegetable Blend	8909

Source: Baiao & Lara, (2005); Ravindran et al. (2016); Wiseman (2003)

2.2 Classification of lipid sources

Fats and oils both are composed of triglycerides which have three FAs with one glycerol backbone. FAs are the simplest type of lipid and consist of long chains of hydrocarbons with a carboxylic acid group (-COOH) at one end. Short chain FAs have 2-5 carbon atoms in their structure. Propionic acid and butyric acid are examples of short chain FAs. Medium chain FAs have 6-12 numbers of carbon atoms in their structure such as capric acid and caprylic acid. The long chain FAs have 13-21 numbers of carbon atoms in their structure such as palmitic acid. There are also very long chain FAs that have above 21 numbers of carbon atoms in their structure (Baiao & Lara, 2005; Pond et al., 2005; Tancharoenrat et al., 2013).

The amount of energy that can be yielded from lipids depends on the different sources' degree of saturation and FAs' chain length. The FAs are divided into groups of saturated FAs and unsaturated FAs, depending on the number of double bonds in the chain (Freeman, 1984).

Saturated FAs do not contain double bonds in their carbonic chain structure. Saturated FAs are mostly solid at room temperature and have a higher melting point than unsaturated FAs. This is due to the lack of double bond in the molecules result in a straight hydrocarbon chain. Which allows the FA chains to pack closely in a parallel arrangement (Baiao & Lara, 2005). Saturated FAs are available in animal fats in large quantities, it is non-polar which means it cannot form micelles spontaneously, so bile salt is not able to emulsify them for preparation of digestion. This makes saturated FAs less absorbable which results in a less amount of energy yield (Tancharoenrat et al., 2013). The melting points of saturated FAs increase with increasing carbon chain length. The melting point of saturated FAs decreases with increasing number of double bonds (Freeman, 1984; Shoaib et al., 2023).

Unsaturated FAs have one (mono-unsaturated) or more numbers (poly- unsaturated) of double bonds in their carbonic chain structure. Unsaturated FAs are typically liquid at room temperature and have a lower melting point than saturated FAs. It is available in vegetable oils in large quantities (Smink, 2012).

The double bond in the unsaturated FAs can be either *cis* or *trans*. Which is determined by the different geometric configuration of the double bonds in the hydrocarbon chains of FAs (Lairon, 2009). The different geometric configuration can have significant effects on the physical properties of the FAs. The hydrogen atoms on the carbon atoms adjacent to the double bond in *cis* unsaturated FAs is on the same side of the carbon chain (van Kuiken and Behnke, 1994). This results the FA chain bend which disrupts the straight linearity of the molecule causing lower melting point and unsaturated FAs to be more fluid compared to saturated FAs with no double bond (Baiao & Lara, 2005; Lairon, 2009). On the other hand, *trans* unsaturated FAs have hydrogen atoms on the carbon atoms adjacent to the double bond on opposite sides of the FA chain. This results a straight hydrocarbon chain with linear molecule similar to the structure of saturated FAs (Baiao & Lara, 2005; Brindley, 1984; Tancharoenrat, 2012; Smink, 2012). *Trans* FAs are produced through hydrogenation which converts vegetable oil in liquid from into solid fats in order to improve shelf life and add texture. *Trans* FAs are present in small amounts naturally in animal products like beef and dairy, they are also found in hydrogenated vegetable oils used in processed foods. High consumption of *trans* FAs has been shown to cause negative health effects, including an increased risk of cardiovascular disease by raising low-density lipoprotein cholesterol levels and decreasing high-density lipoprotein cholesterol levels (Ramŕez

et al., 2001).

Triglycerides include simple triglycerides and mixed triglycerides (Baiao & Lara). Simple triglycerides have the same types of FAs attached to their glycerol backbone. The mixed triglycerides have different types of FAs attached to their glycerol backbone. For example, triglycerides with saturated FAs are triacylglycerol such as animal fat, tallow and lard etc. having all three saturated FAs in their structure (Sklan, 1979). Triglycerides with unsaturated FAs have relatively low melting point. One example being monounsaturated FAs such as vegetable oils, and canola oil etc. with one double bond in their unsaturated FA's structure. Triglycerides with polyunsaturated FAs such as salmon oil have two or more number of double bonds in their unsaturated FA's structure (Scott et al., 1982).

2.3 Digestion and absorption of lipid

Chicken's digestive tract is made up of beak, esophagus, crop, proventriculus, gizzard, duodenum, jejunum and ileum, the main site of lipid digestion occurs in the intestine, particularly in the duodenum (Tancharoenrat, 2012). Fats and oils ingested by birds will undergo intestinal emulsification, digestion, micellar solubilization, cell membrane permeation, intracellular esterification, and after absorption, incorporation into lipoprotein then released to the intestinal fluid (Krogdahl, 1985; Tancharoenrat, 2012). Emulsifier is a substance that stabilises emulsion and has a water loving head (Hydrophilic) and an oil loving tail (Hydrophobic). Emulsion is the process of a mixture of two or more immiscible liquids (Lairon, 2009). After the feed enters the digestive tract, it will be broken down into smaller particle size by grinding and mixing in gizzard. Then, the pendular movement and shuttling between proventriculus and gizzard will further facilitate enzymatic and mechanical digestion (Smulikowska, 1998). After that, proventriculus's contraction will push digesta further down the tract. Pancreatic and intestinal juice is mixed with the digesta. Next, digesta with the presence of bile salts are moved back to the gizzard due to shuttling movement by reverse peristalsis and proventriculus initiates fat emulsification followed by the absorption of fat in duodenum and jejunum (Freeman, 1984; Sklan, 1979). When lipid enters the small intestine, it is broken down into smaller fat droplets of micelle by bile salts which act as an emulsifier (Krogdahl, 1985). The digestion of lipid starts when digesta enters duodenum with the shuttling movement between gizzard and duodenum also allows digesta to fully expose to digestive enzyme and facilitates absorption in the upper part of the small intestine (Lairon, 2009; Tancharoenrat, 2012; Tancharoenrat et al., 2014;).

Bile is secreted by liver and flows into the duodenum; it does not contain enzymes but helps to emulsify fat (Krogdahl, 1985). Bile salts released from gall bladder to emulsify fats in chyme to fat droplets which would be hydrolysed by pancreatic lipase secreted from the pancreas and dispersed into small mixed micelle in the intestinal lumen. Colipase is a co-factor present in pancreatic secretion and is essential for lipase and triglyceride emulsions. It binds to the surface of lipid droplets and facilitates lipase to digest triglycerides. The triglycerides will be hydrolysed to produce free FAs and monoacylglycerol which will form micelles and coupled with bile salts and pancreatic lipase, will be transported to the mucosal surface, and pass through the brush border membrane in jejunum and ileum (Krogdahl, 1985; Tancharoenrat, 2012).

After digestion, the short-chain FA and mono glycerides can be absorbed by passing through intestinal cells to the intestinal lumen and enter the mesentery blood vessels after re-esterification into triacylglycerides. Long chain saturated FAs, diglycerides, fat soluble vitamins and cholesteryl esters need to be solubilised in the core of micelle which is hydrophobic to be transported through to the intestinal cells (Pond et al., 2005).

In the cells, monoglycerides and long chain FAs will be rebuilt into new triglycerides and combined with free esterified cholesterol, lipoprotein and phospholipids to form chylomicron and enter the lymphatic system. It is secreted to portal circulation and transported to various tissue which will later be used to synthesize various compound such as lipoprotein, phospholipid and metablised as source of energy or store in tissue as fat deposits (Krogdahl, 1985; Tancharoenrat, 2012; Scott et al., 1982).

2.4 Factors affecting lipid digestibility

The variation of metabolisable energy value of oils and fats is caused by several factors affecting digestibility. The different composition and structure of the lipid source, for example, the length of FA chain, free FA content, their different saturation degree of FAs and position of double bond will affect the digestibility of lipid and AME (Baiao & Lara, 2005; Ravindran et al., 2016). Different types of triglycerides provided in the diet differ in carbonic chain length, where the longer the FA chain, the harder it is for birds to digest. The increasing degree of unsaturation positively affects the absorption of lipid. The increasing number of double bonds in FAs will increase digestibility (Sklan et al., 1973; Ward and Marquardt, 1983). Hurwitz et al. (1979) reported that in duodenum, absorption of stearic acid with longer chain length is lower than palmitic acid with shorter chain length.

Other factors such as, the composition of the free FAs, whether the triglycerides or the free FA is present, the location of the FA in the molecule and the amount of free FAs, the specific arrangement of saturated and unsaturated FAs on the glycerol backbone (sis and trans), the composition of the diet, and the birds' intestinal condition (Baiao & Lara, 2005). The increased level of free FAs negatively impacts the digestibility and AME of lipid.

Plant-based oils consist of high proportions of unsaturated FAs which tend to be easier for oxidation and can be easily digested by birds. On the other hand, animal-based fats consist of higher proportions of saturated FAs which are more difficult and digested by birds in less amount (Leeson and Summers, 2009). However, animal fats such as poultry fat, fish oil and tallow provide higher levels of energy compared to plant-based oils as animal fats contain more unsaturated FAs that when broken, the bond releases more energy (Tancharoenrat & Ravindran, 2014).

2.4.1 Bird-related factors

The age of the birds can also affect their ability to digest lipids. The secretion of bile salts is essential to the emulsification and micelle formation process. However, young birds do not have fully developed digestive tracts, in which their ability to digest lipid is limited by the amount of lipase and bile salt secreted (Krogdahl, 1985). Young birds' digestibility of animal fat source is poor, although plant source oils are less affected, impaired digestibility of lipids still occur in the first week post-hatch chicks. Their lipid digestibility will increase significantly in the second and third week, with no further increase after week 3 (Tancharoenrat et al., 2013; Ravindran & Abdollahi, 2021).

Table 2.2. Influence of broiler age on the total tract fat digestibility in three lipid sources.

Lipid source	Age (Days)	Fat Digestibility, %
Tallow	7	36.8
	14	65.3
	21	73.6
Soybean oil	7	59.1
	14	89.8
	21	96.5
Poultry fat	7	60.0
	14	84.5
	21	92.8

Source: Ravindran & Abdollahi (2021).

The sex of the birds also affects digestibility of lipid. According to Krogdahl (1985), female birds had significantly higher lipid digestibility than males. However, studies done by Guiguis (1975) and Yaghobar (2001) showed opposite result, the latter showed gender affected the AME of oats, tallow and fish meal with higher value in female chickens, while the latter determined that gender had no effect on the AME of maize for layer birds.

Another factor that can affect lipid digestibility is the different breeds of birds. Although the chicken's lipid digestibility was affected by age, no effect of age on turkey poults on lipid digestibility was observed (Halloran & Sibbald, 1979). Another study done by Martin and Farrell (1998) showed that ducklings have better digestibility of lipids in rice bran than similar age broiler birds. And turkeys and ducks may be more capable of producing bile and lipase more sufficiently than chickens.

Intestinal diseases can also affect the lipid digestibility of birds. Various intestinal diseases such as necrotic enteritis, malabsorption syndromes, and coccidiosis etc. can cause inflammation and damage to the intestinal epithelium which results in reduced production of bile sales and poor absorption of nutrients. Lipid digestion is impaired mostly by epithelial damage as the intestinal mucosa contains the sensors that mediates the release of cholecystokinin which is responsible for stimulating gallbladder contraction and secretion of pancreatic enzymes (Ravindran et al., 2016; Wang & Cui, 2007).

2.4.2 Diet-related factors

The quantity and quality of the dietary lipids can influence the amount of bile salt secreted. Lower inclusion level of lipid in the diet can be utilized by birds more efficiently. In addition, higher inclusion level of lipid, longer FA chain, and higher degree of saturation will result decreasing digestibility of lipid (Ravindran et al., 2016; Ward and Marquardt, 1983).

Non-Starch Polysaccharides (NSPs), are complex carbohydrates found in feed ingredients commonly used in poultry diets. NSPs are mainly present in plant-based feed ingredients such as grains, cereals, and certain oilseeds. NSPs in poultry feed can adversely impact on the birds' nutrient utilization, and overall performance. Large amount of NSPs can result in increased digesta viscosity, wet excreta, and a highly moisturized environment which encourages growth of harmful bacteria (Jacob & Pescatore, 2012). Increased viscosity in the gastrointestinal tract of the birds can also negatively impact the birds' gut health and digestive functions. Study done by (Dänicke, 2001) showed that the presence of NSP can depress the digestibility of lipid and saturated FAs are more affected than unsaturated FAs. This is the result of higher intestinal viscosity of the bird after ingesting diets containing large number of viscous cereals such as, wheat, barley and rye which contains high concentrations of soluble NSPs namely arabinoxylans and β-glucans. Causing the decreased gut motility and impaired the diffusion and transport of fat micelles, bile salts and digestive enzymes

(Smulikowska, 1998). In addition, the increased viscosity can result microbial growth in the birds' small intestine causing higher rate of deconjugation of bile acid. The deconjugated bile acid cannot be reabsorbed and needs to be excreted, this can cause reduced recycling of bile and lowered the concentration of bile salt presented for digestion and poor digestion of lipid (Annison and Choct, 1991). Saturated FAs require conjugated bile salt for the formation of mixed micelle, therefore, is more affected by the NSP present in the diet (Smits and Annison, 1996).

Study done by Antoniou et al. (1980) showed that a rye-based diet containing tallow greatly depressed the performance and lipid digestion of the broiler birds compared to broilers fed with same diet but containing soybean oil. This is consistent with other studies that tallow containing diets depressed the digestibility of lipid, as tallow contains large amount of long-chain saturated FAs (palmitic and stearic acids) and are poorly digested and absorbed by poultry (Renner and Hill, 1961; Scott et al., 1982). Both palmitic and stearic acids are non-polar and cannot spontaneously form mixed micelles. They require the presence of conjugated bile salts and unsaturated FA to form the mixed micelles. Overall, available data overwhelmingly highlight the poor lipid utilisation in tallow by young, growing birds. On the other hand, vegetable oils contain high concentrations of unsaturated FA that are easily emulsified and better digested than tallow (Leeson and Summers, 2009; Sklan, 1979).

Abdollahi et al. (2013) found that different cereal-based diets have various effects on the effect of pelleting on ileal fat digestibility. Pelleting improves the lipid digestibility of maize-based diets, on the contrary, digestibility of lipid is reduced by pelleting in wheat-based diets. Another study done by Abdollahi et al. (2014) discovered that pelleting reduced the coefficient of apparent ileal digestibility (CAID) of fat of broiler starters fed a sorghum-based diet compared to mash form and re-ground pellets. Although re-grinding the pellets vastly improved the lipid digestibility resulting the higher CAID than mash and pellet form. Some experiments suggested that lipid addition after feed processing may become more digestible (Naderinejad et al., 2016).

Feed form and particle size of the diet also affect birds' digestibility of lipid

(Amerah et al., 2007). The traditional view of feed particle size was that smaller particle size has larger surface area that results in higher digestibility of nutrients, as it increased the interaction with digestive enzymes in the gastrointestinal tract (Preston et al., 2001). However, studies found that large particle size aided by structural components is beneficial to gizzard functions and gut development (Hetland et al., 2002). Experiment done by Naderinejad et al. (2016) also investigated the interaction between feed form of mash and pellet, and particle size of fine, medium, and coarse on the CAID of fat in broiler maize-based diets. Feed form was significant for the CAID of fat with pelleting increasing the digestibility compared to mash diets. A tendency ($P = 0.09$) was also observed for the interaction between feed form and particle size. Another study done by Abdollahi et al. (2014) showed that pelleting of maize-based diets tend to increase the proportion of fine particles (<0.075mm) and does not cause much difference to the coarse particles > 2mm. However, the proportion of coarse particles in wheat-based diets was reduced by pelleting and had no effect on the fine particles. Re-grinding resulted in a further decrease in the proportion of coarse particles in both grain types (Abdollahi et al., 2014). The digestibility of lipid was improved by pelleting and regrinding pellets in maize-based diets compared to the mash diet. In contrast, in wheat-based diets, pelleting reduced digestibility of lipid compared to mash diet, re-grinding the pellets restored fat digestibility to higher than the mash diet (Abdollahi et al., 2013a). Overall, feed form and particle size should be taken into consideration when choosing the source of cereal used in the diet.

Another factor that can affect the digestibility of lipid is dietary Ca level. Free FAs are released during the digestion of lipid which can react with minerals forming soluble or insoluble soap. The formation of insoluble soap can cause unavailability of both FA and minerals to the birds (Leeson and Summers, 2009). Ca-phytate is involved in the formation of insoluble metallic soaps which high dietary Ca level can increase the formation of lipophytins and lower the energy yield. Evidence suggested that the type of FA and different amount of dietary Ca can impact soap formation and the retention of lipid and Ca (Atteh and Leeson, 1983). With increasing dietary Ca concentrations, the lipid digestibility is lowered, with saturated animal fats being affected the most (Lin and Chiang, 2010).

2.5 Strategies to improve fat digestion

Fat digestion can be impaired by diets consisting of viscous cereals. Enzymes such as carbohydrases, can be added to poultry feed to break down NSPs and improve the availability of nutrients. Diets based on viscous cereals can be supplemented with enzymes of exogenous glycanases (β-glucanases and xylanases) to neutralize the digesta viscosity and, to improve bird performance by increase the nutrient digestibility and utilization (Choct and Annison, 1992; Smulikowska, 1998). Study done by Dänicke et al. (1997) confirmed that diets supplemented with enzyme have decreased viscosity of ileal digesta and improved lipid digestibility compared to birds fed the unsupplemented diet.

Lowering the Ca level in diet may facilitate the digestion of lipids, especially diets with larger proportions of saturated FAs (Lin and Chiang, 2010). The main Ca source of broiler birds' diet is limestone which has extremely high acid binding capacity and tends to elevate the pH of digesta in the gut. The increase of digesta pH could increase the formation of Ca-phytate complex and lower the activity of lipase. This means that amount of Ca and P for bone health and bird performance need to be re-defined to also take consideration of the maximum utilisation of lipids (Tancharoenrat & Ravindran, 2014).

Several experiments showed that feed processing of cereal grains, such as grinding, steaming, pelleting certain diet may increase accessibility of lipids in cell wall matrix of the cereal and improved lipid digestibility (Ravindran et al., 2016). For example, fat content in maize-based diets is derived from intact fat within the cells, which grinding the cereal grains can increase the accessibility of lipids within the cell wall matrix (Abdollahi et al., 2013b; Vieira et al., 1997). Study done by Jimenez-Moreno et al. (2009) showed steaming may improve lipid digestibility as the cell wall matrix of the cereal grain releasing the encapsulated lipid allowing increased access of digestive enzymes to cellular content. However, the results vary between the different types of cereal grains used, as pelleting tends to increase lipid digestibility in maize based diets but reduced the digestibility of wheat-based diets (Abdollahi et al., 2014).

Raw cereal feed ingredients often have unique physical and chemical properties and often contain various antinutrient factors that can pose challenges during processing (Annison and Choct, 1991; Choct and Annison, 1992). To neutralize the negative impact of ANF on poultry production, different methods of processing can be used. Processing can neutralize the negative impact of ANFs on poultry production, increase digestibility and nutrient availability, alter particle size, prevent spoilage, improve palatability, remove potential allergens, remove specific parts of the seed and improve handling. Processing can be applied physically, chemically, thermically and using bacteria. Different methods of processing can be used, for example, grinding, milling, mechanical (dehulling), extrusion, expansion, steam flaking, roasting, popping, micronizing, heating, chemical treatments, and pelleting. Alternative methods can also neutralize the adverse effect or reduce the impact of ANF, for example, supplementation with enzymes and plant breeding for less ANF present in the feedstuffs. An important thing is that the treatment needs to be carefully controlled, otherwise it can cause adverse effects (Adejumo & Olojede, 2017). Processing such as heat processing techniques like pelleting and extrusion can help break down NSPs and improve nutrient availability. Heat treatment can also reduce the viscosity of digesta, improving gut health.

As discussed previously, digestibility of saturated FAs is low compared with unsaturated FAs. Research has been done to improve the utilisation and digestion of saturated fats, which can be achieved by adding adequate amounts of unsaturated FA relative to saturated FAs in the diet, so the diet consists of a blend of animal fats and vegetable oils with different ratios (Poorghasemi et al. 2013). Several researchers (Lall and Slinger, 1973, Sibbald et al., 1962, Wiseman and Lessire, 1987) suggested that mixing saturated and unsaturated lipids may improve lipid digestion and that there is a synergistic response with such blends. Blending the FAs allowed more absorption of long chain saturated FAs. Research done by Wiseman and Lessire (1987) showed the effect of blends of tallow and rapeseed oil at five ratios in 14-day broilers and adult roosters. The ratio of unsaturated to saturated fats influenced the AME. The increasing proportions of unsaturated fat added tended to have positive effects, as the increasing ratios of more digestible plant-based oil improved the digestibility of palmitic and stearic acids (Murugesan, 2013). Another study by Ketels and De Groote (1989) examined the relationship between the dietary ratio of (unsaturated FAs : saturated FAs) also observed that blending the vegetable oil with animal fat improved the utilisation of saturated FAs (Leeson and Summers, 2009).

2.6 Conclusion

Lipids, as a concentrated source of energy, contribute large amount of energy to broiler diets, aiding in the maintenance and rapid growth demanded by broiler birds. The diverse range of lipid sources, including animal fats, vegetable oils, and other fats, with different FA chain length, free FA content and degree of saturation creates complexity for diet formulation as these factors affect ileal nutrient digestibility, AME and performance parameters Essential fatty acids, particularly linoleic and linolenic acids, play a crucial role in maintaining bird health, growth, and immune function. Bird-related factors, such as age, sex, breed, and health, alongside diet-related factors like composition, processing, and particle size, significantly impact lipid digestibility. Understanding the digestion and absorption processes of lipids in the avian digestive tract is paramount for effective diet formulation. The duodenum emerges as the primary site for lipid digestion, involving emulsification, micellar solubilization, and intracellular esterification. Various strategies that can enhance fat digestion have been explored, including the enzymes supplementation, adjusting the dietary calcium levels, and feed processing techniques. The inclusion of carbohydrases facilitates breaking down non-starch polysaccharides, while lowering dietary calcium levels is beneficial in mitigating the formation of insoluble soaps that hinder lipid absorption. Additionally, feed processing methods, such as pelleting and extrusion, contribute to improved lipid digestibility by altering particle size and reducing antinutrient factors. Overall, the use of fats in poultry birds increases the dietary energy density allowing the high-performing poultry birds meet their requirements more efficiently. However, there is limited research done on using high fat inclusion level in poultry diets with both animal and plant-based lipid types for researching as high inclusion of lipid affect the pellet quality. Hence, the need to evaluate different fat sources and inclusion levels to maximise the use of lipid and utilize the optimum source of lipid is needed. It was therefore, hypothesised that the influence of lipid types on ileal nutrient digestibility, AME and performance parameters differ in different lipid levels. To test this hypothesis, the present experiment was designed to compare the interaction between the lipid types (crude palm oil, soy oil and poultry fat) and lipid level (Low and High) on the CAID of N, DM, fat, starch, GE, and AME and performance parameters of BWG, FI, FCR and mortality rate in broiler starters fed maize-soybean based diet.

Chapter Three

The influence of lipid type and lipid levels on the performance parameters, apparent metabolisable energy and ileal nutrient digestibility in day 1-21 broilers fed maize-soybean based starter diets.

3.1 Introduction

The modern broiler bird strains have been genetically developed to achieve increasing growth potential, and this requires the larger amount of energy intake to support their rapid growth rate. Therefore, diets that are energy dense have become favorable which result in the wider use of fats and oils in broiler diets as the AME is nearly three times higher than other feedstuffs (NRC, 1994).

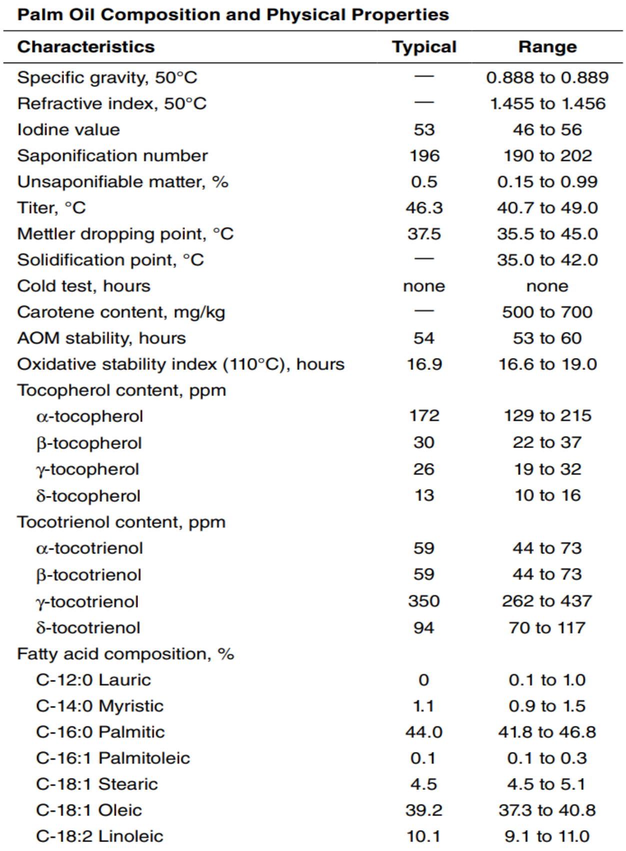
The use of lipid in poultry diets not only can supply large amount of energy to facilitate the rapid growth of broiler birds, but it also improves absorption of lipid-soluble vitamins, decrease dust and amount of wastage, increase the palatability of the pellet, increase the birds' efficiency of consuming energy, and reduce the production cost for energy (Ravindran et al., 2016).

There is a large range of fats and oils that can be used in poultry diets. For example, canola oil, coconut oil, maize oil, soybean oil, poultry fat, lard, beef tallow, fish oil, yellow grease etc. The range of fats and oils differ in physical and chemical properties and the difference in the metabolisable energy can be affected by the characteristics of the fats and oils (Baiao & Lara, 2005). Therefore, research is needed for better understanding of the different characteristics on the effect of birds' performance.

Oil palms trees- *Elaesis guineensis* originated from western Africa, is the highest edible oil-yielding plant. Palm kernel oil is produced from the kernel of an oil palm tree, and the fruit of the oil palm consists of an outer pulp which is used for the production of crude palm oil (CPO) (O'Brien, 2009; Sambanthamurthi et al., 2000). Shown in Table

3.1 and 3.2, CPO consists of similar amounts (approximately 50:50) of saturated and unsaturated FAs (with around 40% monounsaturated FAs and 10% polyunsaturated FAs of total FAs), and unique feature with 10%-16% of the saturated FAs in sn-2 position in triglycerides. The structural properties are similar to tallow. Palm oil consists mainly of triglycerides (high levels of palmitic and oleic FAs) and is semisolid at room temperature. CPO displays an orange red colour due to high concentration of carotene. CPO also contains sufficient amount of tocopherols and tocotrienols, which are natural antioxidants (Kolani et al., 2018; O'Brien, 2009).

Table 3.1. Palm Oil Composition and Physical Properties.

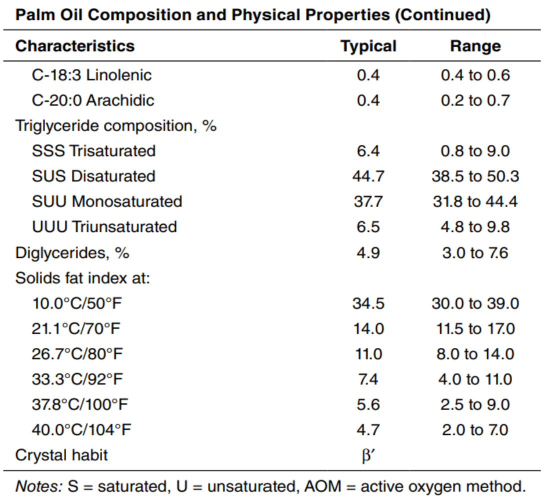


Palm Oil Composition and Physical Properties

Characteristics	Typical	Range
Specific gravity, 50°C	—	0.888 to 0.889
Refractive index, 50°C	—	1.455 to 1.456
Iodine value	53	46 to 56
Saponification number	196	190 to 202
Unsaponifiable matter, %	0.5	0.15 to 0.99
Titer, °C	46.3	40.7 to 49.0
Mettler dropping point, °C	37.5	35.5 to 45.0
Solidification point, °C	—	35.0 to 42.0
Cold test, hours	none	none
Carotene content, mg/kg	—	500 to 700
AOM stability, hours	54	53 to 60
Oxidative stability index (110°C), hours	16.9	16.6 to 19.0
Tocopherol content, ppm		
α-tocopherol	172	129 to 215
β-tocopherol	30	22 to 37
γ-tocopherol	26	19 to 32
δ-tocopherol	13	10 to 16
Tocotrienol content, ppm		
α-tocotrienol	59	44 to 73
β-tocotrienol	59	44 to 73
γ-tocotrienol	350	262 to 437
δ-tocotrienol	94	70 to 117
Fatty acid composition, %		
C-12:0 Lauric	0	0.1 to 1.0
C-14:0 Myristic	1.1	0.9 to 1.5
C-16:0 Palmitic	44.0	41.8 to 46.8
C-16:1 Palmitoleic	0.1	0.1 to 0.3
C-18:1 Stearic	4.5	4.5 to 5.1
C-18:1 Oleic	39.2	37.3 to 40.8
C-18:2 Linoleic	10.1	9.1 to 11.0

(Source: O'Brien, 2009)

Table 3.2. Palm Oil Composition and Physical Properties (Continued).



Palm Oil Composition and Physical Properties (Continued)

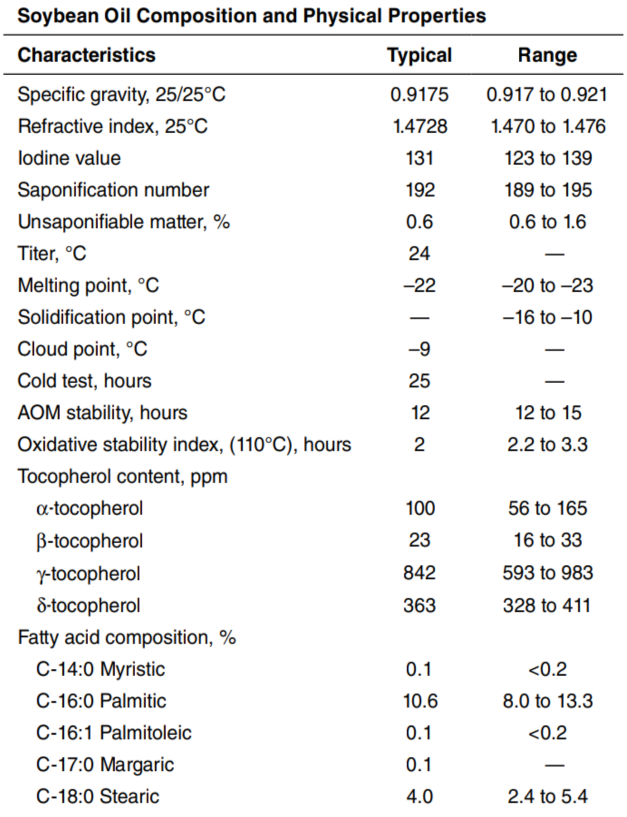
Characteristics	Typical	Range
C-18:3 Linolenic	0.4	0.4 to 0.6
C-20:0 Arachidic	0.4	0.2 to 0.7
Triglyceride composition, %		
SSS Trisaturated	6.4	0.8 to 9.0
SUS Disaturated	44.7	38.5 to 50.3
SUU Monosaturated	37.7	31.8 to 44.4
UUU Triunsaturated	6.5	4.8 to 9.8
Diglycerides, %	4.9	3.0 to 7.6
Solids fat index at:		
10.0°C/50°F	34.5	30.0 to 39.0
21.1°C/70°F	14.0	11.5 to 17.0
26.7°C/80°F	11.0	8.0 to 14.0
33.3°C/92°F	7.4	4.0 to 11.0
37.8°C/100°F	5.6	2.5 to 9.0
40.0°C/104°F	4.7	2.0 to 7.0
Crystal habit	β′	

Notes: S = saturated, U = unsaturated, AOM = active oxygen method.

(Source: O’Brien, 2009)

Soy oil (SO) is obtained from soybeans, *Glycina maxima*, which are grown in many countries around the world. It has become a popular vegetable oil due to its high nutritional quality, abundance, economical value, and wide functionality. Shown in Table 3.3 and 3.4 SO contains a high concentration of polyunsaturated FA. The triglyceride structure of SO is characterized by an almost complete absence of saturated FAs in its sn-2 position of the triglyceride structure, random distribution of oleic and linolenic FAs on all glycerol position and has high level of linolenic FAs in the sn-2 position (O’Brien, 2009). The soybean oil composition shown in Figure 3.4 mainly consist of unsaturated FAs with small proportion of saturated FAs, which is consistent with result shown in study by Saleh et al. (2021).

Table 3.3. Soybean Oil Composition and Physical Properties.

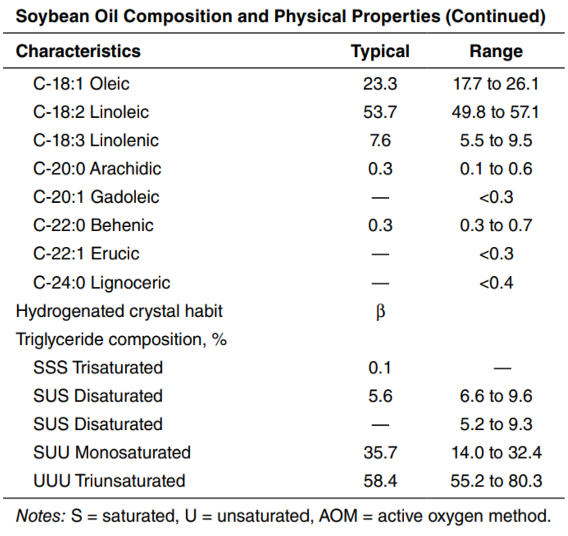


Soybean Oil Composition and Physical Properties

Characteristics	Typical	Range
Specific gravity, 25/25°C	0.9175	0.917 to 0.921
Refractive index, 25°C	1.4728	1.470 to 1.476
Iodine value	131	123 to 139
Saponification number	192	189 to 195
Unsaponifiable matter, %	0.6	0.6 to 1.6
Titer, °C	24	—
Melting point, °C	–22	–20 to –23
Solidification point, °C	—	–16 to –10
Cloud point, °C	–9	—
Cold test, hours	25	—
AOM stability, hours	12	12 to 15
Oxidative stability index, (110°C), hours	2	2.2 to 3.3
Tocopherol content, ppm		
α-tocopherol	100	56 to 165
β-tocopherol	23	16 to 33
γ-tocopherol	842	593 to 983
δ-tocopherol	363	328 to 411
Fatty acid composition, %		
C-14:0 Myristic	0.1	<0.2
C-16:0 Palmitic	10.6	8.0 to 13.3
C-16:1 Palmitoleic	0.1	<0.2
C-17:0 Margaric	0.1	—
C-18:0 Stearic	4.0	2.4 to 5.4

(Source: O’Brien, 2009)

Table 3.4. Soybean Oil Composition and Physical Properties (Continued)



Soybean Oil Composition and Physical Properties (Continued)

Characteristics	Typical	Range
C-18:1 Oleic	23.3	17.7 to 26.1
C-18:2 Linoleic	53.7	49.8 to 57.1
C-18:3 Linolenic	7.6	5.5 to 9.5
C-20:0 Arachidic	0.3	0.1 to 0.6
C-20:1 Gadoleic	—	<0.3
C-22:0 Behenic	0.3	0.3 to 0.7
C-22:1 Erucic	—	<0.3
C-24:0 Lignoceric	—	<0.4
Hydrogenated crystal habit	β	
Triglyceride composition, %		
SSS Trisaturated	0.1	—
SUS Disaturated	5.6	6.6 to 9.6
SUS Disaturated	—	5.2 to 9.3
SUU Monosaturated	35.7	14.0 to 32.4
UUU Triunsaturated	58.4	55.2 to 80.3

Notes: S = saturated, U = unsaturated, AOM = active oxygen method.

(Source: O’Brien, 2009)

Poultry fat (Sirona) derived from various avian species such as chickens and turkeys, is a valuable byproduct of the meat industry. Poultry fat is the birds’ viscera fat and is obtained from extraction of poultry waste or rendering bird feathers which is heated, disinfected, and autoclaved in a percolator tank and expeller. Poultry fat is a complex mixture of lipids that includes saturated FAs, monounsaturated FAs, and polyunsaturated FAs. The specific composition varies based on factors such as the bird's diet, genetics, and processing methods. Study done by Saleh et al. (2021) used poultry fat consisting of around 30.3% saturated FAs, and large proportion of unsaturated FAs around 69.1% (with 37.19 % monounsaturated FAs and 31.94% polyunsaturated FAs of total FA). The saturated FAs in poultry fat generally consist of large amount of palmitic and stearic acids (Scott et al., 1982). Poultry fat contains high concentration of unsaturated FA (Baiao & Lara, 2005). Monounsaturated FAs such as oleic acid and polyunsaturated FAs, including essential omega-3 and omega-6 fatty acids, are also present and contributed to the nutritional value. The source of poultry fat depends on the content of the birds and can vary greatly. The range of energy can be affected by the birds’ sex, weight of the birds, and other factors, which may result less uniformity in the diet nutrient level (Ravindran et al., 2016).

In this experiment, different sources of lipid were incorporated into maize-soybean meal based diet containing similar levels of AME and fed to the birds. Effects of AME, ileal nutrient digestibility of nitrogen (N), dry matter (DM), fat, starch and performance parameters of weight gain, feed intake, feed conversion ratio, and mortality were investigated.

3.2 Material and Methods

3.2.1 Experimental diets

This study used 2 x 3 factorial arrangement of 6 treatments with 8 replicates, and 8 birds per replicate to study the effect of two lipid levels: High (H) and Low (L) and three lipid types: Crude Palm Oil (CPO), Soy Oil (SO) and Poultry Fat (Sirona, PF). Six experimental diets (CPOH, CPOL, SOH, SOL, PFH and PFL) were formulated (Table 3.5). The CPO used in this experiment was supplied by Kemin Industries whereas SO and PF are sourced within New Zealand. The diets were formulated using maize and soybean as the main ingredients. The inclusion levels of the feed ingredients and lipid vary between the diets, achieving different levels of energy and nutrient density. Shown in Table 3.6, the lipid sources provided the birds with similar levels of AME within the same lipid level. Diets were formulated on AME within lipid type (H = L + 100Kcal/kg) (Table 3.6) with L level diets formulated to contain 6% of crude fat. H level diets were formulated with more lipids than the L level diets, with an extra 100Kcal AME. Shown in Table 3.5, H level diets contain 5.98% SO, 6.514% SPO and 5.89% PF individually. The L level diets separately contain 4.015% SO, 3.948% CPO and 3.759% PF. Pellet quality is affected when lipid inclusion is higher than 4% (Abdollahi et al., 2013b). Using 6% crude fat or higher inclusion level in the diet for investigating if the high energy level will make up the low pellet quality.

All six diets were supplemented with mineral and vitamin premixes. Titanium dioxide of 5 g/kg was added to all the diets as indigestible marker to measure ileal nutrient digestibility. The diets were mixed in a single-screw paddle machine, then transferred to pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) for pelleting. The length of the pellet formed for starter diet was measured to be approximately 3.0 mm. Representative samples of steam-pelleted diets were collected and underwent the pellet durability test for pellet durability index (PDI).

Table 3.5. Composition of broiler starter diets (1-21 days) (g/kg as fed).

Ingredients	**Diets**					
	CPOH	**CPOL**	**SOH**	**SOL**	**PFH**	**PFL**
Maize	52.119	55.175	52.749	55.087	52.856	55.393
Soybean meal	37.512	37.021	37.41	37.034	37.392	36.985
Soy oil	-	-	5.98	4.015	-	-
Crude palm oil	6.514	3.948	-	-	-	-
Poultry fat	-	-	-	-	5.89	3.759
Limestone	1.064	1.07	1.065	1.07	1.066	1.07
Dicalcium phosphate	0.775	0.772	0.744	0.772	0.774	0.771
Sodium chloride	0.133	0.128	0.132	0.128	0.132	0.127
Sodium bicarbonate	0.345	0.348	0.346	0.348	0.346	0.349
DL Methionine	0.282	0.278	0.281	0.278	0.281	0.277
Lysine HCl	0.286	0.294	0.288	0.293	0.288	0.294
L Threonine	0.169	0.17	0.169	0.17	0.169	0.17
L Valine	0.022	0.022	0.022	0.022	0.022	0.022
Vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1
Mineral premix	0.1	0.1	0.1	0.1	0.1	0.1
Choline chloride 60%	0.078	0.076	0.077	0.076	0.077	0.076
Titanium dioxide	0.5	0.5	0.5	0.5	0.5	0.5
Phytase	0.007	0.007	0.007	0.007	0.007	0.007
Total	100	100	100	100	100	100

Crude palm oil High=CPOH; Crude palm oil Low=CPOL; Soy oil High=SOH; Soy oil Low=SOL; Poultry fat High=PFH; Poultry fat Low=PFL.
Lipid types= CPO, SO and PF. Lipid levels= H and L.

Table 3.6. Calculated Chemical composition (%, as is) of the broiler starter diets (1-21 days).

Composition	**Diets**					
	CPOH	**CPOL**	**SOH**	**SOL**	**PFH**	**PFL**
AME (kcal/kg)	3113	3013	3163	3063	3136	3036
Crude protein	22	22	22	22	22	22
Crude fat	8.3	6	7.7	6	8	6
Crude fibre	2.79	2.84	2.8	2.83	2.8	2.84
Total calcium (+Phytase Matrix)	0.77 (0.92)	0.77 (0.92)	0.77 (0.92)	0.77 (0.92)	0.77 (0.92)	0.77 (0.92)
Total phosphorus (P)	0.52	0.52	0.52	0.52	0.52	0.52
Non phytate P (+Phytase Matrix)	0.31 (0.46)	0.31 (0.46)	0.31 (0.46)	0.31 (0.46)	0.31 (0.46)	0.31 (0.46)
Phytate P	0.21	0.21	0.21	0.21	0.21	0.21
Chloride	0.19	0.19	0.19	0.19	0.19	0.19
Sodium	0.19	0.19	0.19	0.19	0.19	0.19
Potassium	1.12	1.12	1.12	1.12	1.12	1.12
Choline (mg/kg)	1700	1700	1700	1700	1700	1700
Starch	32.7	34.6	33.1	34.5	33.2	34.7
Digestible lysine	1.15	1.15	1.15	1.15	1.15	1.15
Digestible methionine + cysteine	0.854	0.854	0.854	0.854	0.854	0.854
Digestible threonine	0.773	0.773	0.773	0.773	0.773	0.773
Digestible valine	0.863	0.863	0.863	0.863	0.863	0.863

Crude palm oil High=CPOH; Crude palm oil Low=CPOL; Soy oil High=SOH; Soy oil Low=SOL; Poultry fat High=PFH; Poultry fat Low=PFL.

Lipid types= CPO, SO and PF. Lipid levels= H and L.

3.2.2 Birds and housing

The experiment was conducted at Massey University Poultry Unit. The experiment procedures described in this thesis were approved by the Massey University Animal Ethics Committee (MUAEC approval numbers: MU22/82 and MU22/84) and complied with the New Zealand Revised Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching.

A total of 384 day-old male broilers (Ross 308) were obtained from a commercial hatchery, and individually weighed and allocated on the basis of body weight (to achieve similar average bird weight per cage) to 48 cages with 8 birds per cage in electrically heated battery brooders. The birds were fed the pelleted diet offered *ad libitum* from day 1 to 21 days post-hatch, feed trays were placed inside the cage with pellet mixed with water for the day-old chicks to gain easy access until capable of consuming pellet from feed troughs.

During the first 12 days post-hatch, each cage had 8 birds with the stocking density of 530 cm^2 per bird. While, for the remaining period (13-21 d) the birds were moved to another shed with larger cages, the same number of 48 cages were used, 8 birds per cage and the space allocation per bird was 640 cm^2. This was done to accommodate the increase in bird size, at the same time maintaining the respective replicates. Throughout the experimental period, birds were kept in an environmentally controlled poultry house supplying fluorescent illumination 20 hours per day. The temperature was maintained at 31 °C on day 1, and gradually reduced to 22 °C by 21 days of age. The nipple drinkers and feed troughs were provided in the cages. Fresh and clean water were readily available, and feed was provided *ad libitum* throughout the experiment period.

3.2.3 Pellet durability

Pellet durability index (PDI) of pelleted diets (L and H level diets) was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK). Pellets were weighed to get 100g per sample, then sieved with care to prevent fines from pouring into the tester. Then, air pressure rapidly circulate (Pneumatic tumbling) the sample around a perforated test chamber for thirty seconds. During the test, fines were continuously removed through a 2.0 mm sieve. At the end of the test, pellets were collected out of the tester and weighed manually. The PDI was calculated as the relative proportion by weight of the retained pellets after the test to the pellets before the start (100g).

3.2.4 Performance data and uniformity

Mortality and the probable cause were recorded daily throughout the experimental period. Feed intake and body weight of birds from each pen were recorded at weekly intervals until the end of the trial, with weight recorded when birds first arrived, birds were weighed four times in total during the entire trial. Feed per unit gain was corrected for body weight of any dead bird. Uniformity was determined based on the percentage of birds that were in the range of plus/minus 15 % from the mean average weight in each replicate.

3.2.5 Total tract retention and AME

Feed intake, and excreta output were collected from each cage from day 18 to 21 post-hatch for determination of the DM, nitrogen, gross energy (GE), and fat. The daily collected excreta from each pen were pooled, mixed in a blender, and sub-sampled.

The sub-samples were then freeze-dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand), and ground to facilitate the passing of the sample through a 0.5mm sieve, the grounded samples were stored in airtight plastic bag at 4 °C for chemical analysis.

3.2.6 Ileal nutrient digestibility assay

On day 21 post-hatch, following the finishing of AME assay, all birds were euthanised by intravenous injection (0.5 mL per kg body weight) of sodium pento-barbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand). The digesta were collected from the lower half of the ileum. The ileum was the portion of the small intestine extending from the Meckel’s diverticulum to approximately 40 mm to the ileocecal junction. To collect the digesta, the ileum was removed from the birds’ carcass and divided into halves (proximal and distal ileum). The digesta samples were collected from the lower half of the ileum towards the ileocecal junction by gently flushing using syringes filled with distilled water into plastic bags. The ileal digesta from birds within a cage were flushed into the same bag. After collection, the digesta were frozen immediately and then freeze dried. Freeze dried digesta samples were grounded to pass through a 0.5-mm sieve and stored in airtight plastic bag at 4 °C pending chemical analyses (DM, N, titanium, GE, starch, and fat).

The diets, ileal digesta samples, and excreta samples were analysed for DM, N, GE, titanium, starch, and fat at the Nutrition Laboratory of the Institute of Food Science and Technology, at Massey University.

3.2.7 Chemical analysis

Lipid samples were analysed for lipid quality and profile tests (peroxide value, p-Anisidine value, FFA content, FA profile, iodine value). For the determination of lipid content of the diet, ileal digesta and excreta, the Soxhlet extraction method (method 991.36; AOAC, 2005) was used. Adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) was used to determine gross energy, standardised with benzoic acid. Dry matter content was determined in a convection oven at 105 °C (AOAC 930.15; AOAC 925.10). The samples were tested for Ti on a UV spectrometer in accordance with the method described by Short et al. (1996). The CP content was calculated as N × 6.25. Starch content was measured using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable alpha-amylase and amyloglucosidase. Nitrogen content was determined by Dumas combustion method (method 968.06; AOAC, 2005) using a CNS-200 carbon, nitrogen, and sulphur auto analyser (LECO® Corporation, St. Joseph, MI, USA).

3.2.8 Calculations

The apparent metabolisable energy was calculated using the following formula, with the correction for the differences in dry matter:

$$\text{AME (MJ/kg diet)} = [(\text{FI} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})] / \text{FI}$$

The following formula was used to calculate the coefficient of apparent ileal digestibility (CAID):

$$\text{CAID of diet nutrient} = [(\text{Nutrient/Ti})_{\text{diet}} - (\text{Nutrient/Ti})_{\text{ileal}}] / (\text{Nutrient/Ti})_{\text{diet}}$$

Where $(\text{Nutrient/Ti})_{\text{diet}}$ = ratio of nutrient to Ti in the diet, and

$(\text{Nutrient/Ti})_{\text{ileal}}$ = ratio of nutrient to Ti in the ileal digesta.

3.2.9 Statistical Analysis

To determine the main effect (lipid source and lipid level) data were analysed as a 2 x 3 factorial (2 fixed main effects and their interaction) using the General Linear Models procedure of SAS (version 9.4; 2015). Cage served as the experimental unit. The differences were considered statistically significant at $P < 0.05$, and significant differences between means were separated by Least Significant Difference tests.

3.3 Results

The analysed chemical composition of the diets is presented in Table 3.7 and the FA profile of the different type of lipids used in this study is presented in Table 3.8.

Table 3.7. Analysed chemical composition of the diets (g/100g DM basis).

Composition	**Diet**					
	CPOH	**CPOL**	**SOH**	**SOL**	**PFH**	**PFL**
DM	89.19	88.72	88.85	88.32	89.59	89.05
CP	3.99	3.95	3.96	3.98	4.10	4.15
Fat	10.37	8.21	9.20	8.35	9.91	6.34
Starch	34.80	37.79	36.28	37.37	33.64	35.10
TiO_2, %	0.56	0.56	0.56	0.57	0.56	0.56
GE	19.25	18.72	19.14	18.75	19.09	18.76

Dry matter= DM. Crude protein= CP. Gross energy= GE.
Crude palm oil High=CPOH; Crude palm oil Low=CPOL; Soy oil High=SOH; Soy oil Low=SOL; Poultry fat High=PFH; Poultry fat Low=PFL. Lipid types= CPO, SO and PF. Lipid levels= H and L.

Results in Table 3.8. showed notable variation in the composition of saturated, monounsaturated, and polyunsaturated fats across each lipid source. CPO consists of

39.8% saturated FAs, which is the highest of the three lipid sources. However, CPO constitute mostly of unsaturated FAs, with monounsaturated FAs accounts for the largest proportion (38%) in CPO, and with the combination of 22% polyunsaturated FAs, lead to a total unsaturated FAs of 60%. The level of unsaturated FAs included in CPO is the lowest compared to other lipid sources. On the other hand, SO presented a different profile with the highest levels of unsaturated FAs in all lipid sources, adding to a total of 89.79% with 23.43% monounsaturated fat, and a significant concentration of polyunsaturated fat of 66.36%. Saturated fat is least proportional in SO and when compared to the other two sources at 16.6%. PF consists of 33.01% saturated fats and a higher level of unsaturated FAs of 68.91% with 53.76% monounsaturated fats, and polyunsaturated fat at 15.15%.

Table 3.8. Fatty acid profile of the different lipid types (g/100 g FA).

Fatty Acid Profile g/100g	Crude Palm Oil	Soy Oil	Poultry Fat (Sirona)
C4:0 Butyric	<0.010	-	-
C6:0 Caproic	<0.010	ND	0.008
C8:0 Caprylic	<0.010	<0.010	0.012
C10:0 Capric	<0.010	<0.010	0.020
C11:0 Undecanoic	-	ND	0.005
C12:0 Lauric	0.100	0.015	0.092
C13:0 Tridecanoic	-	ND	0.007
C14:0 Myristic	0.640	0.075	0.739
C14:1n5 - cis-9-Myristoleic	<0.010	ND	0.235
C15:0 Pentadecanoic	0.033	-	-
C15:1n5 - cis-10-Pentadecenoic	<0.010	0.006	ND
C16:0 Palmitic	32.720	10.868	25.204
C16:1n7 - cis-9-Palmitoleic	0.150	0.080	6.962
C17:0 Margaric	0.085	0.146	0.215
C17:1n7 - cis-10-Heptadecenoic	0.028	ND	ND
C18:0 Stearic	3.780	4.581	6.631
C18:1n9t Elaidic	<0.010	0.025	0.288
C18:1n7t Vaccenic	0.850	ND	0.143
C18:1n9c Oleic	35.170	22.650	45.660
C18:1n7c Vaccenic	-	1.126	2.192
C18:2n6t Linolelaidic	-	ND	ND
C18:2n6c Linoleic	19.420	59.672	13.241

C18:2 CLA 9c, 11t	<0.010	-	-
C18:3n3 - cis-9,12,15-Alpha linolenic	1.630	6.646	1.175
C18:3n-4 Octadecatrienoic acid	<0.010	-	-
C18:3n6 - cis-6,9,12-Gamma linolenic	<0.010	ND	0.149
C18:4n-3 Steridonic	<0.010	-	-
C20:0 Arachidic	0.340	0.368	0.061
C20:1 Eicosenoic (total)	0.140	-	-
C20:1n9 - cis-11-Eicosenoic	0.140	0.665	0.440
C20:1n-11,13 Eicosenoic	<0.010	-	-
C20:2n6 - cis-11,14-Eicosadienoic	<0.010	0.041	0.098
C20:3n3 - cis-11,14,17-Eicosatrienoic	<0.010	ND	0.012
C20:3n6 - cis-8,11,14-Eicosatrienoic	<0.010	ND	0.102
C20:44n-3 Eicosatetranoic	<0.010	-	-
C20:4n6 - cis-5,8,11,14-Arachidonic	<0.010	ND	0.253
C20:5n3 - cis-5,8,11,14,17-Epa	<0.010	ND	0.038
C21:0 Heneicosanoic	<0.010	0.037	0.017
C21:5n-3 Heneicosapentaenoic acid	<0.010	-	-
C22:0 Behenic	0.140	0.377	0.007
C22:1n9 - cis-13-Erucic	<0.010	ND	0.016
C22:1n-11,13 Docosenoic	<0.010	-	-
C22:2n6 - cis-13,16-Docosadienoic	-	ND	ND
C22:4n-6 Docosatetraenoic	<0.010	-	-
C22:5n3 - cis-7,10,13,16,19-DPA	0.023	ND	0.052
C22:5n-6 Docosapentaenoic	<0.010	-	-
C22:6n3 - cis-4,7,10,13,16,19-DHA	<0.010	ND	0.033
C23:0 Tricosanoic	-	0.050	ND
C24:0 Lignoceric	0.072	0.136	ND
C24:1n9 - cis-15- Nervonic	0.036	0.006	0.012
Total linoleic acid	19.560	59.67	13.240
Total linolenic acid	1.650	6.650	1.320
Trans fat content	0.200	-	-
Omega 3 total	1.650	6.650	1.310
Omega 6 total	19.420	59.710	13.840
Omega 9 total	35.350	23.350	46.400
Saturated fat	39.800	16.600	33.010
Mono unsaturated fat	38.000	23.430	53.760
Poly unsaturated fat	22.000	66.360	15.150

3.3.1 Pellet durability index

The values of pellet durability index (PDI) are presented in Table 3.9. They were significantly lower for the high level of lipid inclusion than the low level (P<0.0001). And were the highest for PF, and the lowest for SO, the values for CPO were in between (P<0.0001). The interaction between lipid type and level was significant (*P*<0.0001), whereas the difference between the high and the low level was different for the different type of lipid.

Table 3.9. Pellet durability index values of the six types of diets (g/100g).

Lipid type	Level	Pellet Durability Index
CPO	H	9.0^{b}
CPO	L	39.7^{e}
SO	H	6.5^{a}
SO	L	31.9^{d}
PF	H	10.9^{c}
PF	L	43.8^{f}
SEM		0.46
Main Effects		
Lipid type		
CPO		24.4^{b}
SO		19.2^{a}
PF		27.4^{c}
SEM		0.33
Level		
H		8.8^{a}
L		38.5^{b}
SEM		0.27
Probability		
Lipid type		<0.0001
Level		<0.0002
Lipid type x Level		<0.0003

Crude palm oil=CPO; Soy oil=SO; Poultry fat=PF. High= H, Low= L.

Means that have no superscript in common are significantly different from each other (P<0.05).

3.3.2 Growth performance

From day 1 to 21 post-hatch, birds on both lipid levels and different lipid type did not have high mortality rate. Mortality was not influenced by the lipid level, lipid sources and the interaction ($P > 0.05$).

Table 3.10 summarises that both main effects of lipid source and inclusion level can significantly impact birds' body weight gain, as ($P < 0.05$). Weight gain of the birds fed the L level diet was higher than that of the birds fed the H level diets, regardless of the lipid sources. When comparing the lipid sources with the same lipid level, PF resulted in the greatest weight gain in broilers, with slight lower weight gain observed in birds fed SO diets, and birds on CPO had the lowest BWG. However, the lipid sources x lipid level interaction did not influence the weight gain of birds ($P > 0.05$).

The different lipid source x lipid level interaction did not cause significant impact ($P > 0.05$) to the birds' amount of feed intake. However, the different lipid level ($P < 0.05$) significantly impacted the amount of feed intake by birds. Birds fed L level diets consumed more feed than birds on H level diets for every lipid source. There was a tendency ($P = 0.066$) for the different lipid sources to affect birds' feed intake in birds fed diets consisting of PF had slightly increased feed intake than birds on SO and CPO diets.

The interaction between lipid sources and lipid level did not influence the broilers' FCR ($P > 0.05$). However, lipid type had significant effect ($P < 0.05$) on the birds' FCR with birds fed with CPO diets had the highest FCR. FCR was reduced in birds fed the SO diet which was not much different from the birds fed the PF diet. On the other hand, lipid level did not cause significant effect ($P > 0.05$) on the birds' FCR. Although lipid level ($P = 0.056$), there was a tendency for lipid level to influence the FCR, as broilers on L level diets had slightly higher FCR than broilers on H level diets.

Table 3.10. Influence of dietary treatments on the performance parameter of body weight gain (BWG) (g/bird), feed intake (FI) (g/bird), feed conversion ratio (FCR) (g feed/g fain), and mortality (%) in broiler starters (1-21 days post-hatch).

		Performance Parameters			
Lipid type	Level	BWG (g/bird)	FI (g/bird)	FCR	Mortality
CPO	H	976.4	1166.9	1.198	1.563
CPO	L	995.5	1192.0	1.201	4.688
SO	H	989.0	1154.9	1.169	1.563
SO	L	1016.7	1202.6	1.187	1.563
PF	H	1015.9	1189.3	1.182	7.813
PF	L	1040.1	1229.4	1.193	4.688
SEM		12.51	14.50	0.0068	2.3868
Main Effects					
Lipid type					
CPO		986.0 b	1179.5	1.199 a	3.125
SO		1002.8 ab	1178.7	1.178 b	1.563
PF		1028.0 a	1209.4	1.188 ab	6.250
SEM		8.84	10.25	0.0048	1.6877
Level					
H		993.8 b	1170.4 b	1.183	3.646
L		1017.5 a	1208.0 a	1.194	3.646
SEM		7.22	8.37	0.0039	1.3780
Probability					
Lipid type		0.006	0.066	0.013	0.148
Level		0.025	0.003	0.056	1.000
Lipid type x Level		0.941	0.731	0.561	0.432

Crude palm oil=CPO; Soy oil=SO; Poultry fat=PF. High= H, Low= L.

Means in a column not sharing a common superscripts are significantly different ($P < 0.05$).

Mortality (%) was recorded as count of birds' mortality per treatment (8 birds per cage) and 8 replicates per treatment, which resulted in the decimals.

Each value represents the mean of 8 replicates.

Sample collection occurs at weekly intervals (four times) during the 21 days.

3.3.3 Nutrient and energy utilisation

Table 3.11 summarises the apparent ileal digestibility of nutrients and AME in broilers. The coefficient of CAID of N and starch was not affected ($P > 0.05$) by the type of lipid used, lipid level and their interaction.

The lipid level, and the interaction of lipid sources x lipid level did not significantly affect ($P > 0.05$) the CAID of DM, fat, and GE. However, the different lipid sources caused significant effect ($P < 0.05$) to the CAID of DM, fat and GE. The birds fed diets that contain PF had the lowest CAID of DM, fat, and GE, and birds had diet consist of CPO had higher level. Birds on diets that included SO had the highest CAID of DM, fat, and GE compared to the others.

The lipid sources x lipid level interaction did not have a significant effect ($P > 0.05$) on the birds' AME. On the other hand, the different lipid sources and lipid level separately had significant influence ($P < 0.05$) on the birds' AME. Birds on diet containing PF had the lowest CAID of AME, followed by birds fed diet with CPO had increased CAID of AME. Lastly, birds on diet consisting of SO had the highest CAID of AME. Also, broilers on H level diets had higher CAID of AME compared to the broilers on L level diets.

Table 3.11. Influence of dietary treatments on the coefficient of apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), fat, starch, gross energy (GE), and apparent metabolisable energy (AME, MJ/kg DM) in broiler starters (1-21 d).

		Nutrient Digestibility					AME
Lipid type	Level	N	DM	Fat	Starch	GE	AME
CPO	H	0.761	0.660	0.888	0.964	0.709	14.800
CPO	L	0.774	0.651	0.892	0.963	0.704	14.390
SO	H	0.795	0.672	0.925	0.960	0.727	14.900
SO	L	0.775	0.650	0.886	0.962	0.704	14.531
PF	H	0.771	0.629	0.860	0.957	0.684	14.526
PF	L	0.776	0.623	0.854	0.957	0.676	14.186
SEM		0.0107	0.0114	0.0151	0.0029	0.0102	0.0677
Main Effects							
Lipid type							
CPO		0.767	0.655 a	0.89 a	0.963	0.707 a	14.595 a
SO		0.785	0.661 a	0.906 a	0.961	0.715 a	14.715 a
PF		0.773	0.626 b	0.857 b	0.957	0.680 b	14.356 b
SEM		0.0076	0.0081	0.0107	0.0021	0.0072	0.0478
Level							
H		0.775	0.654	0.891	0.961	0.707	14.742 a
L		0.775	0.641	0.878	0.960	0.695	14.369 b
SEM		0.0062	0.0066	0.0087	0.0017	0.0059	0.0391
Probability							
Lipid type		0.265	0.008	0.008	0.088	0.004	<.0001
Level		0.940	0.186	0.284	0.944	0.156	<.0001
Lipid type x Level		0.261	0.763	0.327	0.877	0.644	0.873

Crude palm oil=CPO; Soy oil=SO; Poultry fat=PF. High= H, Low= L.

Means in a column not sharing a common superscripts are significantly different ($P < 0.05$).

Each value represents the mean of 8 replicates.

Ileal digesta collection occurs on day 21 post-hatch. Excreta output were collected from each cage from day 18 to 21 post-hatch.

3.4 Discussion

The data in Table 3.10 and 3.11 showed that all the interactions of different lipid sources x lipid level are not significant. The different lipid sources incorporated with H level diets do not have significantly higher or lower value than with L level diets. The lack of interaction indicated that the fat sourced acted the same regardless of fat level. So, the interaction of lipid sources and lipid level did not significantly affect and differ the birds' ileal nutrient digestibility, AME, and performance.

The high and low lipid level did not significantly affect the nutrient digestibility of the birds for N, DM, fat, starch, and GE. Study done by Whitehouse (2018) showed that added dietary fat in broiler diets can improves the bird's ileal digestibility of nutrients. However, the difference between the levels of lipid included may not be large enough to cause the ileal digestibility of nutrients to differ. Also, the ileal digestibility of nutrients can be affected by the type of cereal grain used in diet, as the same soybean and maize is used for all six diets in this experiment, it may eliminate other factors that may affect ileal digestibility (Zaefarian et al. 2015).

Lipid level had a tendency to affect the birds' FCR which the birds on L level diets have slightly higher value of FCR than birds on H level diets. Which can be explained by research from AL-Jaf et al. (2018), as the result showed adding 4% canola oil to broiler birds' diet significantly improved the birds' FCR, compared to the control group that did not include fats and oils. The 4% difference between lipid inclusion level caused a significant effect, which may justify the tendency of lipid level affecting the birds' FCR as the H and L level diets have 1.965%-2.566% difference between lipid inclusion level.

On the other hand, different lipid level inclusion of H level diets allowed birds to have significantly higher AME than birds on L level diets, which is consistent with other studies (Al-Khalaifah & Al-Nasser,2020; Whitehouse, 2018; Wiseman, 1990). As diets with added lipid had higher digestibility compared to same diets without added lipids. The higher lipid content and improved digestibility likely resulted in the higher AME value in H level diets. In contrast, birds fed L level diets have higher amount of feed intake and body weight gain than birds fed with H level diets. This is due to L level diets have less AME, in order to consume enough energy for maintenance and growth, the birds on L level diets require larger amount of feed to meet their daily energy requirement. This may cause the increased amount of feed intake and body weight gain compared to H level diets, as birds on H level diets do not require large amount of feed because the higher energy content feed allow them to achieve their daily energy intake in less amount of feed (Al-Khalaifah & Al-Nasser,2020; Whitehouse, 2018; Wiseman, 1990). Therefore, the amount of feed intake and body weight gain is less than birds on L level diet, which is consistent with result from Wiseman. (1990) and Tavárez et al. (2011). Also, the higher lipid level can also develop rancid flavour that reduced the palatability of the diets. Which is consistent with the significantly higher feed intake of the L level diet (Tavárez et al., 2011).

The three different types of lipid sources included in the diets (CPO, SO, and PF) significantly affected the birds' nutrient digestibility of DM, fat, and GE. With diet containing SO the highest, CPO less, lastly, diet consist of PF the least, which might be result of compositional and structural difference between plant-based and animal-based fat sources (Scott et al., 1982). Plant-based lipid sources tend to have higher concentrations of unsaturated FAs which can be digested more easily compared to animal-based fat sources (Rodriguez-Sanchez et al., 2019). Animal fat sources contain large proportion of saturated FAs and are more difficult to digest, especially palmitic and stearic acids are poorly digested (Sklan, 1979). SO had the highest CAID might be the result of consisting of the largest level of unsaturated FAs and the least number of saturated FAs. Including lipid in the diet increased digesta retention time in the digestive tract. Lipid sources with long chain FA, such as soybean oil, tend to have longer retention time than short chain FAs (Hurwitz et al. 1979). Comparing the diets as to the three lipid types, CPO diets consist the largest proportion of saturated FAs, and the least proportion of unsaturated FAs. Diets containing PF had the lowest CAID of DM, fat GE and AME, it contains relatively high saturated FAs and low level of unsaturated FAs. Van Kuiken and Behnke (1994) had highlighted that stearic acid is long chain saturated FAs that inhibit the activity of lipase, which by poultry fat has a higher content of stearic acid (6.63%) than CPO (3.78%). In addition, mono- unsaturated FAs are less digestible than poly-unsaturated FAs. Thus, unsaturated FAs content of poultry fat mostly consist of mono-unsaturated FAs which is much more than CPO, this might result the lower digestibility (Scott et al., 1982). However, this is inconsistent with research done by Tancharoenrat (2012) that poultry fat had higher lipid digestibility than palm oil and AME of palm oil is similar to poultry fat.

On the other hand, the different lipid sources did not significantly affect the nutrient digestibility of N and starch. Studies (Allahyari-Bake & Jahanian, 2017; Jimenez-Moreno et al., 2009; Whitehouse, 2018) reported differences in the digestibility of N between diets including soybean oil, palm oil and tallow, with different results of higher digestibility recorded in tallow and other soybean oil. In general, diets that included tallow had the highest N digestibility as lower ability to form micelles cause longer gut transit time for further digestion, which is contradictory with the present result (Whitehouse, 2018). This might be due to CPO is structural similar to tallow consisting large amount long-chain saturated FAs of high levels of palmitic and oleic FAs (Kolani et al., 2018; O’Brien, 2009; Renner and Hill, 1961; Scott et al., 1982). Also, in this case, the PF had high proportion of unsaturated FAs which is less than SO but higher than CPO which did not cause much longer gut retention time for further digestion of N (Whitehouse, 2018). Which can also explain the contradictory to the study done by Jimenez-Moreno et al. (2009) showed that higher starch digestibility was observed in soy oil than yellow grease.

Different sources of lipids did not have a significant effect on the birds’ feed intake and mortality rate. This might be the result that the diets were designed to have similar levels of energy despite using different lipid sources (Wongsuthavas et al., 2007). So, the birds are consuming the same amount of energy which resulted in a similar amount of feed intake. The result of mortality is consistent with study done by Al-

Khalaifah & Al-Nasser (2020), that the use of different lipid source did not affect the birds' health with minimal mortality.

On the other hand, the birds' FCR and BWG are significantly impacted by the different lipid sources used in the diets. Birds with diet consisting of PF had significantly more BWG than other diets. Followed by birds on SO diet, and birds with the lowest BWG value is observed with CPO diet. This contradicted other studies, as plant-based oils such as, corn oil and canola oil, contains the more digestible unsaturated FAs and were shown to have significantly more weight gain than adding animal fats to broilers' diets which consist more of saturated FAs and is less digestible (Sklan, 1979; Scott et al., 1982). Although Lara et al. (2003) observed no significant difference in FCR and BWG of broilers fed diets containing poultry fat and raw soybean oil. This may be caused by the highest pellet durability index of poultry fat diets compared to the other two diets. Therefore, the birds might have more intact pallets which allows them to consume the same amount of energy with less movement and saves their energy for more growth hence, increased weight gain (Parsons et al., 2006).

Birds fed on diets using crude palm oil had the highest FCR with birds fed on diets including poultry fat in between, and the lowest FCR value was recorded with diets consisting of SO. The lower FCR indicates higher efficiency, which might be the result of CPO having the highest proportion of saturated FAs and lowest proportion of unsaturated FAs, followed by poultry fat in between, and with the least proportion of saturated FAs and highest unsaturated FAs proportion in SO. This confirmed that poultry utilizes unsaturated FAs more efficiently than saturated FAs. It is consistent with the study done by (AL-Jaf et al., 2018), that the highest FCR value was from plant-based corn oil. Research done by Saleh et al. (2021) investigated the effect of using poultry fat for partial or complete replacement of soybean oil. Results showed that partial or full replacement did not cause significant difference to the birds' FCR. However, the full replacement of soybean oil using poultry fat showed slightly higher FCR (Saleh et al., 2021).

According to Abdollahi et al., (2013b), the inclusion level of animal fat or plant oil as source of energy is maximum of 4%. Using more than 4% in the diet may cause difficulty in pellet quality. All of the diets used in this study contain higher lipid content even in the low lipid level diets. Although including lipid in poultry diet can reduce the amount of dust in pellets and decrease the amount of wastage. High lipid content diets can result in the opposite effect and cause an increasing chance of pellet breakage and create increased amount of dust. In this study, due to the high lipid content in all of the diets, the pellets are less able to hold their form which resulted in a low pellet durability index and large amount of dust, this resulted the pellet form diets fed to the birds were mostly mash form. This may influence the broilers performance parameters of feed intake and body weight gain. In addition, to minimize the influence of the birds' age affecting the result of ileal nutrient digestibility (Tancharoenrat et al., 2013; Ravindran & Abdollahi, 2021), the majority of the literatures looked at were studies based on starter broiler birds to ensure the data and result reported is relevant and appropriate for this study.

The result from this work suggested that the fat sources acted the same regardless of fat level, which did not affect similarly on the broiler birds' performance parameters of BWF, FI, FCR, mortality, ileal nutrient digestibility of N, DM, fat, starch, GE, and AME. However, even though FCR was not influenced by lipid level, FI and BWG is significantly higher in L level diets. Which indicates that using 6% crude fat has less impact on pellet quality and resulted better performance (Tancharoenrat et al., 2013; Ravindran & Abdollahi, 2021). This means that using fat to formulate energy dense diets need to take consideration of the pellet quality, which requires further research for possible alternative methods, namely, spray-coating lipid for increasing lipid in pellet form diet (Amin & Sobhi, 2023).

3.5 Conclusion

The current study showed that different lipid sources of crude palm oil, soy oil and poultry fat used on a maize-soybean based diet influenced the performance parameters and ileal nutrient digestibility of broilers. Poultry fat diets improved body weight gain of the broiler birds the most, and diet used crude palm oil had the lowest BWG. Feed conversion ratio is highest in crude palm oil diets, with poultry fat at similar level and soy oil had the best efficiency of lowest FCR value. Ileal digestibility of dry matter, fat, gross energy, and AME were affected by the lipid type. Plant-based lipid sources of soy oil and crude palm oil displayed higher digestibility than animal-based lipid source of poultry fat, with highest digestibility observed in soy oil diets. The two different high and low inclusion levels of lipids also impacted the birds' performance parameter and ileal digestibility of AME. Low lipid inclusion level improved the broilers' body weight gain and feed intake. In contrast, birds on diets with high inclusion level of lipids showed increased digestibility of AME. Further study can be carried out to investigate the optimum lipid inclusion level in relation to pellet quality for optimum performance and processing methods, which with production merit, include the largest proportion of lipid in the diet with good pellet durability index.

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