Calculating apparent metabolisable energy (AME) of different oils with and without lysophospholipids for broilers

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***Magister Scientiae Agriculturae* - MSc. (Agric) Animal Science (Animal Nutrition)**

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DECLARATION

I, Zancia Swart declare that the dissertation, which I hereby submit for the degree MSc. (Agric) Animal Science (Animal Nutrition) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Zancia Swart

DATE: June 2018

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ABSTRACT

Calculating apparent metabolisable energy (AME) of different oils with and without lysophospholipids for broilers

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With increasing raw material costs, feed formulations are becoming more expensive. Formulating a less energy dense diet and adding an emulsifier to improve lipid digestion and absorption is an option to try and decrease feed costs. Different lipid sources are available on the market but due to their unknown quality it is not always certain what the energy value of the lipid source is and this might lead to over or under supplying of energy to the animal.

This study evaluated two commonly used lipid sources in South Africa, soya oil and F10 oil (an unsaturated blend of animal fats and vegetable oils with a maximum of 10% FFA content) which were supplied by Energy Oil (165 Tedstone Road, Wadeville, Gauteng). Both oils were chemically analysed and their AME values were calculated with the Wiseman equation corrected for moisture, impurities and unsaponifiables (MIU), before diets were formulated and the effect of the addition of LYSOFORTE EXTEND dry (LEX) on digestible and performance parameters were investigated.

A metabolic study in broilers was conducted to investigate the effect of 2 different oil sources (soya oil and F10 oil) and varying dosage levels of LEX on diet digestibility and apparent metabolisable energy (AME). The study contained 10 treatments: 5 treatments included 3% soya oil and LEX at increasing dosages (0, 0.25,

0.50, 0.75 and 1 g/kg) and 5 treatments included 3% F10 oil and LEX at increasing dosages (0, 0.25, 0.50, 0.75 and 1 g/kg). Diet digestibility parameters were improved for soya oil treatments with the addition of 0.25 g/kg LEX with significant differences for crude protein (CP) digestibility (69.88%), crude fat (EE) digestibility (84.49%) and AME (10.95 MJ/kg). Digestibility parameters for F10 oil treatments were improved with 0.75 g/kg LEX addition, with significant differences for DM digestibility (94.10%) and EE digestibility (84.79%).

Following the metabolic study, a 35-day broiler performance trial was conducted to evaluate if the addition of LEX can overcome a 0.42 MJ/kg energy decrease in final feed. The trial included 10 treatments. Five treatments comprised of a basal diet with added soya oil, the positive control contained 3% soya oil (PC) and the negative control (NC) contained 1.8% soya oil with LEX addition at 0, 0.25, 0.50 or 0.75 g/kg, respectively. The other five treatments comprised of a basal diet with added F10 oil, the positive control contained 3% F10 oil (PC) and the negative control (NC) contained 1.8% F10 oil with LEX addition at 0, 0.25, 0.50 or 0.75 g/kg, respectively. Performance parameters including body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were measured weekly during the trial. Growth parameters for both soya oil and F10 oil treatments were improved with the addition of 0.25 g/kg LEX compared to the NC, and this was more noticeable for the F10 oil treatments. A significant difference was noticed for FCR for F10 oil NC (1.63) and F10 oil NC + 0.25 g/kg LEX (1.59) at the end of the 35-day performance period.

It was concluded from the metabolic study that an “on top” application of LEX at 0.25 g/kg for a soya oil demonstrated an improved CP digestibility, EE digestibility and AME value for these diets. For diets containing a blended oil an improvement was noticed for DM digestibility, and CF digestibility and AME value when 0.75 g/kg LEX was added. It is recommended to use LEX in an “on top” application at 0.25 g/kg for pure vegetable oils and at 0.75 g/kg for blended oils.

For the broiler performance trial, diets were formulated to contain 0.42 MJ/kg less than the PC. Broilers that received soya oil containing diets supplemented with 0.50 g/kg LEX showed a significant improvement in body weight at 28 days of age. Feed conversion ratio at 35 days of age showed a significant with the inclusion of 0.50 g/kg LEX. It is recommended to use LEX in an “on top” application at 0.50 g/kg for both pure vegetable oils and blended oils during commercial broiler farming.

LIST OF ABBREVIATIONS

AME	Apparent Metabolisable Energy
ANOVA	Analysis of Variance
BW	Body Weight
˚C	Degree Celsius
Ca	Calcium
CF	Crude Fibre
CMC	Critical Micelle Concentration
CP	Crude Protein
DM	Dry Matter
EE	Ether Extract (Crude Fat)
FA	Fatty Acid
FAME	Fatty Acids Methyl-Esters
FCR	Feed Conversion Ratio
FFA	Free Fatty Acids
FI	Feed Intake
g	Gram
GE	Gross Energy
kJ	Kilojoule
Kg	Kilogram
LCFA	Long Chain Fatty Acid
LEX	LYSOFORTE EXTEND dry
LPA	Lysophosphatidic Acid
LPL	Lysophospholipid
LPC	Lysophosphatidylcholine

MJ	Megajoule
ME	Metabolisable energy
meq	Milliequivalent
MCFA	Medium Chain Fatty Acid
mg	Milligram
MIU	Moisture, Impurities and Unsaponifiables
MUFA	Mono Unsaturated Fatty Acid
NRC	National Research Council
NSP	Non-Starch Polysaccharides
OM	Organic Matter
PA	Palmitic Acid
PL	Phospholipid
PUFA	Poly Unsaturated Fatty Acid
SA	Stearic Acid
SAS	Statistical Analysis Systems
SCFA	Short Chain Fatty Acid
SFA	Saturated Fatty Acid
SD	Standard Deviation
SEM	Standard Error of the Mean
TG	Triglyceride
Ti	Titanium
TiO_2	Titanium Dioxide
UFA	Unsaturated Fatty Acid
U/S	Unsaturated:Saturated
VFA	Volatile Fatty Acids

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CHAPTER 1
GENERAL INTRODUCTION

Worldwide there is an increase in feed costs that is resulting in the search for more cost-effective feed utilisation techniques, without compromising on nutritive quality. Fats and oils are the most concentrated sources of energy that are used as dietary energy-yielding ingredients in diets for poultry (Wiseman *et al*, 1992). An increase in diet energy concentration will lead to an improvement in growth performance and assist in achieving industry standards (Blanch *et al.*, 1996).

During fat digestion in monogastric animals, fats and oils are enzymatically hydrolysed to form fatty acids (FA) and monoglycerides that are water insoluble. Due to the aqueous environment of the small intestine, FA needs to be absorbed as a hydrophobic component by the formation of micelles. Micelle formation is naturally mediated by emulsifiers such as bile salts and phospholipids.

Young animals do not have sufficient lipolytic enzymes and this limits the effectiveness of fat digestion and absorption (Leeson & Atteh, 1995; Melegy *et al.*, 2010). Bile salts and lipase secretion are limited in young birds until they reach gastrointestinal tract maturity around 10-14 days of age (Noy & Skaln, 1998). Studies have shown that dietary supplementation of bile salts (Polin *et al*., 1980; Kussaibati *et al*., 1982) and a biosurfactant (Emmert *et al*., 1996; Huang *et al*., 2007; Zhang *et al*, 2011; Murugesan, 2013) improved fat digestion and absorption, and growth performance of broilers.

Lysophospholipids (LPL) are natural bio surfactants that is naturally secreted by the gallbladder and are known to enhance the absorption of oils, fats and fat-soluble vitamins (Melegy *et al.*, 2010; Zhang *et al.*, 2011). Enhancing the rate and efficiency of fat absorption by LPL is due to:

1. Formation of smaller triglyceride droplets in the small intestine, providing more surface area for lipase activity.
2. Easier micelle formation due to a lower critical micellar concentration (CMC).
3. Increasing cell membrane fluidity and in this way increasing passive and active transport across the enterocytes.

Lysophosphatidylcholine (LPC) is an example of a LPL that has a CMC of 0.02–0.2 mM/L, which was shown to be 20–200 times more effective than bile (CMC = 4 mM/L) and lecithin (CMC = 0.3–2 mM/L) (Zubay, 1983).

There is a wide range of fats and oils used as high energy-yielding ingredients, but due to their chemical composition and processing conditions they have variable nutritive values (Murugesan, 2013). Chemical

variation includes the degree of saturation of the constituent FA, fatty acid chain length and the proportion of free fatty acids (FFA) present within a blend (Wiseman *et. al.*, 1992). Fatty acids from fat sources high in unsaturated fatty acids (UFA) are better utilised by chickens than from fats high in saturated fatty acids (SFA) (Wiseman *et al*., 1991; Leeson & Atteh, 1995; Smits *et al*., 2000). During processing and refining of fats and oils, heat treatment is invariably employed and this may have undesirable effects upon their subsequent nutritive value.

Soya oil is currently the preferred fat source being used in poultry feed. Due to its high demand, the price of soya oil is higher than that of blended oils. The main reason why blended oils are rarely used in poultry feed is that the fat composition and quality are unknown and variable, and they mostly have a lower energy value than soya oil.

The first objective of this study was to determine the AME of two different fat sources for broilers (soya oil and blended oil), and the effect of a LPL product in combination with these oils at varying doses by means of a digestibility study. The second objective was to determine how two different lipid sources (soya oil and blended oil), without and with LPL supplementation, would impact the performance of commercial broiler chickens. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were the parameters measured in this performance study.

CHAPTER 2
LITERATURE REVIEW

2.1. Use of lipids in poultry diets

2.1.1. Introduction

The terms lipid, fat and oil are used interchangeably and describe a diverse variety of compounds that are insoluble in water. Lipids, fats and oils are normally used as an energy source in poultry diets due to its high energy density. Fat energy is at least twice that of protein and carbohydrates (NRC, 1994), and metabolisable energy values are around 37.7 kJ for 1 gram of fat, 16.7 kJ for 1 gram of protein and 16.7 kJ for 1 gram of carbohydrates (FAO, 2003). Lipid addition has other benefits including a reduction in dustiness and binding of other nutrients (Baião & Lara, 2005), improved palatability and lubrication of equipment during food processing (Thomas *et al.*, 1998; Firman *et al*., 2008), which improves pellet quality and durability.

2.1.2. Definition, composition and classification of lipids

2.1.2.1. Definition

According to the lipid library of the American Oil Chemists' Society (AOCS, 2015) lipids are defined as fatty acids and their derivatives (*e.g.* triglycerides), and substances related biosynthetically (*e.g.* lipoproteins) or functionally (*e.g.* cholesterol) to these compounds. Additionally, the terms fat, oil and lipid are often used interchangeably. The term fat can also be considered as that subgroup of lipids that are solid at room temperature, while oils are considered as that subgroup of lipid mixtures that are liquid at room temperature (Baião & Lara, 2005; Fellows, 2009).

2.1.2.2. Composition

The main composition of fats and oils are triglycerides (triacylglycerol). Triglycerides are esters of glycerol with three FA (Figure 2.1). Glycerol consists of a hydrocarbon chain of three carbon atoms with a hydroxyl group bound to each of the three carbon atoms. A FA is an organic acid with a hydrocarbon chain.

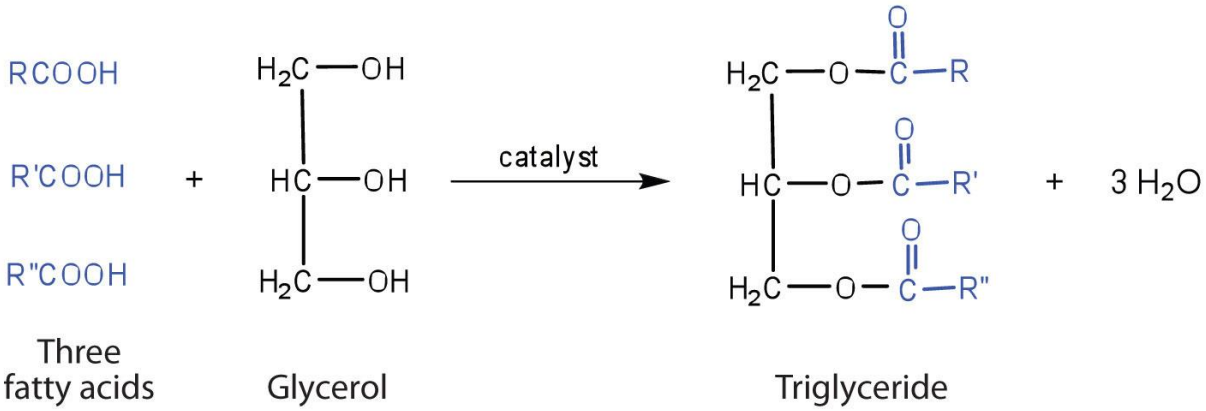


Figure 2.1. Structure of triglycerides. R, R' and R" represent the hydrocarbon chains of the respective fatty acids (Ball *et al.*)

Fatty acids can be saturated or unsaturated (Figure 2.2). A SFA has no double bonds between the carbon atoms of the hydrocarbon chain, whereas an UFA has one or more double bonds between the carbon atoms of the hydrocarbon chain (Raven *et al.*, 2005). Fatty acids with only one double bond are known as monounsaturated fatty acids (MUFA) while fatty acids with more than one double bond are known as polyunsaturated fatty acids (PUFA).

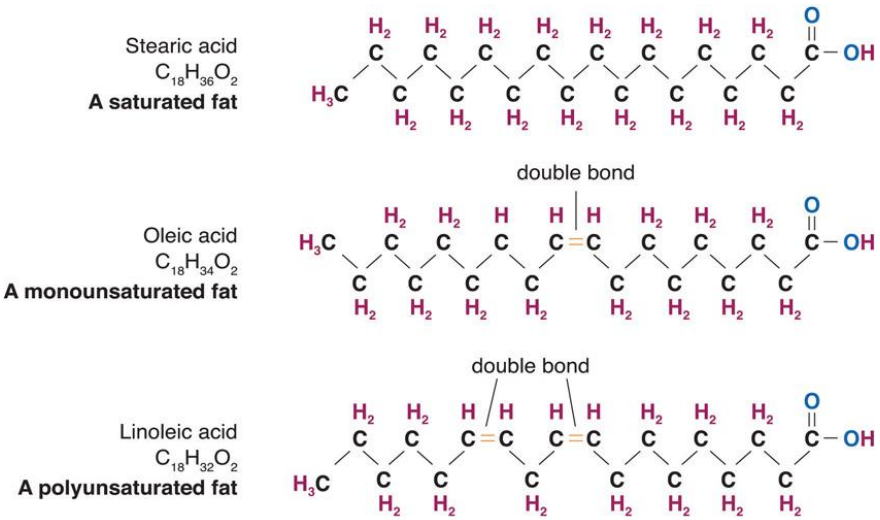


Figure 2.2. Structures of saturated, monounsaturated and polyunsaturated fat (Zimmerman & Snow, 2012)

Fatty acids also differ in the length of the hydrocarbon chain, and are divided into short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and long chain fatty acids (LCFA). Short chain fatty acids are fatty acids with hydrocarbon chains of less than eight carbon atoms, MCFA consist of eight to twelve carbon atoms and LCFA consists of more than 12 carbon atoms in their tail. An overview of the names, number of carbons and double bonds of fatty acids most common in fats and oils are shown in Table 2.1.

Trans-fatty acids are UFA with the hydrogen atom on the opposite side of double bond, whereas cis-fatty acids have the hydrogen atom on the same side of the double bond. Trans-fatty acids are less commonly found in nature, but are mostly formed during the processing of fats and oils (AOAC, 2005).

Table 2.1. Overview of the most common fatty acids encountered in fats and oils (adapted from AOCS, 2015)

Abbreviated designation	Fatty acid	Carbon atoms	Double bonds
C4:0	Butyric acid	4	0
C6:0	Caproic acid	6	0
C8:0	Caprylic acid	8	0
C10:0	Capric acid	10	0
C12:0	Lauric acid	12	0
C14:0	Myristic acid	14	0
C16:0	Palmitic acid	16	0
C16:1	Palmitoleic acid	16	1 (ω - 7)
C18:0	Stearic acid	18	0
C18:1	Oleic acid	18	1 (ω - 9)
C18:2	Linoleic acid	18	2 (ω - 6)
C18:3	α-Linolenic acid	18	3 (ω - 3)
C20:0	Arachidic acid	20	0
C20:4	Arachidonic acid	20	4 (ω - 6)
C20:5	Eicosapentaenoic acid	20	5 (ω - 3)
C22:1	Erucic acid	22	1 (ω - 9)
C22:6	Docosahexaenoic acid	22	6 (ω - 3)

2.1.2.3. Classification

Two classification systems have been proposed by Small (1986) and Fahy *et al.* (2005). The first classification system proposed by Small (1986) are mostly used in the field of nutrition. Lipids are categorised into (1) non-polar, neutral and hydrophobic and (2) polar lipids, which are further subdivided into three classes as shown in Table 2.2.

Table 2.2. Classification of polar lipids (adapted from Small, 1986)

Class I	Class II	Class III
Neutral, hydrophobic lipids	Amphipathic lipids	Surface active lipids
Form stable monolayer in the oil-water interphase	Form stable monolayer in the oil-water interphase	Limited solubility in water
Triglycerides, diglycerides, protonated free fatty acids, cholesteryl esters, Vitamins A, D, E & K	Monoglycerides, phospholipids	Deprotonated free fatty acids, lysophospholipids, bile salts

Fahy *et al*. (2005) classified lipids based on their chemical structure, their hydrophobic and hydrophilic elements, resulting in the following eight categories: fatty acyls, glycolipids, glycophospholipids, sphingolipids, sterol lipids, phenol lipids, saccharolipids and polyketides. An online database with number identification (LIPID MAPS, 2015) has been established with this classification system, and is frequently used in the field of biochemistry.

2.1.3. Lipid digestion and absorption

2.1.3.1. Digestion of lipids

The digestive tract (Figure 2.3) of poultry consists of the beak, the oesophagus which widens into the crop, the lower oesophagus, proventriculus, gizzard, duodenum, jejunum and ileum. The gizzard is connected to the proventriculus via a narrow and short isthmus, and to the duodenum via a narrow pylorus. The pancreatic and bile ducts open into the distal end of the duodenal loop (Duke, 1986). The gizzard reduces feed particle size mechanically by grinding and mixing, these movements are pendular and are followed by contractions of the proventriculus (Smulikwoska, 1998). Digesta moves between the proventriculus and gizzard to increase the enzymatic and mechanical digestive actions, and is then pushed through the pylorus into the duodenum (Klasing, 1999). This digesta movement, or intestinal reflux, occurs continuously in chickens. The reflux pattern allows the reverse passage of the intestinal digesta that contains pancreatic and intestinal juice, and bile into the gizzard and the proventriculus (Sklan *et al.*, 1978). The first step of lipid digestion is the release of lipids from the feed matrix and initiation of lipid emulsification by the gizzard and proventriculus. Secondary emulsification is established by the bile salts and is important for the following stages of digestion and absorption in the duodenum and jejunum. The digesta movement between the gizzard and duodenum increases the time the feed is in contact with digestive enzymes (Smulikowska *et al.*, 1998).

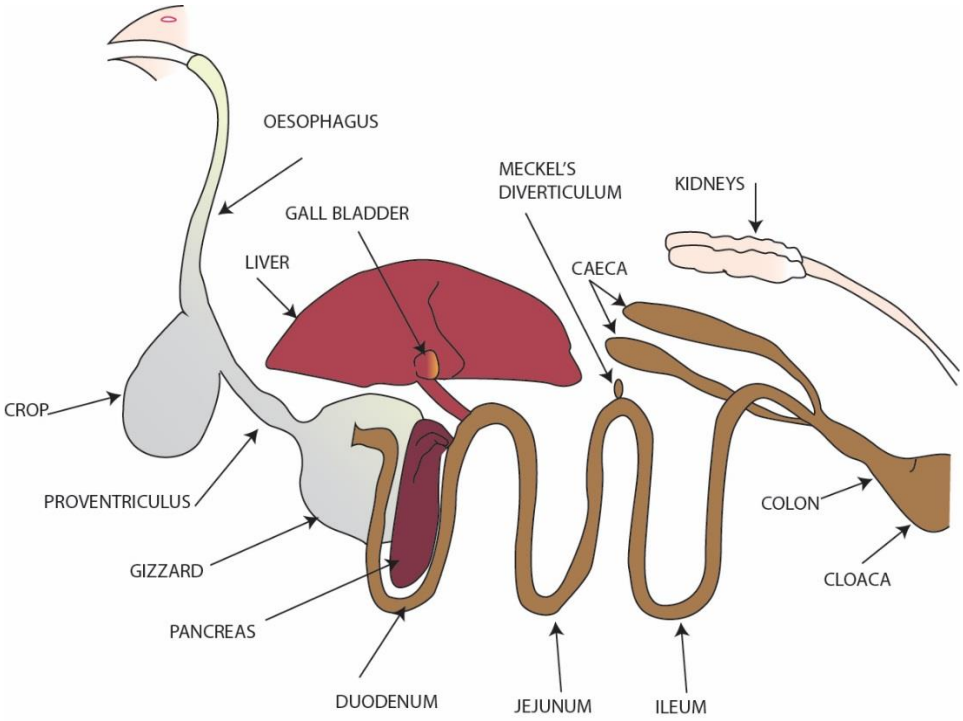


Figure 2.3. Schematic overview of the broiler's digestive system (http://www.poultryhub.org/wp-content/uploads/2012/07/Mingan_anatomy_diagram.jpg)

When lipid enters the duodenum, cholecystokinin secretion is stimulated which in turn regulates the secretion of pancreatic enzymes and bile (Krogdahl, 1985). The gall bladder releases bile salt to emulsify lipid in the chime, and the pancreas secretes pancreatic lipase that hydrolyses lipid with the aid of co-lipase (Erlanson *et al*., 1971). The activity of lipase can be inhibited by a high concentration of bile salts (Bosc-bierne *et al*., 1984) and the activity can be restored by colipase.

Colipase is a co-factor released from the pancreas and consists of hydrophobic and hydrophilic amino acids. Colipase is needed for the action of lipase on triglyceride emulsion and aids in maintaining the lipase in an active form at the lipid-water interface. The colipase acts as an anchor for lipase by binding to the surface of a lipid droplet and assists the lipase to digest triglycerides (Borgström, 1980).

Figure 2.4 demonstrates the hydrolysis of triglycerides by pancreatic lipase and the resulting products are FFA and monoacylglycerol. These products form mixed micelles with the conjugated bile salts (Figure 2.5). The micelles are then transported to the mucosal surface where it passes through the brush border membrane (Krogdahl, 1985).

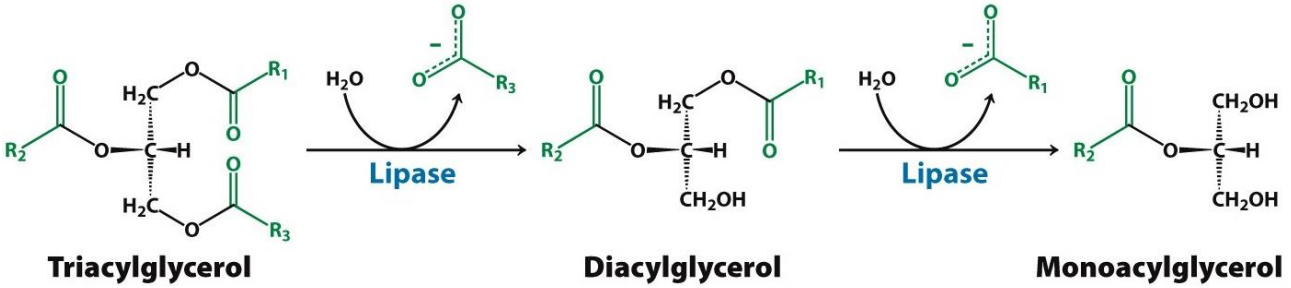


Figure 2.4. Triglycerol hydrolysis via pancreatic lipase (Berg *et al*., 2011)

Lipase action is inhibited by the accumulation of FFA in the vicinity of the enzymes, while lipase enzyme is more active on long chain PUFA (Van Kuiken & Behnke, 1994). Lipase activity was also inhibited by the LCFA; stearic acid (Van Kuiken & Behnke, 1994).

Adequate amounts of bile salts, pancreatic lipase and colipase are needed for the complex process of fat digestion. The absence of any one of these essential substances will impair fat digestion and absorption.

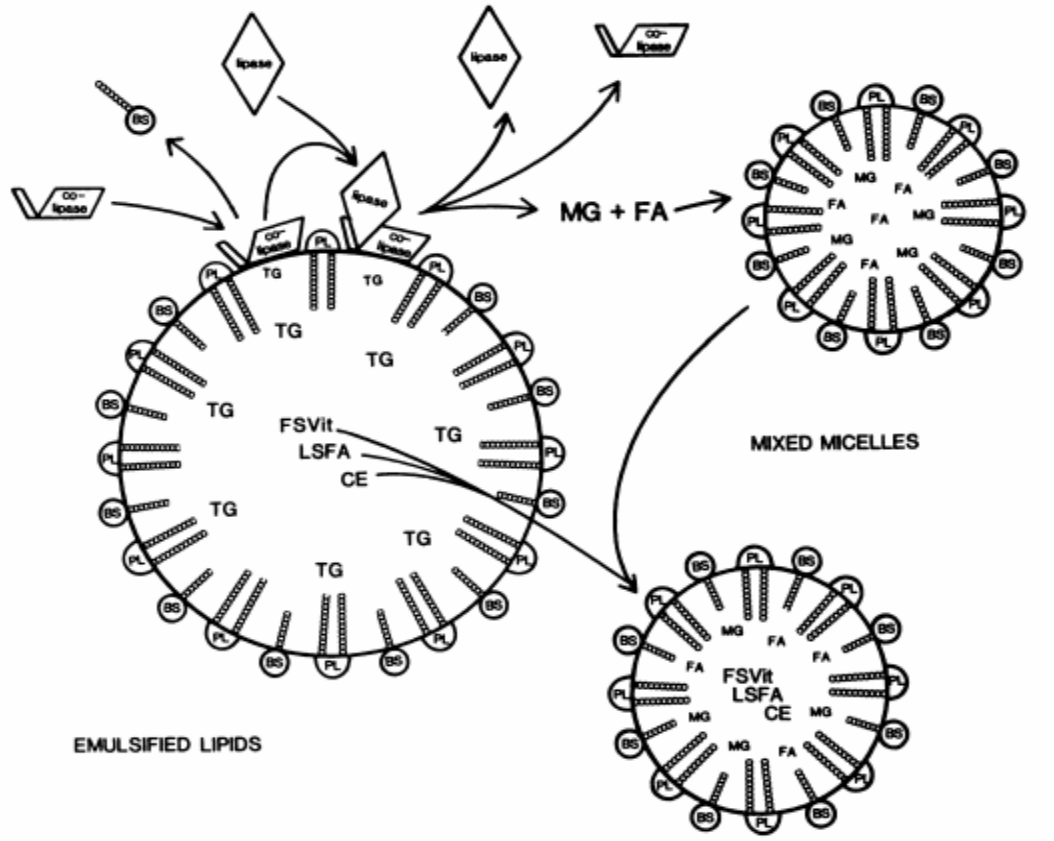


Figure 2.5. Possible sequence of events during intestinal lipolysis in poultry (bile salts (BS), cholesteryl ester (CE), free fatty acid (FFA), fat-soluble vitamins (FSVit), long-chain saturated fatty acids (LSFA), monoglyceride (MG), phospholipid (PL), triacylglyceride (TG)) (Wiseman *et al.*, 1991)

2.1.3.2. Absorption of lipids

Fat digestion and absorption occur mainly in the small intestine (Hurwits *et al*., 1973; Freeman, 1976; Krogdahl, 1985), while absorption in the caeca and large intestine are negligible (Renner, 1965). The main site of fat absorption in the small intestine is in the upper jejunum and continues in the ileum (Hurwitz *et al*., 1973). Figure 2.6 illustrates the process of fat digestion and absorption.

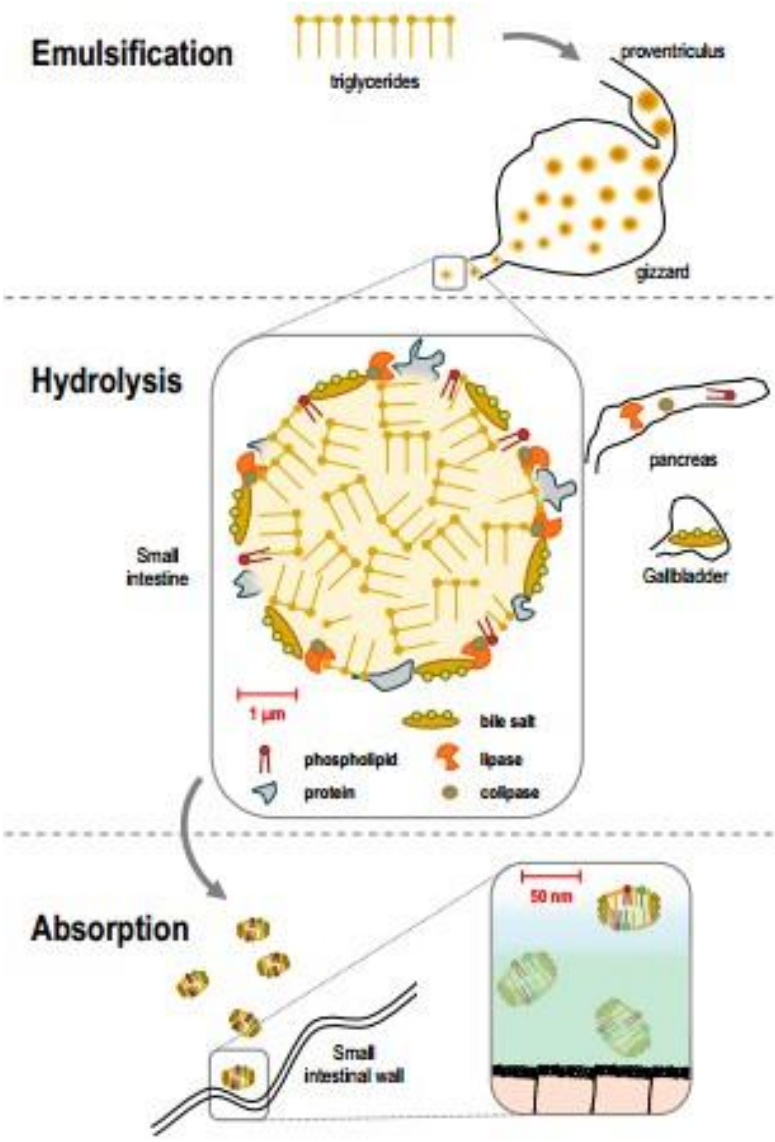


Figure 2.6. Simplified schematic overview of the three crucial steps in lipid digestion: emulsification, hydrolysis and absorption (Jansen, 2015)

Short-chain fatty acids and monoglycerides, two products of lipid digestion, are passively absorbed across the intestinal cells and need no emulsification (Pond *et al*., 2005). Assembly of LCFA, diglycerides, fat soluble vitamins and cholesteryl esters into mixed micelles is required to be transported to the intestinal cells

(Davenport, 1980), where these substances are solubilised within the hydrophobic cores of mixed micelles (Figure 2.5).

Monoglycerides and FA are rebuilt into triglycerides within the intestinal cells. Chylomicrons are formed by the combination of triglycerides with free and esterified cholesterol, lipoprotein and phospholipids (PL) and secreted into the blood. In poultry, chylomicrons are directly secreted into the portal circulation and thus are termed portomicrons (Hermier, 1997). Portomicrons are utilised for the synthesis of lipoprotein and phospholipids, metabolised as an energy source or stored as fat deposits. The major site of synthesis of portomicrons is the liver (Scott *et al*., 1982).

2.2. Factors influencing lipid digestion

2.2.1. Introduction

Dietary lipid digestibility and AME are influenced by several factors (Krogdahl, 1985; Wiseman, 1990; Leeson & Atteh, 1995; Baião & Lara, 2005; Tancharoenrat *et. al.*, 2010) and can be categorised into two major factors, namely diet-related and broiler-related factors. Diet-related factors include the composition, inclusion level and quality of the fats, dietary calcium levels, non-starch polysaccharides and processing of the diet, while broiler-related factors include age, strain and gender and lastly the animal's gut microbiota.

2.2.2. Diet-related factors

2.2.2.1. Lipid quality and inclusion level

Degree of saturation and chain length of fatty acids

Fats and oils are commonly added to poultry feed to produce high energy and nutrient dense diets. These fats and oils include vegetable oils, restaurant greases, rendering by-products, acid oils, hydrogenated fats and acid soap stocks (McDonald *et al*., 2002; Leeson & Summers, 2005; Kellems & Church, 2010). Not all lipid sources are equally utilised by the animal due to the difference in fatty acid profile, unsaturated/saturated (U/S) ratio and oxidative state and these chemical structure of lipid influences the energy-yielding potential of lipid (Freeman, 1984; Krogdahl, 1985). Digestion and absorption of lipids are also influenced by the carbon chain length, the degree of saturation and the position of the double bonds in the fatty acids (Renner & Hill, 1961; Baião & Lara, 2005). Saturated fatty acids, especially LCFA have a lower digestibility and absorption in broilers compared to SCFA, MCFA and UFA. This difference was clearly noticed in a trial performed by Tancharoenrat & Ravindran (2014) where oleic and linoleic acids (UFA) were better digested and absorbed than stearic acid (SFA). A synergistic effect was seen where the presence of UFA improved the digestibility of SFA, and this has led to the use of blends of saturated and unsaturated lipid sources (Baião & Lara, 2005; Leeson & Summers, 2005; Tancharoenrat *et al*., 2013). During micelle formation, unsaturated LCFA are more easily incorporated into the micelles, due to it being more polar than saturated LCFA (Krogdahl, 1985).

Fellows (2009) and Baião & Lara (2005) postulated that the arrangement of the fatty acids on the glycerol backbone affects the physiochemical properties of the fatty acid and thus the digestibility of the lipid. Diets formulated with lipids also show an increase in utilisation of other dietary nutrients due to the slower passage rate through the gastrointestinal tract which allows for better nutrient absorption (NRC, 1994; Swennen *et al*., 2004; Baião & Lara, 2005).

Plant lipid sources have a higher U/S ratio than animal fats, and are thus considered to be better utilised by the animal. A higher solubility is noticed with more polar UFA, and a reduction in monoglyceride levels are correlated to lower digestibility during the micelle formation phase. Table 2.3 shows the relationship between different lipids and their AME value, PUFA content and palmitic acid (PA) + stearic acid (SA) content (Scheele *et al*., 1997).

Table 2.3. AME value, poly-unsaturated fatty acid (PUFA) content and palmitic acid (PA) + stearic acid (SA) content of fats in 4-week-old broiler chickens (adapted from Scheele *et al*., 1997)

Fats/oils	AME, MJ/kg	PUFA, %	PA+SA, %
Soybean oil	35.4[a]	60.0	15.3
Safflower oil	35.0[a]	76.2	10.0
Grapeseed oil	35.0[a]	69.9	9.3
Linseed oil	34.0[ab]	74.7	8.4
Rapeseed oil	33.5[abc]	32.7	6.9
Olive oil	32.5[bc]	18.3	15.8
Coconut oil	31.6[bc]	10.2	4.0
Groundnut oil	31.5[cd]	33.5	15.7
Poultry oil	30.1[d]	16.4	23.3
Mixed animal fat	28.1[e]	9.3	31.6
Palm oil	25.8[f]	11.0	45.6
Tallow	24.5[f]	7.9	39.9

[a-f]Means within a column without a common superscript differ significantly ($P < 0.05$)

Free fatty acid

Free fatty acids are by-products of lipid digestion, and high FFA levels are generally seen in by-product oils and restaurant greases. Free fatty acids are highly prone to rancidity and may cause corrosion to some equipment. In Figure 2.7 a reduction in digestibility was seen with an increase in FFA content (Wiseman *et al*., 1991). Free fatty acids are thought to negatively influence micelle formation and bile secretion, resulting in a lowered lipid digestibility (Freeman, 1976; Sklan, 1979) and lowered ME (Wiseman *et al*., 1991).

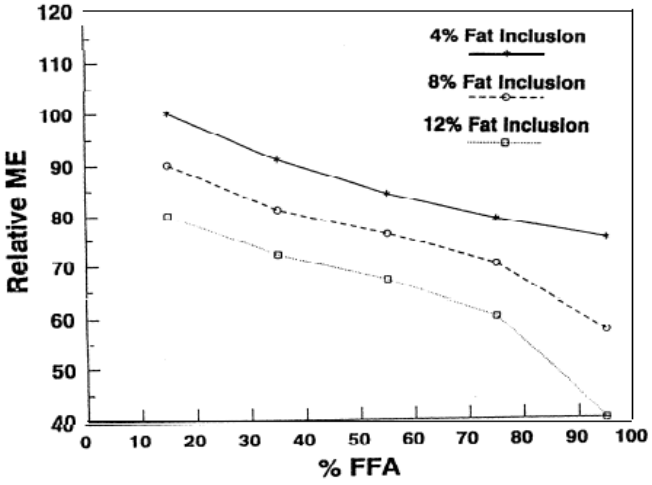


Figure 2.7. Fat saturation and relative ME related to fat inclusion for young birds (Vasanthakumari *et al.*, 2011)

Rancidity and oxidation

Oxidative rancidity reduces lipid quality, causes rancid odour, effect product colour, leads to off flavours, and decreases the nutritive value of the lipid (Baião & Lara, 2005). Oxidation can also destroy both lipids and fat-soluble nutrients of other diets and body reserves, and negatively affect energy value of fats and oils. Oxidation consist of the degradation of double bonds presents in UFA, resulting in the formation of free radicals. These free radicals get converted to peroxide free radicals in the presence of oxygen (Sherwin, 1978) and acts as a catalyst for oxidation. Jensen *et al.* (1997) demonstrated the negative effects of oxidised lipids on animal performance, reduced feed intake due to a reduction in palatability and meat quality.

Lipid inclusion level

Increasing lipid levels in poultry diets lead to a decrease in lipid digestibility (Wiseman *et. al.*, 1991; Blanch *et al*., 1996; Sanz *et al.*, 2000; Villaverde *et al.*, 2006; Smink *et al.*, 2010), but to optimise lipid digestion a minimal level of added lipid, 10 g/kg, is necessary (Leeson & Summers, 2005). The decrease of lipid digestibility is due to a limited availability of lipase and bile salts for the increasing amounts of lipid (Krogdahl, 1985) and this reduction is more pronounced in young broilers (Wiseman *et al*., 1991).

Moisture, impurities and unsaponifiables

Moisture, impurities and unsaponifiables (MIU) are pure diluting factors with no energy benefit for the animal. The maximum accepted level for moisture is 1.0%, since moisture interferes directly with the energy content of fat (Butolo, 2002). Impurity is the percentage of the insoluble fraction of the fat in petroleum ether and contents should be lower than 1%. Unsaponifiables (steroids, pigments and hydrocarbons) that form soaps when mixed with caustic soda comprise the unsaponifiable matter. These substances are indigestible and are

soluble in common solvents for oils. Therefore, the higher their percentage, the lower the energetic value of the oil or fat. The maximum level of unsaponifiable matter admitted in oils and fats is also 1% (Butolo, 2002; Baião & Lara, 2005).

2.2.2.2. Dietary calcium levels

Hydrolysis of triacylglycerides leads to monoglycerides and FFA, and these FFA can react with other nutrients to form soluble and insoluble soaps. Insoluble soaps cause the fatty acid and the mineral it is bounded with to be unavailable to the animal (Leeson & Summers, 2005). Ca-phytate has been identified as a substrate during the formation of insoluble metallic soaps in the gastrointestinal gut (Tancharoenrat & Ravindran, 2014). Dietary calcium level and type of fatty acid impacts calcium metabolism and soap formation.

Atteh & Leeson (1983) fed broilers different supplemental fatty acids and two levels of calcium. It was found that increasing calcium levels led to a reduction of lipid retention in birds fed palmitic acid, while the fatty acid type affected calcium retention with palmitic and stearic acid resulting in a lower retention than the UFA. Atteh & Leeson (1984) investigated the effect of SFA and UFA and three calcium levels on lipid retention and soap formation in broilers. Diets containing palmitic acid and calcium levels above 8 g/kg resulted in higher soap formation and faecal soap excretion, indicating that SFA are more prone to soap formation than UFA.

A recent study by Tancharoenrat & Ravindran (2014) investigated the effect of three levels of dietary calcium with three inclusion levels of tallow on fat digestibility. Again, the study demonstrated that with an increase in calcium there was an increase in calcium soap formation and a decrease in both calcium and fat digestibility.

2.2.2.3. Non-starch polysaccharides (NSP)

Non-starch polysaccharides are found in wheat, barley and rye and are known to reduce lipid digestibility (Choct & Annison, 1992) and exhibit anti-nutritional effects in poultry (Choct & Annison, 1992; Lee *et al*., 2004; Meng *et al*., 2005; Smeets, 2015). Intestinal viscosity is increased by NSP, e.g. arabinoxylans and β-glucans (Iji *et al*., 2001; Smeets, 2015), leading to a slower gut motility and decreased lipid droplet transportation within the intestinal lumen (Smulikowska, 1998). Meng *et al*. (2005) demonstrated that with the supplementation of carbohydrase a reduction in the intestinal viscosity occurs with a resulting improvement in lipid and NSP digestion. The microbial population in the gut is also influenced by NSP, which directly affect lipid digestion (Rodríguez et al., 2012).

2.2.2.4. Processing of the diet

In short, the pelleting process is as follows: some raw materials, like maize are ground or flaked, then all raw materials are milled and mixed together resulting in the mash phase of feed processing. The mash is then transported to a conditioner where steam is added, after which the conditioned feed is compressed through a die in the pellet mill to shape the pellets. Lastly, pellets go through a cooler to decrease pellet surface

temperature to ambient temperature. Final pellets are cylindrical and have a diameter between 1.5 and 4 mm and a length between 3 and 6 mm, depending on the age of the bird being fed (Cerrate *et al.*, 2009; Abdollahi, 2011; Abdollahi *et al.*, 2013b). Crumbles are sometimes fed to birds, which is achieved by an additional step where the pellets pass through rollers to break the pellets into smaller pellets.

Some researchers (Amerah *et al.*, 2008; Abdollahi *et al.*, 2013a; Abdollahi *et al.*, 2013b; Lv *et al.*, 2015) have shown that pelleting of feed positively affect BWG and FCR in broilers. The reasoning is that during the pelleting process the high heat and friction resulting from the die lead to cell wall disruption, increasing the access of the cellular contents to digestive enzymes (Abdollahi *et al.*, 2013b). However, some negative effects on fat digestibility have been noticed for pelleting. Engberg *et al*. (2002) demonstrated that pelleted feed resulted in smaller gizzards and pancreas than in birds fed mash diets. A lower pancreatic lipase activity was noticed in pelleted fed birds. An increase in intestinal viscosity was also noticed by Abdollahi *et al.* (2013a) and De Vries *et al.* (2014), resulting in a decreased fat digestibility.

2.2.3. Bird-related factors

2.2.3.1. Age

Lipid metabolism is not fully developed in young animals (Kroghdahl, 1985; Wiseman, 1990; Baião & Lara, 2005; Tancharoenrat *et al*., 2013), and bile secretion seems to be first limiting followed by lipase secretion (Krogdahl, 1985). Meng *et al.* (2005) attributed the lowered lipid utilisation by young chickens to the low bile salt concentration in the intestine, and low bile salt secretion is due to the lowered synthesis of bile salts (Kroghdahl, 1985; Smits *et al*, 1998). Polin *et al*. (1980), Kroghdahl (1985) and Maisonnier et al. (2003) demonstrated that lipid utilisation improved with dietary supplementation of bile salts.

Further studies (Roy *et al*., 2010) reported that low lipase secretion, low lipase activity level and low bile salt synthesis rate lead to the lowered metabolism of lipids by young chickens. An increase in lipase, trypsin and amylase secretion is seen between 4 and 21 days post hatch (Noy & Sklan, 1995).

The addition of lipids also improves the absorption of fat-soluble vitamins and essential fatty acids (Villaverde *et al*., 2004; Baião & Lara, 2005). Fat-soluble vitamins include A, D, E and K and essential fatty acids include linoleic acid, linolenic acid and arachidonic acid (Watkins, 1991). Due to animals not being able to synthesise essential fatty acids, a deficiency can lead to a reduction in growth, lower testis weight, decreased egg size and a delay in the development of secondary sexual characteristics (Wiseman *et al.*, 1991). A minimum inclusion of 10 g/kg lipids and a maximum of 30-40 g/kg lipids for poultry diets has been suggested by Leeson & Summers (2005) and values above 40 g/kg lipids showed negative effects on pellet quality (Thomas *et al*., 1998; Wiseman, 1999).

Most studies demonstrated that lipid digestion and absorption increased with an increase in bird age (Renner & Hill, 1960; Carew *et al*., 1972; Tancharoenrat *et al*., 2013). Due to the limited bile secretion during the first

weeks after hatching, overall lipid metabolism is not optimal (Krogdal, 1985). Table 2.4, adapted from Tancharoenrat *et al*. (2013), shows that lipid digestibility increases with age, with the main increase during the first two weeks of age. Renner & Hill (1960) investigated the utilisation of tallow in chickens and showed that tallow absorption increased from 70% at 2 weeks of age to 82% at 8 weeks of age. Carew *et al.* (1972) determined the absorbability of maize oil and beef tallow during the first two weeks of age and found an increase in maize oil absorbability from 84% to 95% and 40% to 79% in beef tallow.

Table 2.4. Fat digestibility in broilers relevant to age (adapted from Tancharoenrat *et al*., 2013)

Age	**Fat Digestibility%**
1 week	53.2
2 weeks	80.7
3 weeks	85.9
5 weeks	85.7

It is due to the impact of bird age on fat digestion, AME values of the fats also differ with age. Wiseman & Salvador (1989) evaluated the AME of different lipid sources (vegetable oil and tallow) for broilers at different ages. Both lipid sources' AME values increased between two and four weeks of bird age, with the highest AME increase seen in tallow. Wiseman (1990) then studied the AME value of two dry emulsified lipids (tallow and a blend of soya oil and tallow) and again found that the AME of both lipids were higher in older birds. Poor emulsification rather than lower lipase activity was attributed to the lower AME value for younger birds compared to older birds. Scheele *et al.* (1997) looked at both the effect of different ages on lipid digestibility and AME in Table 2.5.

Table 2.5. AME of added lipid and total diet lipid digestibility in broilers (adapted from Scheele *et al*., 1997)

Age	**Lipid AME (MJ/kg)**	**Total lipid digestibility (%)**
Week 2	27.96[a]	62.5[a]
Week 4	29.02[b]	66.9[b]
Week 6	32.40[c]	72.0[c]
Week 8	33.19[d]	73.4[c]

[a-d]Means within a column without a common superscript differ significantly ($P < 0.05$)

Tancharoenrat *et al*. (2010) also demonstrated the effect of age on lipid metabolism by formulating with different lipid sources (tallow, soya oil, 50:50 blend of tallow and soya oil, poultry fat and palm oil) and noticed a significantly lower AME value for lipids in the first week of life compared with the following weeks. In

another study performed by Wiseman (1999) the effect of U/S ratio, FFA and bird age (1.5 weeks and 6 weeks) were combined into equations to predict AME of lipids. The following was noticed:

1. AME increased curvilinearly as the US increased from 0.9 to 4.1
2. AME was higher for a 10% FFA lipid than a 40% FFA lipid
3. AME was higher in 6-week-old than 1.5-week-old broilers

2.2.3.2. Gender and strain

Limited studies are available to support the statement that gender and strain influence lipid digestion in broilers. Most studies were performed more than 25 years ago and only recently new studies have been executed to investigate the effect of strain and gender on lipid digestion and metabolism.

Guirguis (1975; 1976) found lipid digestibility to be higher for female broilers, while Slinger *et al*. (1995) showed that male broilers had a better growth performance over female broilers due to their superior ability for lipid digestion. Female broilers tend to have a higher fat deposition while male broilers have higher growth rate and feed efficiency (Becker *et al.*, 1981; Shalev & Pasternak, 1998; Huang *et al.*, 2008; Abdullah *et al.*, 2010). Zelenka (1997) and Yaghobfar (2001) did not observe any difference between male and female broilers in their ability to digest lipid.

The effect of broiler strain is also not clear-cut and are mainly attributed to genetic variation in nutrient digestibility and absorption and varying results have been observed. While Grunder *et al.* (1987) and Huang *et al.* (2008) did notice a difference between broiler strains for abdominal fat deposition, Becker *et al.* (1981) and Sonaiya & Benyi (1983) observed no difference. Marcato *et al*. (2008) investigated the growth rate and body nutrient composition in Ross and Cobb birds, and discovered that Cobb birds had a higher growth rate and earlier protein and ash deposition. A higher energy utilisation per unit of feed was observed for White Leghorn chicks compared to White Rock chicks (Sibbald & Slinger, 1963) and an improvement in tallow and maize oil absorption was observed for New Hampshire chicks over White Leghorn chicks (Katongale & March, 1980). However, no differences were apparent for energy utilisation and fat digestibility between White Plymouth Rock and crossbred Rhode Island Red and Barred Plymouth Rock chicks (Young *et al.,* 1963). With continuous genetic selection, further studies will be needed to investigate gender and strain effects on lipid metabolism.

2.2.3.3. Microbiota

Dietary components such as lipids can alter a broiler's microbial community (Knarreborg *et al*., 2002; Yang *et al*., 2009; Van der Hoeven-Hangoor *et al*., 2013) and affect lipid digestibility. The microbiota is directly involved in the conversion of primary bile salts to secondary bile salts via microbial deconjugation and dihydroxylation. This results in a more hydrophobic bile salt which decreases the bile salt's effectiveness in lipid digestion (Krogdahl, 1985; Drackley, 2000).

2.3. Emulsifiers

2.3.1. Introduction

Due to the insolubility of lipids in water, they first need to be emulsified before lipolytic enzymes can start with digestion. Emulsification is dependent on the lipid characteristics which include chain length, fatty acid positioning and saturation (Jansen, 2015). Enhanced emulsification is seen with LPL, increasing their importance for oil in water emulsions in the gastro-intestinal tract, as demonstrated for PL in Figure 2.8. Lysophospholipids are more hydrophilic than PL due to the presence of only one FA residue on the molecule compared to two FA for PL (Figure 2.9).

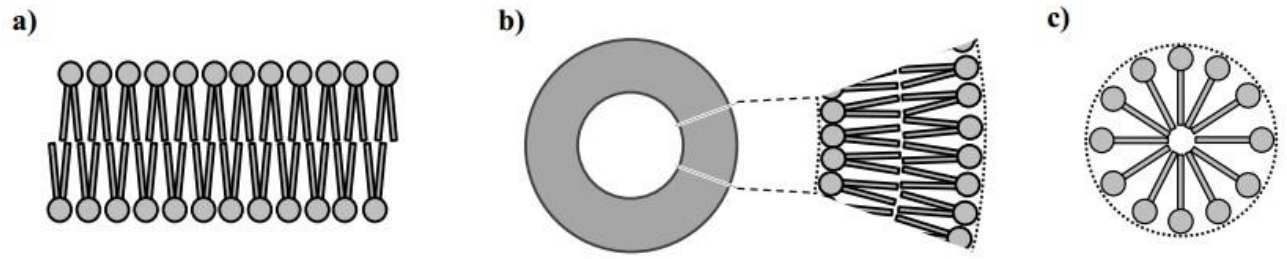
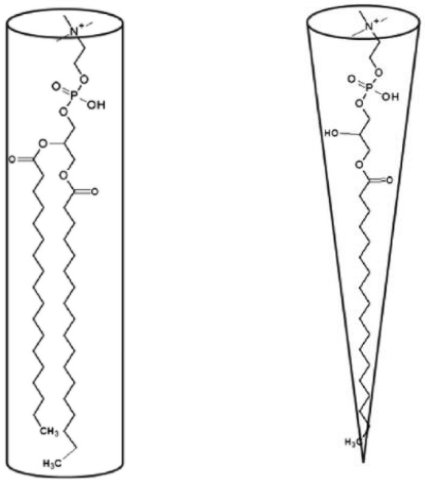


Figure 2.8. Assembly of phospholipids and lysophospholipids in an aqueous environment. Phospholipids will either form (a) a phospholipid bilayer (*e.g.* surrounding a cell) or (b) a liposome. Lysophospholipids have the tendency to form (c) micelles. (Jansen, 2015)



Phospholipid Lysophospholipid

Figure 2.9. Structures of cylindrical phosphatidylcholine and cone-shaped lysophosphatidylcholine (Grzelzcyk & Gendaszewska-Darmach, 2013)

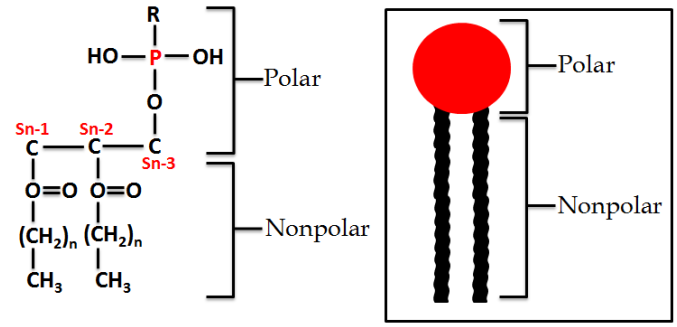
Lysophosphatidylcholine, in combination with linoleic acid, leads to the formation of smaller and more stable ovalbumin protein emulsions (Mine *et al*., 1993; Jansen, 2015). A smaller (lower micellar mass) and more stable micelle would lead to an improvement in lipid absorption across the unstirred water layer in the GIT. Van Barneveld *et al.* (2003) demonstrated in 45kg pigs that ileal amino acid digestibility was increased with the supplementation of a LPL-based emulsifier to the diet. It should be noted that this improvement may also be due to the enhanced enzyme access to the protein during a faster digestion and absorption of lipids. Carter & Henman (2003) demonstrated improved weaner growth performance, while Carter & Perez-Maldonada (2007) demonstrated an improvement in weight gain for broilers when LPL were added to the diet.

2.3.2. Phospholipids

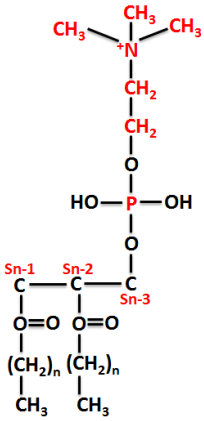
Few studies (Dowhan, 1997; Vance & Vance, 2002; Vares *et al*., 2003) have focused on PL due to them being essential constituents of cellular membranes and being amphipathic. Additional areas of PL application as an emulsifier is in pharmaceuticals, food and preparation of liposomes for cosmetics and drug delivery (Gabizon *et al.*, 1997; Uhumwangho & Okor, 2005).

Phospholipids are characterised by a glycerol backbone and a linked polar phosphodiester group at the *sn*-3 carbon. Phospholipids can be divided into three structural regions according to AOCS, 2015 (Figure 2.10):

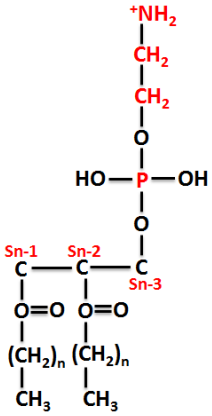
1. a polar hydrophilic headgroup which resides at the lipid-water interface
2. interfacial region which is of intermediate polarity
3. hydrophobic tail region



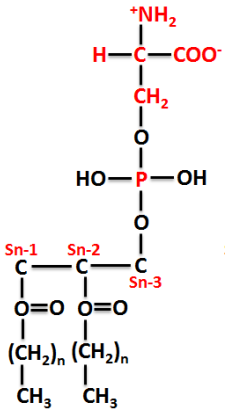
Phospholipid



Phosphatidylcholine



Phosphatidylethanolamine



Phosphatidylserine

Figure 2.10. Chemical structures of phospholipids (adapted from AOCS, 2015)

2.3.3. Lysophospholipids

Lysophospholipids are a result of enzymatic hydrolysis of PL (Figure 2.11) and are constructed with a monoacylglycerol in either position *sn*-1, 1-lysophospholipids (1-LPL) or *sn*-2, 2-lysophospholipids (2-LPL) and a phosphate residue in position *sn*-3. Lysophospholipids are found in small amounts in cellular membranes (Birgbauer & Chun, 2006), they are good emulsifiers and solubilising agents and are used in foods, cosmetics and pharmaceuticals such as PL (Reblova & Pokorny, 1995; Dennis *et al.*, 2006). Lysophospholipids are also important during reproductive physiology (Parrill, 2008), vascular development (Karliner, 2004), and nervous system physiology (Chun, 2005) due to their presence and their receptors in various tissues and cell types.

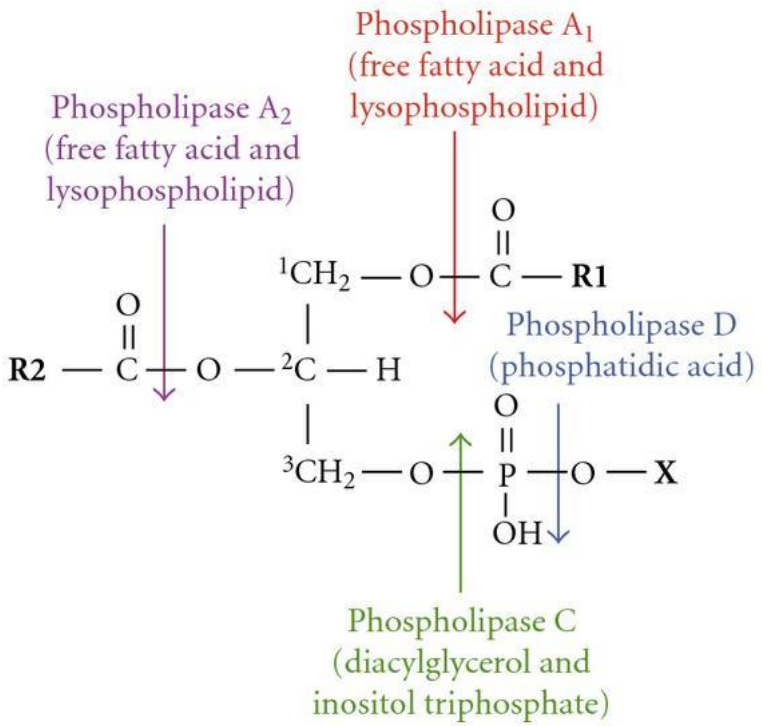


Figure 2.11. Hydrolysis of phospholipids by phospholipases. Arrows indicate the sites of attack for hydrolytic cleavage of phospholipases type A1, A2, C, and D. The main products generated by their action are also shown. R1/R2: free fatty acids in sn-1 or sn-2 positions; X: choline, ethanolamine, serine, inositol, and others. (Belaunzaran, *et al.*, 2011)

2.3.4. Lysophosphatidylcholine

Lysophosphatidylcholine, a product of phosphatidylcholine hydrolysis, is the most abundant LPL (Figure 2.12) and can be found in most animal and plant tissues at trace amounts (AOCS, 2015). Lysophosphatidylcholine is the most investigated LPL and have been demonstrated to influence gene transcription, mitogenesis and smooth muscle relaxation (Yan *et al.*, 1996; Prokazova *et al.*, 1998).

Lysophospholipids gets converted to lysophosphatidic acid (LPA), a highly potent inducer of cell proliferation, migration and survival (Daleau, 1999; Van Leeuwen *et al.*, 2003), by lysophospholipase D. Moolenar (2004) showed that LPA is important in transmembrane signal transduction and cell proliferation stimulation.

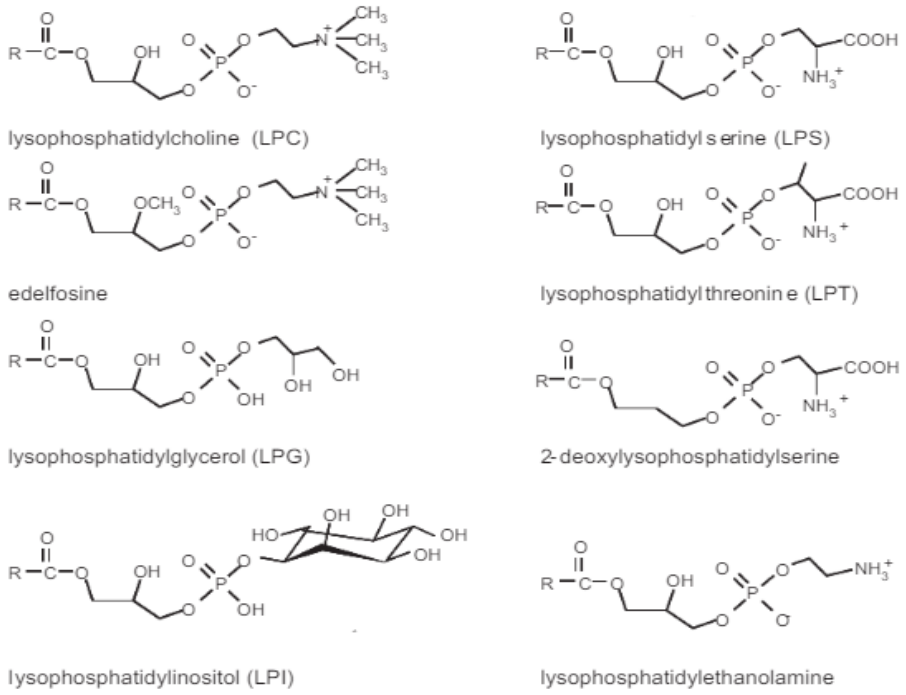


Figure 2.12. Chemical structures of lysophospholipid derivatives (Grzelzcyk & Gendaszewska-Darmach, 2013)

2.3.6. Modes of action of lysophospholipids

2.3.6.1. Emulsification and hydrolysis

Both PL and LPL have active surface properties due to their hydrophilic head group and a hydrophobic tail (fatty acid chains). Lysophospholipids have better oil-in-water emulsifying properties than PL because of the removal of one FA during hydrolysis (Joshi *et al.*, 2006; Liu & Ma, 2011). Zhang *et al.* (2011) postulated that LPL act as emulsifiers in combination with bile salts during the initiation of lipid digestion. Smaller lipid droplets will be a result of the improved emulsification, creating a bigger interphase area. Lipase attachment is facilitated by a bigger available interphase area, improving lipid hydrolysis. Keep in mind that lipase absorption and activity are affected by the surface-active compounds, which include PL and LPL (Dahim & Brockman, 1998; Reis *et al.*, 2008; Mandalari *et al.*, 2009; Reis *et al.*, 2010; Malaki *et al.*, 2011; Maldonado-Valderrama *et al.*, 2011; Verrijsen, 2015).

By removing monoglycerides and FFA from the lipid interphase, lipid hydrolysis will be improved, creating another potential mode of action of lysophospholipids (Zhang *et al.*, 2011). Biosurfactants (emulsifiers) are required to remove these products to the aqueous gut lumen and this is achieved by the formation of mixed micelles with the assistance of LPL.

2.3.6.2. Lipid absorption

Phospho- and lysophospholipids play a role in cell membrane structures and in cell signalling. Lundbaek & Andersen (1994), Wendel (2000) and Lundbaek (2006) demonstrated that LPL increase the fluidity and permeability of cell membranes. Lysophospholipids also have a direct or indirect effect on membrane protein formation and function (Lundbaek & Andersen, 1994; Maingret *et al.*, 2000; Lundbaek, 2006), thus influencing the uptake of lipids across enterocytes in the small intestine.

Due to LPL incorporating monoglycerides and FFA into mixed micelles, transportation through the unstirred water layer is improved. Lundbaek (2006) concluded that by increasing the LPL content in the lumen, smaller micelle will be formed, and micelle transportation and lipid absorption will be improved.

2.3.6.3. Immunology

A few studies (Ojala *et al.*, 2007; Cunningham *et al.*, 2008; Olofsson *et al.*, 2008; Gonçalves *et al.*, 2012; Domeij *et al.*, 2013) focused on a LPC induced inflammatory response in atherosclerosis in humans. Studies demonstrated an increased plasma LPC in patients with atherosclerosis (Lavi *et al.*, 2007). The LPC fatty acid composition influenced its inflammatory properties (Huang *et al*., 2010). Drzazga *et al.* (2014) showed that SFA (C14:0 and C16:0) are mainly associated with pro-inflammatory properties while PUFA (C20:4 and C22:6) were linked to anti-inflammatory properties.

There is currently little broiler data available on the inflammatory properties of LPL, but by extrapolation of studies performed in humans, the interaction with the bird's immune system might be another possible mode of action for phospholipids.

2.4 Prediction equation for supplemental lipids

During the current trials, the energy prediction equation of Wiseman (1989) was used:

$$\text{Dietary energy (MJ/kg fat)} = A + B \times FFA + C \times e^{(D \times U/S)}$$

This prediction equation incorporates both U/S ratio and FFA content at different ages for poultry. The symbols A, B, C and D are constants with different values (Table 2.6) for young and old birds. Wiseman's prediction equation is utilised for determining AME values for dietary lipids.

Table 2.6. Functions utilised to predict the dietary energy value of lipids (Wiseman & Salvador, 1989)

Constant	**Poultry (broilers)**	
	Young[a]	Old[b]
A	38.112 ± 1.418	39.025 ± 0.557
B	-0.009 ± 0.002	-0.006 ± 0.001
C	-15.337 ± 2.636	-8.505 ± 0.746
D	-0.506 ± 1.186	-0.403 ± 0.088
PV[c]	0.816	0.925

[a]1.5 weeks of age and 15kg live weight for poultry and pigs, respectively. [b]7.5 weeks of age and 30-85kg live weight, respectively, for poultry and pigs. [c]Proportion of variance accounted for by function.

2.5 Aim

The aim for this project was to determine the inclusion level of LEX to improve nutrient metabolism and bird performance. To achieve this aim, the following was performed:

1. A metabolic study was done to measure the effect of different LEX inclusion levels on nutrient digestibility of two different lipid sources.
2. Excreta was collected during the metabolic study for nutritional analysis.
3. A performance study was done to determine the application dose of LEX for maximum animal performance.
4. Performance parameters (BW, FI and FCR) were collected on a weekly basis.

2.6 Hypothesis

The hypotheses for the current trials were:

H_0: Addition of lysophospholipids at recommended levels to feed, will not improve the digestibility and energy contribution of the oil in the feed.
H_1: Addition of lysophospholipids at recommended levels to feed, will improve the digestibility and energy contribution of the oil in the feed.

H_0: Increasing the level of added lysophospholipids above recommended levels will not increase oil digestibility and energy contribution.
H_1: Increasing the level of added lysophospholipids above recommended levels will increase oil digestibility and energy contribution.

H_0: No correlation exists between *in vivo* broiler performance and *in vitro* AME values of different oils.
H_1: A correlation exists between *in vivo* broiler performance and *in vitro* AME values of different oils.

CHAPTER 3

The effect of lysophospholipids and two different oil sources on digestibility and AME of broiler diets

3.1. Introduction

This metabolic broiler study was conducted at the University of Pretoria. The study evaluated the effect of 2 different lipid sources (soya oil and F10 oil) on diet digestibility and apparent metabolisable energy (AME). The study also investigated the effect of an added lysophospholipid (LEX) and a possible dose response within each lipid source. Birds were kept in metabolic cages for the duration of the test period. The trial was approved by the Animal Ethics Committee of the University of Pretoria (EC058-16).

3.2. Materials and Methods

Lipids and diets

Oils used in the formulations were provided by Energy Oil (165 Tedstone Road, Wadeville, Gauteng):

1. Refined soya oil
2. F10: an unsaturated blend of animal fats and vegetable oils with a maximum of 10% FFA content

Both oils were analysed at Chem Nutri Analytical Laboratory (4 Porcelain Road, Clayville, Johannesburg, Gauteng) for free fatty acids (FFA), total saturated fatty acids (SFA), total unsaturated fatty acids (USFA) and moisture, impurities and unsaponifiables (MIU). The AME for young and older animals were calculated from these results by the Wiseman equation (Wiseman & Salvador, 1989). The following methods were used:

FFA	AOAC Ca 5a-40
SFA	AOAC 977.17
USFA	AOAC 977.17
Moisture	AOCS Ca 2c-25
Insoluble Impurities	AOCS Ca 3a-46
Unsaponifiable Matter	AOCS Cs 6b-53

The AME values of both lipids (Table 3.1) were determined before diets were formulated using the Wiseman equation (Wiseman & Salvador, 1989):

$$\text{Dietary energy (MJ/kg fat)} = A + B \times FFA + C \times e^{(DxU/S)}$$

Table 3.1. Chemical analysis and AME calculation for soya oil and F10 oil used in the metabolic study

	Soya Oil	**F10 Oil**
FFA[a] (% of Oleic Acid)	7.94	3.25
Total Saturated Fatty Acids	11.26	25.73
Total Unsaturated Fatty Acids	88.73	74.28
Unsaturated/Saturated ratio	5.06	2.45
MIU[b]	0.82	1.32
AME 0-21 days broilers (MJ/kg)	35.92	32.94
AME >21 days broilers (MJ/kg)	37.13	35.20

[a]Free fatty acids
[b]Moisture, impurities, unsaponifiables

The basal diets (Table 3.2) were formulated to meet ROSS Breeder standards. For all treatments, a 3% oil was added to the basal diet, with the pre-calculated AME value from the Wiseman equation. Poultry AME value were formulated to be the same for both basal diets by increasing the maize inclusion with 1.5% for the F10 oil basal diet to compensate for the 8% energy difference between the two oil sources.

Table 3.2. Raw material inclusion and calculated nutrient levels for soya oil and F10 oil treatments in the metabolic study

Ingredients (%)	**Soya oil basal diet**	**F10 oil basal diet**
Yellow maize	53.52	55.02
Soya oilcake	22.16	22.53
Bran	10.41	8.527
Sunflower oilcake	7.50	7.50
Soya Oil	3.00	-
F10 Oil	-	3.00
Limestone	1.06	1.06
Methionine hydroxy analogue (MHA)	0.30	0.30
Salt	0.21	0.21
Avatec	0.06	0.06
Olaquindox 10%	0.02	0.02
Axtra Phy 10000 TPT	0.01	0.01
Calculated Nutrient Concentration		
Moisture (%)	11.65	11.65
AME Poultry (MJ/kg)	12.19	12.19
Protein (%)	20.00	20.00
Fat (%)	5.92	5.81
Fibre (%)	4.91	4.80
Ash (%)	4.91	4.88

A lysophospholipid product, LEX (Kemin Industries, Sub-Sahara Africa) was added on top in increasing increments to evaluate a dose response. Lastly, an indigestible marker (titanium dioxide) was added to all diets at 3 g/kg. Dietary treatments are shown in Table 3.3.

Table 3.3. Treatments used in the metabolic study with one of two lipid sources (soya oil and F10 oil) and increasing dosages of a lysophospholipid product (LYSOFORTE EXTEND dry)

Treatment 1	Basal diet + 3% Soya oil
Treatment 2	Basal diet + 3% Soya oil + 0.25 g/kg LYSOFORTE EXTEND dry
Treatment 3	Basal diet + 3% Soya oil + 0.50 g/kg LYSOFORTE EXTEND dry
Treatment 4	Basal diet + 3% Soya oil + 0.75 g/kg LYSOFORTE EXTEND dry
Treatment 5	Basal diet + 3% Soya oil + 1 g/kg LYSOFORTE EXTEND dry
Treatment 6	Basal diet + 3% F10
Treatment 7	Basal diet + 3% F10 + 0.25 g/kg LYSOFORTE EXTEND dry
Treatment 8	Basal diet + 3% F10 + 0.50 g/kg LYSOFORTE EXTEND dry
Treatment 9	Basal diet + 3% F10 + 0.75 g/kg LYSOFORTE EXTEND dry
Treatment 10	Basal diet + 3% F10 + 1 g/kg LYSOFORTE EXTEND dry

Animals and housing

Broiler rearing: 0-21 days of age

The study was conducted at the broiler facility on the Experimental Farm, Hatfield, University of Pretoria. Housing and care of the birds were done in such a way as to represent as far as possible commercial conditions. Day old male and female broiler chicks (ROSS 308) of good quality were bought from Eagles Pride Hatchery in Pretoria. On receiving the chicks, all birds were sexed and placed sex-separate in pens. Chicks were reared in an environmentally controlled broiler house in floor pens for the first 21 days. Prior to placing the day-old chicks, the broiler house was washed, disinfected, and pre-heated to the comfort zone of the chicks of 36ºC ambient temperature and at least 34 ºC litter (floor) temperature. Clean pine shavings were spread on the floor of the pens to absorb waste and to help with insulation from the floor. 30 birds were housed per pen (total of 40 pens) of 2.25m^2 resulting in a stocking density of 13.33 birds/m^2. This was considerably less than the commercial accepted norm of 22 birds/m^2.

All birds had *ad libitum* access to feed and water, provided by a tube feeder and bell drinkers, respectively. Automatic heaters provided the optimum temperature to keep the bird in their desired comfort zone. Ventilation were controlled manually to ensure optimum oxygen supply and removal of ammonia and carbon dioxide. Up to 7 days of age, birds were provided with 1-hour darkness and 23 hours' light, thereafter the birds received 8 hours of darkness continuously in a 24-hour period.

For the first 18 days, all birds were fed a single common starter feed and from 19 days a single common grower feed formulated to achieve optimum performance for all birds and according to commercial standard specifications recommenced by ROSS Breeder company. Feed was mixed by Pennville Animal Feeds and delivered to the broiler house.

At 21 days of age, all birds were weighed individually and divided into 6 groups per weight. Only the middle 4 groups representing the median and ± 2 standard deviation in individual BW were selected for the digestibility trial. Remaining birds not selected for the trial (falling more than 2 standard deviation from the median) were returned to floor pens and reared to 35 days of age on commercial feed. The objective of this selection was to reduce variation within and between pens for the digestibility measurements, thus requiring fewer replicate pens of birds and fewer total birds in the trial.

Metabolic study: 22-27 days of age

The metabolic study was conducted with 10 treatments and 9 replicates (metabolic cages) per treatment. Eight birds (4 males and 4 females) of 22 days of age were placed in each metabolic cage of 0.675m^2 resulting in a stocking density of 11.85 birds/m^2. Birds were allocated to metabolic cages such that the average BW and range in BW between cages were similar. The metabolic cages were in a closed building (Experimental Farm, University of Pretoria) fitted with lights, ventilation and air conditioning. Temperature were closely controlled for optimum bird comfort.

Birds (4 males and 4 females) were placed in the metabolic cages at the start of day 22 and received the allocated experimental diets for an adaptation period of 3 days. On day 25, the experimental period started after cages had been cleaned and all excreta has been removed from the trays, and birds continued with the test diet for another 3 days.

Before the start of the experimental period, a 200 gram representative feed sample of all treatments were collected for analysis with the excreta samples at the end of the experimental period. During the three-day experimental period, the feed intake was recorded and a representative excreta sample was collected from the trays under the metabolic cages at the end of the period. At excreta collection, all birds were removed from the cages, and the excreta trays were individually placed on a table where contaminants like feathers and spilt feed were removed. A homogeneous and clean sample of the mixed wet excreta, avoiding dried excreta samples, were freeze-dried before being analysed. The freeze-dried excreta samples, together with samples of the feed, were ground to pass through a 0.5 mm sieve and stored in air tight containers. Samples were oven-dried at a

low temperature (50ºC) for 72 hrs for drying and then analysed for gross energy, dry matter, moisture, protein, fat, fibre, ash and inert marker (titanium dioxide).

The titanium dioxide (TiO_2) tracer was determined using the method of Short *et al*. (1996) with modifications according to Myers *et al*. (2004). The final feed and freeze-dried excreta samples were analysed at Chem Nutri Analytical Laboratory (4 Porcelain Road, Clayville, Johannesburg, Gauteng) for dry matter (DM), gross energy (GE), crude protein (CP) and ether extract (EE) by the following methods:

DM EC (1971),
GE ISO (1998), 9831
CP ISO (2005), 5983-2, N × 6.25 (Kjeldahl)
EE ISO (1999), 6492
CF ANKOM Technology AOCS Approved Procedure Ba 6a-05

All chemical analyses were performed by Chem Nutri Analytical (Table 3.4).

Table 3.4. Chemical analysis of experimental diets used in the metabolic study

Treatment	**DM**	**CP (as is)**	**EE (as is)**	**CF (as is)**	**AME (as is)**	**TiO_2**
	g/100g	**g/100g**	**g/100g**	**g/100g**	**MJ/kg feed**	**Mg/kg**
Treatment 1	88.13	18.34	5.48	4.73	12.66	2571.09
Treatment 2	88.88	18.35	5.93	4.59	12.78	2816.40
Treatment 3	88.87	18.81	5.36	5.11	12.72	2774.00
Treatment 4	88.11	19.27	5.76	5.02	12.65	2612.76
Treatment 5	88.24	18.93	5.46	4.79	12.65	2635.27
Treatment 6	88.80	18.39	5.59	4.98	12.73	2682.79
Treatment 7	88.94	18.49	5.48	4.50	12.78	2646.40
Treatment 8	88.43	18.93	5.61	4.98	12.68	3174.10
Treatment 9	88.44	18.70	5.51	4.65	12.68	2562.60
Treatment 10	88.92	18.90	5.50	4.58	12.82	2530.14

The AME values of the experimental diets were calculated from their respective titanium dioxide ratios and corresponding gross energy (GE) contents, as shown in the following equation:

$$AME\ (MJ/kg) = \frac{GE_{diet} - \left\{GE_{faeces}\ x\ \frac{TiO_{2\ diet}}{TiO_{2\ excreta}}\right\}}{1000}$$

Where: GE_{diet} and GE_{faeces} are the analysed GE values of the diet and excreta samples (Megajoule/kilogram).

At the end of the 28-day experimental period, all birds were weighed and returned to the grow-out floor pens. They received a common broiler finisher feed (28-33 days) that was formulated to commercial standards. The trial was terminated at 35 days of age. At the termination of the trial, birds were sold to Eagles Valley abattoir where the birds were humanely euthanised and slaughtered as per industry standard. Birds were monitored daily by the principal investigator as well as students and staff on the farm to ensure optimum growing conditions and bird comfort throughout the 35 days trial period.

Statistical analysis

Statistical analysis on data was done with the statistical software program SAS (Statistical Analysis System, 2014). The significance between treatments was determined by an analysis of variance with the general linear model (GLM). Means, standard error and significance of differences between means were determined by Fischer's test (Samuels, 1989) at the 95% confidence level. In all cases the level of statistical significance was $P<0.05$. Differences between treatments for mortalities were calculated with a Chi square.
Repeated Measures Analysis of Variance with the GLM model (SAS, 2014) were used for repeated period measures. Means and standard error of means for the different treatments were calculated and significant differences ($P<0.05$) between means were determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$Y_{ij} = \mu + T_i + H_j + TH_{ij} + e_{ij}$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

H_j = effect of the j^{th} house

TH_{ij} = effect of the ij^{th} interaction between treatment and house

e_{ij} = error associated with each Y

3.3. Results

Digestibility of DM, CP and ether extract, as well as AME of feed containing either soya oil of F10 oil are shown in Table 3.5. Dry matter digestibility differed significantly between soya oil and F10 oil treatments. This can be due to the lower MIU value (Table 3.1) of 0.82 for soya oil versus the 1.32 value for the F10 oil. Significant differences for AME of feed containing the two lipid sources were noticed, with soya oil treatments having a higher AME value of 10.95 MJ/kg versus 10.79 MJ/kg for F10 oil treatments. The difference in dietary energy was expected taking into consideration the difference in oil AME in Table 3.1.

Table 3.5. Least square means of dry matter digestibility, crude protein digestibility, crude fat digestibility and apparent metabolisable energy (AME) of experimental diets containing either soya oil or F10 oil

Oil source	Dry matter digestibility (%)	Crude protein digestibility (%)	Crude fat digestibility (%)	Energy (AME) (MJ/kg feed)
Soya oil	93.51[a]	67.85	82.27	10.93[a]
F10 oil	93.05[b]	66.16	82.86	10.79[b]
Standard error of means	0.137	0.613	0.599	7.369

[ab]Values within columns without a common superscript differ significantly ($P<0.05$)

A dose response of the added emulsifier (LEX) was investigated, irrespective of the lipid source (Table 3.6). DM digestibility percentage were significantly different between 0.75 g/kg LEX and 1 g/kg LEX inclusion, with a decrease in DM digestibility for the 1 g/kg LEX inclusion at 92.95%. No dose response was noticed for DM digestibility of feed with increasing levels of LEX.

CP digestibility of feed was the highest for 0.25 g/kg LEX inclusion and significantly differed from the lowest CP digestibility of feed containing 0 g/kg LEX (65.82%) and 1 g/kg LEX (66.08%). CP digestibility did not show a clear dose response, and it was noticed that at the highest LE inclusion of 1 g/kg, CP digestibility seemingly started to drop again.

Crude fat digestibility of feed was the highest for 0.75 g/kg LEX inclusion (84.28%), but did not differ significantly from the CF digestibility of feed containing no LEX. However, feed that contained 1 g/kg of LEX had a significantly lower CF digestibility (80.12%) compared to 0.25 g/kg LEX and 0.75 g/kg LEX.

AME demonstrated that an emulsifier can improve dietary energy since the treatments with 0 g/kg LEX inclusion was significantly lower than the feed containing 0.75 g/kg LEX. As with the other metabolic parameters, AME at 1 g/kg LEX inclusion showed a negative effect.

Table 3.6. Least square means of increasing inclusion levels of LYSOFORTE EXTEND dry on dry matter digestibility, crude protein digestibility, crude fat digestibility and apparent metabolisable energy (AME) of experimental diets

LYSOFORTE Extend Dry Inclusion	Dry matter digestibility (%)	Crude protein digestibility (%)	Crude fat digestibility (%)	Energy (AME) (MJ/kg)
0 g/kg LEX	93.03^{ab}	65.82^{b}	82.61^{ab}	10.78^{b}
0.25 g/kg LEX	93.54^{ab}	69.06^{c}	83.31^{a}	10.91^{ab}
0.50 g/kg LEX	93.26^{ab}	66.63^{abc}	82.50^{ab}	10.89^{ab}
0.75 g/kg LEX	93.62^{a}	67.41^{abc}	84.28^{a}	10.92^{a}
1 g/kg LEX	92.95^{b}	66.08^{ab}	80.12^{b}	10.79^{ab}
Standard error of means	0.134	0.582	0.689	6.999

$^{a-c}$Values within columns without a common superscript differ significantly (P<0.05)

Each fat source was individually investigated for a dose response effect for LEX inclusion level. Table 3.7 show the soya oil and F10 oil treatments with increasing inclusion levels of the emulsifier. The soya oil treatments showed no significant differences for DM digestibility, but numerically 0 g/kg and 1 g/kg LEX inclusion had the lowest values of 92.78% and 92.75%, respectively.

For soya oil treatments, feed containing 0.25 g/kg LEX had a significantly higher CP digestibility (69.88%) than all other dietary treatments. A dose response was not noticed for CP digestibility in diets containing soya oil as the lipid source.

Crude fat digestibility for soy oil treatments, was the highest (84.49%) for feed containing 0.25 g/kg LEX inclusion which was significantly different from the 1 g/kg LEX inclusion at 80.22%. Numerically, EE digestibility was improved at 0.25 g/kg LEX compared to 0 g/kg, 0.50 g/kg and 0.75 g/kg LEX treatments containing soya oil.

Feed containing soya oil with 0.25 g/kg LEX inclusion had significantly higher AME values (10.95 MJ/kg) compared to the feed with 0 g/kg LEX (10.68 MJ/kg) and 1 g/kg LEX (10.71 MJ/kg). Numerically, 0.25 g/kg LEX soya oil treatments had a higher AME value than 0.50 g/kg and 0.75 g/kg LEX treatments containing soya oil.

The F10 oil treatments showed significant differences for DM digestibility for 0.75 g/kg LEX at the highest digestibility, 94.10% and 1 g/kg LEX inclusion with the lowest digestibility of 93.14%. The F10 oil treatment containing 0.75 g/kg LEX showed numerically higher DM digestibility that 0 g/kg, 0.25 g/kg and 0.75 g/kg LEX inclusion.

No significant differences were noticed for the F10 oil treatments for CP digestibility. A numerical difference was noticed with 0.75 g/kg LEX inclusion having the highest value of 69.78% and 1 g/kg LEX inclusion having the lowest value of 66.76%.

F10 oil treatment containing 0.75 g/kg LEX had the highest EE digestibility of 84.79% which are significantly different from 1 g/kg LEX inclusion with the lowest EE digestibility of 80.03%. There was also a numerical difference for EE digestibility at 0.75 g/kg LEX inclusion compared to the lower inclusions of 0 g/kg, 0.25 g/kg and 0.75 g/kg LEX.

LYSOFORTE EXTEND dry inclusion showed no significant difference for AME between different inclusion levels of LEX for F10 oil treatments. A numerical difference was noticed for feed containing 0.75 g/kg LEX which had the highest value at 11.04 MJ/kg. The lowest AME values were noticed for 0 g/kg and 1 g/kg LEX inclusion at 10.87 MJ/kg and 10.88 MJ/kg, respectively.

Table 3.7. Least square means of increasing inclusion levels of LYSOFORTE EXTEND dry to soya oil and F10 oil treatments on dry matter digestibility, crude protein digestibility, crude fat digestibility and energy (AME) of experimental diets

Soya oil treatments	Dry matter digestibility (%)	Crude protein digestibility (%)	Crude fat digestibility (%)	Energy (AME) (MJ/kg)
Soya Oil + 0 g/kg LEX	92.78	64.54^{b}	83.05^{ab}	10.68^{b}
Soya Oil + 0.25 g/kg LEX	93.44	69.88^{a}	84.49^{a}	10.95^{a}
Soya Oil + 0.50 g/kg LEX	93.13	65.90^{b}	82.77^{ab}	10.80^{ab}
Soya Oil + 0.75 g/kg LEX	93.15	65.04^{b}	83.78^{ab}	10.79^{ab}
Soya Oil + 1 g/kg LEX	92.75	65.41^{b}	80.22^{b}	10.71^{b}
F10 Oil + 0 g/kg LEX	93.28^{ab}	67.11	82.16^{ab}	10.87
F10 Oil + 0.25 g/kg LEX	93.65^{ab}	68.24	82.13^{ab}	10.86
F10 Oil + 0.50 g/kg LEX	93.39^{ab}	67.36	82.23^{ab}	10.98
F10 Oil + 0.75 g/kg LEX	94.10^{b}	69.78	84.79^{b}	11.04
F10 Oil + 1 g/kg LEX	93.14^{a}	66.76	80.03^{a}	10.88
Standard error of means	0.126	0.590	0.503	8.753

^{ab}For each oil treatment, values within columns without a common superscript differ significantly (P<0.05)

3.4. Discussion

The objective of this study was to investigate the effect of two different oils and the addition of a LPL in increasing amounts on feed digestibility. Scheele *et al.* (1997) clearly showed differences in AME and chemical composition for different oils. Results from the current study support this finding with marked differences noted in calculated oil energy value for soya oil and F10 oil at different ages for broilers (Table 3.1). Soya oil had a higher energy at both ages compared to F10 oil with 35.92 MJ/kg at 0-21 days of bird age and 37.13 MJ/kg at >21 days of age versus F10 oil energy value of 32.94 MJ/kg at 0-21 days of age and 35.20 MJ/kg at >21 days of age.

This shows the importance of analysing an oil before formulating any feed, as provided tabulated AME values are not always reliable. According to the CVB (2012) soya oil has a poultry AME value of 34.95 MJ/kg and does not take into consideration the effect of bird age, while in this current trial values of 35.92 MJ/kg at 0-21 days of bird age and 37.13 MJ/kg at >21 days of age were calculated with the Wiseman equation. An accurate AME value will improve feed formulation and eliminate over or under formulating energy of a diet. This difference in oil energy value is also noticeable in the digestibility parameters, with the significant lower DM digestibility and energy in the feed supplemented with F10 oil compared to soya oil treatments.

High digestibility values were observed in the current digestibility trial, which could be a possible reason why so few statistical significant differences were noticed.

LYSOFORTE EXTEND dry supplementation of feed containing either soya oil of F10 oil at increasing levels, did not show a dose response for nutrient digestibility or AME value of the feed. Statistical differences for soya oil treatments were noticed when feed was supplemented with 0.25 g/kg LEX versus the control. Numerical differences for the different digestibility parameters were noticed when feed was supplemented with 0 0.75 g/kg LEX compared to the control. LYSOFORTE EXTEND dry inclusion of 0.25 g/kg demonstrated a marked improvement for CP digestibility (69.06%) versus the control, irrespective of the lipid source. At the inclusion of 0.75 g/kg LEX a significant improvement was noticed for AME (10.92 MJ/kg) versus the control, irrespective of the lipid source.

It is necessary to remember that fat energy is twice that of protein and carbohydrates (NRC, 1994) with an AME value of 0.038 MJ per 1 gram of fat. This makes fat an attractive option to increase diet energy densities, especially where feed cost plays an important role. Dietary AME can be improved with the inclusion of an emulsifier as was demonstrated by Melegy *et al.* (2010) and Zhang *et al*. (2011) and the current trial supported these findings with the addition of LEX in soya oil treatments at 0.25 g/kg LEX and F10 oil treatments at 0.75 g/kg LEX.

A linear dose response to LEX inclusion was not seen, and at 1 g/kg LEX inclusion a negative effect was noticed for both soya and F10 oils, possibly due to the decrease in fat digestibility, with 80.22% EE digestibility for soya oil and 80.03% for F10 oil. Further investigation needs to be performed to determine the reason for the decrease in digestibility parameters at 1 g/kg LEX. Thus, it was concluded that 1 g/kg LEX inclusion would not improve fat digestibility and absorption and the maximum LEX dose to show a positive response was 0.75 g/kg. It should be mentioned that the expected dose response was not clearly noticed as the 0.50 g/kg LEX inclusion did not fit into the positive dose response that was expected and would need further investigation.

Soya oil treatments with 0.25 g/kg LEX showed the best response for crude protein digestibility, crude fat digestibility and diet AME, making this the recommended dose of LEX for a diet containing soya oil as energy source. F10 oil did not show this increased improvement for any parameter at 0.25 g/kg LEX but rather at a higher inclusion of 0.75 g/kg LEX, with significant differences noticed for DM digestibility and EE digestibility. It can be concluded, due to the differences between the blended oil (F10) and soya oil, that more

of a LPL will be needed to improve lipid digestibility and absorption in F10 oil. The metabolic study was followed up by a broiler performance trial to investigate the effects of either a soya oil or a F10 oil in combination with LEX on growth performance in broilers.

3.5. Conclusion

Results from the metabolic study have shown that energy content differs between lipid sources and evaluating a lipid source before feed formulation can assist in more efficient utilisation of raw materials and precision feeding of animals. This study also demonstrated that an addition of 0.25 g/kg LEX to a diet containing 3% soya oil delivered optimal results for CP digestibility and AME. For a blended oil (F10) of lower quality a higher inclusion of 0.75 g/kg LEX will be required to improve DM and EE digestibility due to the variability of a blended oil quality can content. Further investigation is needed to determine the reason for a negative results when adding LEX at 1g/kg.

CHAPTER 4

The effect of lysophospholipids and two different oil sources on broiler performance

4.1. Introduction

A 35-day broiler performance study was conducted at the University of Pretoria. The study evaluated the efficacy of a lysophospholipid (LEX) to breach an energy deficit created by lowering the oil content, and decreasing the diet AME with 0.42 MJ/kg feed. Two oils were used during the trial to investigate if a cheaper and lower quality blended oil (F10) can replace soya oil without adversely affecting performance. Lysophospholipids was added to diets at increasing levels to determine the optimum dose of the product. Performance parameters including body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were measured weekly during the trial. The trial was approved by the Animal Ethics Committee of the University of Pretoria (EC058-16).

4.2. Materials and Methods

Lipids and diets

Oils used in the formulations were provided by Energy Oil (165 Tedstone Road, Wadeville, Gauteng):

1. Refined soya oil
2. F10: an unsaturated blend of animal fats and vegetable oils with a maximum of 10% FFA content

Both oils were analysed at Chem Nutri Analytical Laboratory (4 Porcelain Road, Clayville, Johannesburg, Gauteng) for free fatty acids (FFA), total saturated fatty acids (SFA), total unsaturated fatty acids (USFA) and moisture, impurities and unsaponifiables (MIU). The AME for young and older animals were calculated from these results by the Wiseman equation (Wiseman & Salvador, 1989). The following methods were used:

FFA	AOAC Ca 5a-40
SFU	AOAC 977.17
USFA	AOAC 977.17
Moisture	AOCS Ca 2c-25
Insoluble Impurities	AOCS Ca 3a-46
Unsaponifiable Matter	AOCS Cs 6b-53

The AME values of both oils (Table 4.1) were determined before diets were formulated using the Wiseman equation (Wiseman & Salvador, 1989):

$$\text{Dietary energy (MJ/kg fat)} = A + B \times FFA + C \times e^{(DxU/S)}$$

Table 4.1. Chemical analysis and AME calculation for soya oil and F10 oil used in the performance trial

	Soya oil	**F10 oil**
FFA[a]	0.36	1.17
Total Saturated Fatty Acids	12.08	31.38
Total Unsaturated Fatty Acids	87.92	68.62
Unsaturated/Saturated ratio	7.28	2.21
MIU[b]	0.62	2.67[c]
AME 0-21 days broilers (MJ/kg)	37.47	32.13
AME >21 days broilers (MJ/kg)	38.32	34.53

[a]Free fatty acids
[b]Moisture, impurities, unsaponifiables
[c]1.86% was due to moisture content

The basal diets (Table 4.2, Table 4.3 and Table 4.4) were formulated to meet industry standards for ROSS 308 broiler chickens. The positive control (PC) diets for both soya oil and F10 oil consisted of 3% oil, whereas the negative control (NC) diets consisted of 1.8% oil which resulted in a 0.42 MJ AME/kg decrease.
Three feed phases were included during the grow-out period of 35 days, starter, grower and finisher. Birds were fed a starter diet from 0-14 days, grower diet from 15-18 days and a finisher diet from 29-35 days. The starter feed was in a crumble from whereas the grower and finisher diets were pelleted.

Table 4.2. Raw material inclusion and calculated nutrient levels for starter diets supplemented with either soya oil or F10 oil

Ingredients (%)	**Soya oil PC[1]**	**Soya oil NC[2]**	**F10 oil PC[1]**	**F10 oil NC[2]**
	Starter	**Starter**	**Starter**	**Starter**
Yellow maize	45.40	45.16	47.30	46.30
Soya oilcake	26.33	25.59	26.82	25.88
Bran	6.50	8.69	4.11	7.26
Sunflower oilcake	5.00	5.00	5.00	5.00
Soya Oil	3.00	1.80	-	-
F10 Oil	-	-	3.00	1.80
Limestone	1.42	1.43	1.41	1.43
Methionine hydroxy analogue (MHA)	0.34	0.34	0.34	0.34
Salt	0.46	0.46	0.46	0.46
Avatec	0.06	0.06	0.06	0.06
Olaquindox 10%	0.02	0.02	0.02	0.02
Axtra Phy 10000 TPT	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration				
Moisture (%)	9.68	9.78	9.69	9.79
AME Broiler (MJ/kg)	11.50	11.08	11.5	11.08
Crude protein (%)	22.00	22.00	22.00	22.00
Ether extract fat (%)	6.59	5.45	6.49	5.40
Crude fibre (%)	5.42	5.61	5.23	5.49
Ash (%)	3.93	4.01	3.84	3.96

[1]PC = Positive Control: formulated with 3% additional lipid and an AME of 11.5 MJ/kg

[2]NC = Negative Control: formulated with 1.8% additional lipid and an AME of 11.08 MJ/kg (0.42 MJ/kg less than PC)

Table 4.3. Raw material inclusion and calculated nutrient levels for grower diets supplemented with either soya oil or F10 oil

Ingredients (%)	**Soya oil PC[1]**	**Soya oil NC[2]**	**F10 oil PC[1]**	**F10 oil NC[2]**
	Grower	**Grower**	**Grower**	**Grower**
Yellow maize	51.22	51.03	53.11	52.16
Soya oilcake	18.85	18.29	19.32	18.57
Bran	6.89	9.57	4.47	8.12
Sunflower oilcake	7.50	7.50	7.50	7.50
Soya Oil	3.00	1.80	-	-
F10 Oil	-	-	3.00	1.80
Limestone	1.35	1.36	1.34	1.35
Methionine hydroxy analogue (MHA)	0.30	0.30	0.30	0.30
Salt	0.45	0.45	0.45	0.45
Avatec	0.06	0.06	0.06	0.06
Olaquindox 10%	0.02	0.02	0.02	0.02
Axtra Phy 10000 TPT	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration				
Moisture (%)	9.61	9.72	9.63	9.72
AME Broiler (MJ/kg)	11.75	11.33	11.75	11.33
Crude protein (%)	20.00	20.00	20.00	20.00
Ether extract fat (%)	6.68	5.55	6.59	5.49
Crude fibre (%)	5.67	5.88	5.48	5.76
Ash (%)	3.59	3.68	3.50	3.62

[1]PC = Positive Control: formulated with 3% additional lipid and an AME of 11.5 MJ/kg

[2]NC = Negative Control: formulated with 1.8% additional lipid and an AME of 11.08 MJ/kg (0.42 MJ/kg less than PC)

Table 4.4. Raw material inclusion and calculated nutrient levels for finisher diets supplemented with either soya oil or F10 oil

Ingredients (%)	**Soya oil PC[1]** **Finisher**	**Soya oil NC[2]** **Finisher**	**F10 oil PC[1]** **Finisher**	**F10 oil NC[2]** **Finisher**
Yellow maize	60.17	59.87	62.06	61.00
Soya oilcake	12.86	12.281	13.33	12.56
Bran	5.44	7.52	3.06	6.09
Sunflower oilcake	10.00	10.00	10.00	10.00
Soya Oil	3.00	1.80	-	-
F10 Oil	-	-	3.00	1.80
Limestone	1.28	1.29	1.27	1.29
Methionine hydroxy analogue (MHA)	0.27	0.27	0.27	0.26
Salt	0.45	0.45	0.45	0.45
Avatec	0.06	0.06	0.06	0.06
Olaquindox 10%	0.02	0.02	0.02	0.02
Axtra Phy 10000 TPT	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration				
Moisture (%)	9.56	9.67	9.57	9.68
AME Broiler (MJ/kg)	12.25	11.83	12.25	11.83
Crude protein (%)	18.01	18.06	18.01	18.06
Ether extract fat (%)	6.74	5.60	6.64	5.55
Crude fibre (%)	5.69	5.88	5.50	5.77
Ash (%)	3.13	3.22	3.04	3.16

[1]PC = Positive Control: formulated with 3% additional lipid and an AME of 11.5 MJ/kg

[2]NC = Negative Control: formulated with 1.8% additional lipid and an AME of 11.08 MJ/kg (0.42 MJ/kg less than PC)

A lysophospholipid product, LEX (Kemin Industries, Sub-Sahara Africa) was added at increasing increments to each of the oils to determine if there was a dose response. Dietary treatments are shown in Table 4.5.

Table 4.5. Treatments used in the broiler performance trial with two lipid sources (soya oil and F10 oil). And increasing dosages of a lysophospholipid product (LYSOFORTE Extend Dry).

Treatment 1	Positive Control (PC[1]) Basal diet + 3% Soya oil
Treatment 2	Negative Control (NC[2]) Basal diet + 1.8% Soya oil (-0.42 MJ/kg)
Treatment 3	NC[2] (soya oil) + 0.25 g/kg LYSOFORTE EXTEND dry
Treatment 4	NC[2] (soya oil) + 0.50 g/kg LYSOFORTE EXTEND dry
Treatment 5	NC[2] (soya oil) + 0.75 g/kg LYSOFORTE EXTEND dry
Treatment 6	Positive Control (PC[1]) Basal diet + 3% F10
Treatment 7	Negative Control (NC[2]) Basal diet + 1.8% F10 (-0.42 MJ/kg)
Treatment 8	NC[2] (F10 oil) + 0.25 g/kg LYSOFORTE EXTEND dry
Treatment 9	NC[2] (F10 oil) + 0.50 g/kg LYSOFORTE EXTEND dry
Treatment 10	NC[2] (F10 oil) + 0.75 g/kg LYSOFORTE EXTEND dry

[1]PC = Positive Control: formulated with 3% additional lipid and an AME of 11.5MJ/kg

[2]NC = Negative Control: formulated with 1.8% additional lipid and an AME of 11.08MJ/kg (0.42 MJ/kg less than PC)

Animals and housing

The study was conducted in a broiler facility on the Experimental Farm, Hatfield, University of Pretoria. Housing and care of the birds were done in such a way as to represent as far as possible commercial conditions. 1800 day-old male ROSS broiler chicks from the same breeder flock were acquired from Eagles Pride Hatchery (Pretoria). Male birds were selected via feather sexing, with a weight of between 35 and 37 grams to minimise variation. 20 male birds were placed in floor pens (pen size 2.25 m^2) resulting in a stocking density of 8.88 birds/m^2. Pen treatment designation followed a completely randomised block design to minimise the influence of variations in the house environment on treatments. 90 pens were used during the trial with 9 replicates per treatment.

Prior to placing the day-old chicks, the broiler house was washed, disinfected and pre-heated to the comfort zone of chicks of 36˚C ambient temperature and at least 34˚C litter (floor) temperature. Clean pine shavings were spread on the floor of the pens to absorb waste and to help with insulation from the floor.

All birds had *ad libitum* access to experimental feed and water, provided by tube feeders and a water nipple line with three nipples per pen. Automatic heaters provided the optimum temperature to keep the birds in their desired comfort zone. Ventilation were controlled automatically to ensure optimum oxygen supply and removal of ammonia and carbon dioxide. Up to a weight of 160 g, birds were provided with 1-hour darkness and 23 hours' light, thereafter the birds received 8 hours' darkness in a 24-hour period.

Performance measurements

All birds were weighed weekly until the end of the trial at 35 days of age. Feed intake (FI) were also measured weekly and body weight gain (BWG) and feed conversion ratio (FCR) corrected for mortalities, were calculated. The trial was terminated at 35 days of age. At the termination of the trial birds were sold to Eagles Valley abattoir where the birds were humanly euthanised and slaughtered as per industry standard. Birds were monitored daily by the principal investigator as well as students and staff on the farm to ensure optimum growing conditions and bird comfort throughout the 35 days trial period.

Statistical analysis

Statistical analysis on data was done with the statistical software program SAS (Statistical Analysis System, 2014). The significance between treatments was determined by an analysis of variance with the general linear model (GLM). Means, standard error and significance of differences between means were determined by Fischer's test (Samuels, 1989) at the 95% confidence level. In all cases the level of statistical significance was $P<0.05$. Differences between treatments for mortalities were calculated with a Chi square.
Repeated Measures Analysis of Variance with the GLM model (SAS, 2014) were used for repeated period measures. Means and standard error of means for the different treatments were calculated and significant differences ($P<0.05$) between means were determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$Y_{ij} = \mu + T_i + H_j + TH_{ij} + e_{ij}$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

H_j = effect of the j^{th} house

TH_{ij} = effect of the ij^{th} interaction between treatment and house

e_{ij} = error associated with each Y

4.3. Results

Before the start of the performance study the two oils under investigation were chemically analysed (Table 4.1) and AME was calculated via the Wiseman equation (Wiseman & Salvador, 1989) with a correction for MIU content. Free fatty acid content was low for soya oil and F10 oil at 0.36% and 1.17%, respectively. Soya oil had a much higher U/S ratio (7.28) than F10 oil (2.21), which positively influenced the AME value of the soya oil. For young broilers, 0-21 days of age, there was a 5.34 MJ/kg AME difference between the two oils demonstrating the differences between oils and their chemical composition (Murugesan, 2013). This AME difference was smaller for older broilers of >21 days of age (3.79 MJ/kg) confirming the effect of age on fat

digestion and absorption (Leeson & Atteh, 1995; Melegy *et al.*, 2010). A low MIU value was noticed for soya oil (0.62), while F10 oil had a high MIU value of 2.67% due to the high moisture content of 1.86% that was present in this oil. This moisture content of F10 oil was over the maximum acceptable limit of 1% (Butolo, 2002), which negatively influenced the oil's AME value. The calculated AME values were used during the feed formulation, 37.47 MJ/kg for soya oil and 32.13 MJ/kg for F10 oil. These values differed from tabulated values. According to the CVB (2012) the AME content of soya oil is 34.95 MJ/kg. No tabulated AME values were available for a blended oil due to the unknown chemical composition of such an oil.

During the performance study, BWG and FI were measured weekly, and FCR (corrected for mortalities) calculated. No statistical differences for FI, BWG or FCR were noticed for either of the oils at the end of the 35-day performance trial. Numerically, the birds that consumed the F10 oil treatments had a higher feed intake compared to the birds that consumed the soya oil treatments (3131 grams versus 3120 grams). Body weight gain was numerically higher for birds consuming soya oil treatments than for the birds consuming F10 oil treatments with 1962 grams versus 1956 grams.

Table 4.6. The effect of supplementation of feed with either soya oil or F10 oil on feed intake, body weight gain and feed conversion ratio (FCR) of broilers at the end of a 35-day performance trial

Lipid source	**Feed intake (g/bird)**	**Body weight gain (g/bird)**	**FCR**
Soya oil	3120	1962	1.59
F10 oil	3131	1956	1.60
Standard error of means	5.780	2.697	0.005

During the performance study, the NC diets were formulated to have 0.42 MJ/kg less AME compared to the PC diet (Table 4.5). LYSOFORTE EXTEND dry was added to the NC diets at increasing levels, irrespective of the lipid source. No statistical differences were noticed for either FI or BWG, while numerically FI was the highest for the NC and NC + 0.25 g.kg LEX treatments, 3156 g/bird and 3151 g/bird respectively, and the lowest for the PC (3085.34 g/bird). Statistical differences were noticed between FCR for the PC (1.57) versus NC and NC + 0.25 g/kg LEX at 1.61 for both treatments.

Table 4.7. The effect of increasing inclusion levels of LYSOFORTE EXTEND dry (LEX) in feed on feed intake, body weight gain and feed conversion ratio (FCR) irrespective of lipid source during the performance trial

Inclusion	Feed intake	Body weight gain	FCR
PC	3085	1966	1.57[b]
NC (-0.42 MJ/kg)	3156	1957	1.61[a]
NC + 0.25 g/kg LEX	3151	1963	1.61[a]
NC + 0.50 g/kg LEX	3130	1962	1.60[ab]
NC + 0.75 g/kg LEX	3104	1947	1.59[ab]
Standard error of means	13.667	3.418	0.007

[ab]Values within columns without a common superscript differ significantly ($P<0.05$)

Each lipid source was individually investigated to determine if there was a dose response for FI. Table 4.8 show FI for both soya oil and F10 oil treatments, with the increasing inclusion levels of LEX on the NC diets. The soya oil treatments showed no significant differences for FI during the period of the trial. Only at the end of the 35-day performance study, a significant difference for FI was noticed for soya oil PC (3046 g/bird) and soya oil NC + 0.25 g/kg LEX (3188 g/bird). Overall a negative response in FI was noticed when LEX was added to the diet.

Statistical differences for FI were noticed for F10 oil treatments at 14 days and 28 days. At 14 days of bird age, the highest FI of 541.2 g/bird was noticed for the F10 oil NC treatment, that was expected due to the lowered diet AME, and the lowest FI was noticed for F10 Oil PC (513.8 g/bird) and F10 oil NC + 0.75 g/kg LEX (317.7 g/bird). The lower FI noticed for the PC is due to the diet AME meeting the bird's energy requirements, as feed formulation was based on breed standard, while the NC + 0.75 g/kg LEX showed a positive effect compared to the NC. At 28 days of bird age, there was a significant difference in FI between F10 NC and F10 NC + 0.25 g/kg LEX with 1860 g/bird and 1785 g/bird, respectively. At the end of the 35-day performance trial only a numerical difference was noticed for the F10 oil treatments, with the lowest FI for F10 oil + 0.75 g/kg LEX (3098 g/bird) and the highest FI for F10 oil NC (3200 g/bird). This demonstrated an improvement in FI with LEX addition compared to the NC.

Table 4.8. Least square means of increasing inclusion levels of LYSOFORTE EXTEND dry (LEX) to soya oil and F10 oil on feed intake (g/bird) on a 0.42 MJ/kg energy deficient diet (NC) during the performance trial

	0-7 days	**0-14 days**	**0-21 days**	**0-28 days**	**0-35 days**
3% Soya oil (PC)	163.6	525.9	1130	1790	3046[b]
1.8% Soya oil (NC)	167.1	529.5	1129	1808	3113[ab]
Soya oil NC + 0.25 g/kg LEX	167.0	529.2	1135	1825	3188[a]
Soya oil NC + 0.50 g/kg LEX	160.9	528.4	1157	1820	3142[ab]
Soya oil NC + 0.75 g/kg LEX	160.6	514.0	1111	1824	3109[ab]
3% F10 oil (PC)	166.5	513.8[b]	1146	1819[ab]	3125
1.8% F10 oil (NC)	169.8	541.2[a]	1144	1860[a]	3200
F10 oil NC + 0.25 g/kg LEX	165.4	519.8[ab]	1137	1785[b]	3115
F10 oil NC + 0.50 g/kg LEX	166.8	529.9[ab]	1142	1831[ab]	3118
F10 oil NC + 0.75 g/kg LEX	164.3	517.7[b]	1114	1808[ab]	3098
Standard error of means	0.914	2.715	4.495	6.731	13.827

[a-b]Values within columns without a common superscript differ significantly (P<0.05)

Statistical significant differences for BWG (Table 4.9) were noticed for treatments containing soya oil at 28 days of age. The lowest BWG was noticed for soya oil NC treatments (1328 g/bird) while the highest BWG was noticed for soya oil NC + 0.50 g/kg LEX (1380 g/bird). Numerical differences were noticed at the end of the 35-day performance study, with the lowest BGW for soya oil NC (1947 g/bird) and the highest BWG for soya oil NC + 0.50g/kg LEX (1987 g/bird).

Treatments containing F10 oil demonstrated statistical significant differences for BWG at 14, 21 and 28 days of age. At 14 days of age numerical differences where noticed for the lowest BWG was noticed for F10 oil NC + 0.50 g/kg LEX (349.4 g/bird) with the highest BWG noticed for F10 oil PC (371.1 g/bird) and F10 oil NC + 0.25 g/kg LEX (371.2 g/bird). The lowered BWG at 14 days for F10 NC + 0.50 g/kg LEX demonstrated a negative effect on weight gain significantly compared to PC and F10 NC + 0.25 g/kg LEX. At 21 days of age the lowest BWG was noticed for F10 oil NC + 0.50 g/kg LEX (781.9 g/bird) and F10 oil NC + 0.75 g/kg LEX (784.0 g/bird) while the highest BWG was noticed for F10 oil PC at 825.9 g/bird and was significantly different. At 28 days of age the lowest BWG was for F10 oil NC + 0.75 g/kg LEX (1325 g/bird) and the highest BWG for F10 oil PC (1377 g/bird), which was also significantly different. At the end of the 35-day performance trial only numerical differences were noticed. The lowest BWG at 35 days of age was noticed for F10 oil NC + 0.50 g/kg LEX (1937 g/bird) and F10 oil NC + 0.75 g/kg LEX (1939 g/bird) and F10 oil PC with the highest BWG of 1976 g/bird. The depression in BWG with the addition of LEX was unexpected, due to research that a LPL improve digestibility and absorption, further investigation is needed to determine what the cause of this depression can be.

Table 4.9. Least square means of increasing inclusion levels of LYSOFORTE EXTEND dry (LEX) to soya oil and F10 oil on body weight gain (g/bird) on a 0.42 MJ/kg energy deficient diet (NC) during the performance trial

	0-7 days	0-14 days	0-21 days	0-28 days	0-35 days
3% Soya oil (PC)	117.3	351.1	801.5	1358^{ab}	1956
1.8% Soya oil (NC)	119.1	349.4	777.7	1328^{a}	1947
Soya oil NC + 0.25 g/kg LEX	126.2	364.3	780.1	1348^{ab}	1964
Soya oil NC + 0.50 g/kg LEX	120.4	359.4	802.2	1380^{b}	1987
Soya oil NC + 0.75 g/kg LEX	120.9	360.0	792.8	1362^{ab}	1954
3% F10 oil (PC)	124.8	371.1^{a}	825.9^{b}	1377^{b}	1976
1.8% F10 oil (NC)	123.6	360.2^{ab}	795.6^{ab}	1364^{ab}	1967
F10 oil NC + 0.25 g/kg LEX	127.4	371.2^{a}	809.6^{ab}	1360^{ab}	1963
F10 oil NC + 0.50 g/kg LEX	122.2	349.4^{b}	781.9^{a}	1349^{ab}	1937
F10 oil NC + 0.75 g/kg LEX	118.2	354.4^{ab}	784.0^{a}	1325^{a}	1939
Standard error of means	1.088	2.567	4.809	5.808	4.962

^{ab}Values within columns without a common superscript differ significantly (P<0.05)

A few tatistical significant differences for FCR (Table 4.10) were noticed for both the lipid sources during the performance study period. Soya oil treatments showed significant differences for FCR at 14, 21 and 35 days of age. At 14 days of age the lowest FCR of 1.43 was for soya oil NC + 0.75 g/kg LEX treatment and the highest FCR of 1.53 was noticed for soya oil NC, demonstrating a positive effect of LPL addition to the diet of young birds. At 21 days, the lowest FCR was still for the soya oil NC + 0.75 g/kg LEX treatment at 1.40, while the highest FCR changed to soya oil NC + 0.25 g/kg LEX treatment at 1.46. At the end of the 35-day performance trial the FCR was the lowest at 1.56 for soya oil PC treatment and the highest at 1.63 for soya oil NC + 0.25 g/kg LEX treatment, not demonstrating any positive effect with the addition of LEX for older birds. Treatments containing F10 oil demonstrated statistical differences for FCR throughout the whole performance period. At 7 days of age the lowest FCR was noticed for F10 oil NC + 0.25 g/kg LEX (1.31) and the highest for F10 oil NC + 0.75 g/kg LEX (1.40), demonstrating that young birds with an energy deficient diet can improve FCR with the addition of LEX at 0.25 g/kg at a negative effect is seen at higher inclusion levels. At 14 days of age this changed to lowest values for F10 oil PC (1.39) and F10 oil NC + 0.25 g/kg LEX (1.40) and the highest FCR values for F10 oil NC (1.51) and F10 oil NC + 0.50 g/kg LEX (1.52). At 14 days of age for birds with treatment F10 NC + 0.25 g/kg LEX to have the same FCR can be interpreted that the LEX overcame the energy deficiency created in the diet for young birds with immature gastrointestinal tracts. At 21 days of age a negative effect with the addition of LEX was noticed at 0.50 g/kg inclusion with the highest FCR value of 1.46. At 28 days of age, F10 oil NC + 0.25 g/kg LEX again demonstrated the positive effect of LEX in

overcoming an energy deficiency with the lowest FCR of 1.31 while the highest FCR values were noticed for F10 oil NC (1.37) and F10 oil NC + 0.75 g/kg LEX (1.37). The same FCR was noticed for F10 NC and F10 NC + 0.75 g/kg leading to the conclusion that LEX at this inclusion level had no effect on improving lipid metabolism. At the end of the 35-day performance trial the FCR was the lowest for F10 oil PC (1.58) and F10 oil NC + 0.25 g/kg LEX (1.59) and the highest for F10 oil NC (1.63). For F10 treatments it was concluded that LEX at 0.25 g/kg has a positive effect on FCR for diets lowered in AME.

Table 4.10. Least square means of increasing inclusion levels of LYSOFORTE EXTEND dry (LEX) to soya oil and F10 oil on feed conversion ratio (FCR) on a 0.42 MJ/kg energy deficient diet (NC) during the performance trial

	0-7 days	**0-14 days**	**0-21 days**	**0-28 days**	**0-35 days**
3% Soya oil (PC)	1.40	1.50^{ab}	1.41^{ab}	1.32	1.56^{c}
1.8% Soya oil (NC)	1.41	1.52^{a}	1.45^{ab}	1.36	1.60^{ab}
Soya oil NC + 0.25 g/kg LEX	1.33	1.46^{ab}	1.46^{a}	1.35	1.63^{a}
Soya oil NC + 0.50 g/kg LEX	1.34	1.47^{ab}	1.44^{ab}	1.32	1.58^{bc}
Soya oil NC + 0.75 g/kg LEX	1.33	1.43^{b}	1.40^{b}	1.34	1.59^{abc}
3% F10 oil (PC)	1.34^{ab}	1.39^{b}	1.39^{a}	1.32^{ab}	1.58^{b}
1.8% F10 oil (NC)	1.38^{ab}	1.51^{a}	1.44^{ab}	1.37^{a}	1.63^{a}
F10 oil NC + 0.25 g/kg LEX	1.31^{a}	1.40^{b}	1.41^{a}	1.31^{b}	1.59^{b}
F10 oil NC + 0.50 g/kg LEX	1.37^{ab}	1.52^{a}	1.46^{b}	1.36^{ab}	1.61^{ab}
F10 oil NC + 0.75 g/kg LEX	1.40^{b}	1.46^{ab}	1.42^{ab}	1.37^{a}	1.60^{ab}
Standard error of means	0.011	0.015	0.008	0.007	0.007

$^{a-c}$Values within columns without a common superscript differ significantly (P<0.05)

4.4. Discussion

The objective of this study was to investigate the effect of two different lipids (soya and F10 oils) and increasing inclusion of LEX in diets with a 0.42 MJ/kg energy deficit on broiler performance (FI, BWG and FCR). The study showed that lipid source is important to determine correct AME value of an oil, as per Murugesan (2013) and Wiseman *et. al.* (1992). Soya oil was a high-quality oil with low FFA, SFA and MIU values and high USF value which all improved the oil's AME value. The F10 oil was of a lower quality due to the high moisture content of 1.86%, that was above the maximum limit of 1% (Butolo, 2002), which acted as a diluting factor, as was seen in the lowered AME value for this oil. F10 oil also had a high SFA constant of 31.38% versus soya oil at 12.08% and as Wiseman *et al.* (1991), Leeson & Atteh (1995) and Smits *et al.* (2000) all demonstrated a high SFA content decrease fat utilisation by the bird. Van Kuiken & Behnke (1994)

demonstrated that lipase activity is inhibited in the presence of long chain SFA and this could also be a reason for the lowered FCR results for the F10 oil diets versus the soya oil diets.

Lipid digestion and absorption occur mainly in the small intestine (Freeman, 1976; Hurwits *et al*., 1973; Krogdahl, 1985) and the gastrointestinal tract of a young bird only mature around 14 days of age (Noy & Skaln, 1998). This was apparent in the FI, BWG and FCR results, especially for F10 oil treatments. With statistical differences noticed at 7, 14 and 21 days for FI, BWG and FCR for F10 oil treatments, these differences became less apparent at 28 days for FI and BWG and was lost at 35 days of age. The positive effect of LEX at 0.25 g/kg in F10 oil treatments for FCR was seen throughout the 35-day study period.

Pond *et al.* (2005) and Davenport (1980) stated that emulsification of LCFA is needed for transportation into the intestinal cells. Emulsification is improved by the addition of a LPL (Mine *et al.,* 1993) and could be the reason for the statistical significant improvements that was noticed for FI on the NC diets at 14 days of age with the addition of 0.75 g/kg LEX to F10 oil treatments and at 28 of age days with 0.25 g/kg LEX addition to F10 oil treatments. Improved emulsification could also have accounted for the improvement that was noticed for FCR on the NC diets at 14 days of age for 0.75 g/kg LEX addition for soya oil treatments and for 14, 28 and 35 days of age at varying LEX additions to F10 oil treatments.

LYSOFORTE EXTEND dry supplementation successfully overcame the 0.42 MJ/kg decrease in AME of the NC diets. LYSOFORTE EXTEND dry assisted the birds to optimally utilise energy in the diets by improving digestion and absorption of lipids (Melegy *et al.,* 2010; Leeson & Atteh, 1995).

4.5. Conclusion

In conclusion, broiler performance was significantly improved with the addition of an emulsifier to energy deficient diets, adding up to 0.42 MJ dietary AME to the diet. From the results, it seems as if 0.25g/kg of LEX is the most optimal inclusion level in diets that contain both high quality oils (soya oil) or lower quality oils (F10 blended oil).

CHAPTER 5

GENERAL CONCLUSION

With the constant increase in feed raw material costs, it is of critical importance to decrease feed costs without negatively influencing nutrient value. This is accomplished with high energy dense diets that positively influence growth performance of birds (Blanch *et al.*, 1996). This high energy dense diets are accomplished by the addition of fat that is the most energy dense component of feed (NRC, 1994) at 0.038 MJ per gram of fat versus the 0.017 MJ per gram of protein and carbohydrate. There are various energy sources available for poultry feed. Soya oil is the most commonly used due to its high lipid quality and digestibility, but unfortunately due to its high demand, soya oil prices are constantly increasing. Thus, alternative oils need to be investigated for the utilisation in poultry feed.

Scheele *et al.* (1997), Wiseman (1999) and Tancharoenrat *et al.* (2009) showed the importance of knowing the chemical composition of an oil as it directly influences the AME value of an oil. F10 oil is a locally produced unsaturated oil blend of vegetable oils and animal fats with a maximum FFA content of 10%. F10 is significantly cheaper than soya oil, as soya oil is also used in human food and pet food production. With a known AME value for a blended oil, the feed formulation can be adapted to ensure correct diet energy value to meet the birds demand, and decrease the feed costs. Wiseman *et al.* (1991) created an equation that incorporates FFA content and U/S ratio to calculate an AME value for an oil without a laborious digestible study, shortening the time before an oil with a known AME value can be used in feed formulation.

Adding oil to a diet increase the energy density, but due to the immature nature of a bird's gastrointestinal tract (Kroghdahl, 1985; Wiseman, 1990; Baião & Lara, 2005; Tancharoenrat *et al.*, 2013) an emulsifier will be needed to optimally utilise the fat in the diet. Zhang *et al*. (2011) postulated that lysophospholipids act as an emulsifier in combination with bile salts, during the beginning phases of lipid digestion. Lysophospholipids also influence cell membrane permeability (Wendel, 2000; Lundbaek, 2006) which influences fat adsorption.

LYSOFORTE EXTEND dry is a nutritional emulsifier consisting of hydrolysed lecithins and is enriched in lysophospholipids. The aim of the two studies was to investigate if LEX improve fat digestibility, diet AME and growth parameters. A positive effect was seen with LEX addition at 0.25 g/kg for soya oil containing diets during the digestibility trial and at 0.75 g/kg for F10 oil containing diets. The increase in digestibility at different inclusion levels for the different oils could be due to the difference in oil quality. Soya oil had a higher FFA and U/S ratio compared to F10 oil, and these parameters are important during fat metabolism. The lower LEX dose for soya oil was noticed due to the better oil quality, the higher dose needed for F10 oil treatments are due to the lower quality of the oil.

Some growth parameters were also positively influenced in the NC diets that had a 0.42 MJ AME deficit, when supplemented with LEX. At 35 days of age birds fed the soya oil NC diets had the same BWG as the soya oil PC diet, demonstrating the compensatory effect of LEX on a lowered energy dense diet compared to a more expensive energy dense diet. This is evidence that LEX can effectively utilise all energy available in a diet containing soya oil, up to 0.42 MJ AME. In conclusion LEX can be added at 0.25 g/kg to both soya oil and F10 oil containing diets with a 0.42 MJ energy deficit, and maintain growth parameters at the end of a 35-day period, thus effectively decreasing feed cost without negatively influencing growth performances.

CHAPTER 6

CRITICAL EVALUATION AND RECOMMENDATION

During the metabolic trial, the soya oil and F10 oil that was to be utilised in the trial treatments, were collected a few weeks before feed formulation occurred. This was necessary to perform the chemical analyses, and for future trials this time should be kept to a minimum. The oils should also be stored in a cool dry place, out of direct sunlight as this can initiate oxidation of the lipids in the oils, and decrease the AME value of the oil due to higher FFA formation. Feed formulation was based on AME Poultry (MJ/kg) and it is recommended to use the more correct AME for broilers when it is a broiler study being run.

Final feed for all treatments during the metabolic trial were analysed for nutrient concentrations to ensure formulation and mixing was done according to the provided formulation. From the results it was evident that the final feeds' nutrient values were close to the formulation specification.

A lower than standard industry norm for stocking density was used to adhere to the Ethics Committee. In future it would be recommended to keep stocking density to commercial standard as the results from such an academic trial is not always favourably received by commercial farmers. Furthermore, handling of birds in the metabolic house was labour intensive. The only concern was water supply to the birds in the metabolic cages as this process is done manually, and in the summer months a water shortage is possible when birds are not constantly observed. To prevent a negative effect on feed intake, it would be worthwhile to look into an automatic watering system to keep birds hydrated at all times.

Even though handling of birds were kept to a minimum, the transport of birds between the grow-out house and the metabolic house can lead to increased stress, especially during increasing environment temperatures. Bird transportation should be kept to early mornings and to the shortest time possible. The adaptation period in the metabolic house did assist in alleviating this stress factor on bird performance.

In this trial, total excreta collection was not done, due to the many factors that can influence total collection like water, feed and feather contamination. Titanium dioxide was used as an inert marker and would be recommended to be used in future trials. Excreta sampling was a labour-intensive process, but with cooler boxes at hand and a speedy delivery to the laboratory the same day, this setup worked in this trial. Pre-arranging with the laboratory that will be analysing the faecal samples is very important as the drying process takes 3 days and they need to prepare for this time, especially if a high number of samples are taken. Close communication with the Laboratory Manager ensured that the samples that was taken on a Friday was dried over the weekend under supervision, minimising risks of enzymatic decomposition of the faecal samples if they were left to stand over the weekend till the Monday.

With “on top” LEX trials, it is recommended to be clear on the objectives beforehand, as was in this trial. The trial did not focus on growth performance and only investigated digestibility improvement with the aid of an emulsifier. Since a dose response was investigated during this trial and no other data was available up to date, it was unexpected to see a negative response at the highest inclusion level of LEX (1 g/kg). This led to the conclusion that a nutritional emulsifier can show negative effect at certain dosages.

Lipid quality differs between vegetable, animal and blended oils. Hence the protocol incorporated a pure vegetable oil and a blended oil that are commonly used in Sub-Sahara Africa. Results clearly reinforced this knowledge, as feed digestibility containing the soya oil was improved at 0.25 g/kg LEX, while maximum benefit for feed containing F10 oil was only attained at 0.75 g/kg LEX inclusion.

For a next metabolic trial, I would recommend a significant energy deficit in the feed, to investigate to what extend LEX can overcome the energy deficiency by just increasing the feed nutrient digestibility. For the current trial it was apparent that nutrient digestibility was very high, and to expect a significant improvement in digestibility was not plausible.

Further investigation into the negative effect that was noticed at 1 kg LEX inclusion is required. It can be hypothesised that the decrease in response at this higher dose could be due to the composition of LEX.

During the broiler performance trial, the soya oil and F10 oil that was to be utilised in the 35-day performance trial treatments, were collected a few weeks before feed formulation occurred. This was done to ensure the same oil sources were used during the whole 35-day trial for all feeding phases (Starter, Grower and Finisher). Both oil sources were also chemically analysed to ensure that there was an AME and lipid quality difference between the oils. Keeping the oil sources for such a long period is not ideal as oxidation becomes more of a problem, but for academic reasons the aim was to use the same oil throughout the 35-day trial to minimise variables as much as possible. The oils should also be stored in a cool dry place, out of direct sunlight as this can initiate oxidation of the lipids in the oils, and decrease the AME value of the oil due to higher FFA formation.

For the broiler performance trial AME Broilers (MJ/kg) was used as it was specific a broiler trial, and the energy value was more specific. Treatments in this trial had a 0.42 MJ/kg energy deficiency and it was hypothesised that this deficit would be severe enough to negatively influence broiler performance. Unfortunately, what was noticed that irrespective of the oil source used there was no significant difference for FI and BGW between the PC and the NC. This might be an indication that in Sub-Sahara regions, nutritionist tend to over formulate out of fear that birds will not get the necessary nutrients for performance.

When evaluating the results per oil source and just looking at the PC and NC treatments it was again very apparent that there was no significant difference between the treatments for both the soya oil treatments and

the F10 oil treatments. The only significant difference was for the F10 oil treatment where the NC had a significantly higher FI at 14 days of age than the PC. NC for FCR at 14 days of age did show a significant difference for F10 oil treatments compared to the PC, and the same significant difference was noticed for both soya oil treatments and F10 oil treatments at 35 days of age.

In future trials it would be recommended that the standard feed formulation is not an over formulation, as this just lead to a loss of nutrients in the faeces and can lead birds reaching their full performance potential making significant differences less likely to be noticeable. Trials going forward must ensure that a proper energy deficit is present to ensure a negative effect on growth performance to test the true effect of a semi-emulsifier. Even with a very small day-old chick weight, at 36 gram average, birds still performed to ROSS 308 standards, reaching their full genetic potential for nutrient utilisation and growth.

From these trials it became evident that metabolically soya oil treatments performed better with a lower lysophopholipid dosage, whereas F10 oil treatments needed a higher inclusion levels. On the other hand, when looking just at growth performance a 0.50 g/kg dose for both oil sources showed the best performance compared to the NC.

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