EFFECT OF DIETARY LIPID SATURATION ON LAYER PRODUCTION AND EGG QUALITY

by

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DECLARATION

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Ernest John King

Bloemfontein

2 July 2012

DEDICATED TO MY FAMILY

- To my parents, David and Coreen King, for all the guidance, love and support in the past three years. Thank you for the opportunity you gave me to be able to study and encouraging me throughout my studies.

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ACRONYMS AND ABBREVIATIONS

AME	Apparent metabolisable energy
AME_n	Apparent metabolisable energy corrected for nitrogen
ANOVA	Analysis of variance
BE	Blunt end
BHT	Butylated hydroxytoluene
Ca	Calcium
CLA	Conjugated linoleic acid
CP	Crude protein
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
EFA	Essential fatty acid
EFC	Extractable fat content
EPA	Eicosapentaenoic acid
EQ	Equilibrium
ESA	Eggshell surface area
FA	Fatty acid
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
FE	Feed efficiency
FFA	Free fatty acids
FFDM	Fat free dry matter
GIT	Gastro intestinal tract
HDL	High density lipoprotein
HO	High oleic acid
HSD	Honest significant difference
HU	Haugh units
LCT	Lower critical temperature
LDL	Low density lipoprotein
LPL	Lipoprotein lipase

LSD	Least significant difference
ME	Metabolisable energy
MUFA	Monounsaturated fatty acid
MUFA / SFA	Monounsaturated fatty acid to saturated fatty acid ratio
n-3	Omega-3 fatty acids
n-6	Omega-6 fatty acids
n-6 / n-3	Omega-6 fatty acids to omega-3 fatty acids ratio
n-9	Omega-9 fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate
OM	Organic matter
PUFA	Polyunsaturated fatty acid
PUFA / SFA	Polyunsaturated fatty acid to saturated fatty acid ratio
PV	Peroxide value
RCM	Red-crab meal
SE	Sharp end
SFA	Saturated fatty acid
ST	Shell thickness
SWUSA	Shell weight per unit surface area
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TMA	Trimethylamine
TME	True metabolisable energy
U/S	Unsaturated fatty acid to saturated fatty acid ratio
UCT	Upper critical temperature
UFA	Unsaturated fatty acid
UFA / SFA	Unsaturated fatty acid to saturated fatty acid ratio
VLDL	Very low density lipoprotein

CHAPTER 1
GENERAL INTRODUCTION

In South Africa, cardiovascular disease (CVD) claimed the lives of 195 people every day in the seven year period between 1997 and 2004 (Steyn, 2007). Although coronary heart disease could be genetically inherited, 80% of all heart diseases can be avoided by choosing a healthier lifestyle through improving fitness as well as a change in diet. Within South Africa, it is suggested that one out of five children (20%) is stunted while one out of ten children (10%) is malnourished. In contrast, as indication of the severe diversity in spendable income on food, one out of ten (10%) children in South Africa is obese (DBSA, 2008). Since both these conditions (malnutrition as well as obesity) have some relation to dietary choice and food availability, a number of people in South Africa have a high risk to suffer from some form of cardiovascular malfunction later in life due to obesity, while others face the consequences related to malnourishment, which included poor growth and development as well as a weakened immune system.

The relationship between cholesterol and CVD is well known and one of the main reasons why health practitioners advise patients to try and avoid food with high levels of cholesterol and/or saturated fats. However, various authors (Leskanish & Noble, 1997; McNamara, 2000; Simopoulos, 2000) illustrated that CVD is rather related to the fatty acid composition of the specific food source, rather than to the cholesterol content itself. Consumption of the correct ratio of omega-6 to omega-3 (n-6 / n-3) fatty acids and the presence of sufficient essential fatty acids (EFAs) could reduce the risk of cardiovascular disease in humans (Ros, 2008; Lecerf, 2009; Keefer, 2011). Furthermore, Basmacioglu *et al*. (2003) confirmed that both saturated fatty acids (SFAs) and *trans*-fatty acids cause negative effects on human health and concluded that polyunsaturated fatty acids (PUFAs) have a positive effect on human health as related to a decrease in coronary heart diseases. Essential fatty acids are classified as long chain PUFAs that can only be supplied through dietary consumption while some of the most commonly known EFAs are α-linolenic, linoleic and arachidonic acid.

Omega-3 (n-3) fatty acids received a lot of attention in recent years due to its properties associated with reducing the incidence and risk of cardiovascular disease as well as reducing blood pressure (Simopoulos, 1999; Lecerf, 2009). These fatty acids are also important for

normal growth as well as the development of brain and nervous tissues in foetuses and infants (Kirubakaran *et al*., 2011). Omega-3 fatty acids assist in reducing blood platelet aggregation, decrease oxidative radicals and increase the concentration of high density lipoproteins (HDL) which are responsible for removing cholesterol and lowering triglyceride content in plasma (Lecerf, 2009). People suffering from obesity are at risk of being insulin resistant, while high insulin levels interfere with the breakdown of carbohydrates which leads to the storage of excess carbohydrates as fat in the storage depots of the body. Insulin resistance can effectively be reduced through an increased dietary n-3 fatty acids intake, thereby lowering the insulin levels and increase the utilisation of calories more effectively. Keefer (2011) reported that elevated levels of n-3 fatty acids in the bloodstream of obese people resulted in a decreased food consumption with a consequent reduction in calorie intake.

Diets of most people in the developed western world are very high in omega-6 (n-6) fatty acids with n-6 / n-3 ratios ranging between 20 / 1 and 30 / 1, which is directly related to food production and -preparation methods (Simopoulos, 1999). Intensifying production systems on a large scale has led to feeding animals predominantly maize and soybean meal based diets, which have high concentrations of n-6 fatty acids. The consequence of this practise is the production of meat, milk and eggs, which are higher in n-6 and lower in n-3 fatty acids (Simopoulos, 1999). Lipid inclusion in poultry diets is a common technique used to increase the energy density and reduce dustiness of the diets while supplying the animal with EFAs. Various authors (Caston & Leeson, 1990; Jiang *et al*., 1991; Scheideler & Froning, 1996; and Cachaldora *et al*. 2008) illustrated that supplementary lipid sources such as olive-, fish-, flaxseed- linseed-, soybean oil, lard, tallow and palm oil (varying in lipid saturation from highly unsaturated to highly saturated) could be used very effectively to enrich eggs with specific fatty acids in concurrence with the dietary fatty acid profile. Since these supplemental lipid sources differ in terms of their fatty acid profile, the fatty acid methyl esters (FAME) of eggs are altered in a similar manner. The Food and Agricultural Organization (FAO, 2010) of the United Nations recommend that the daily dietary intake of n-3 fatty acids should contribute between 0.5 and 2.0% of an adult's daily dietary energy consumption and between 5 to 9% for n-6 fatty acids. Since most human food sources are high in saturated and/or polyunsaturated n-6 fatty acids, the dietary consumption of n-3 fatty acids by humans is very limited, especially in diets of lower income groups due to the cost of n-3 enriched food products. The enrichment of eggs with n-3 and n-6 fatty acids by means of dietary intervention is one of the alternatives to increase the consumption of these EFA as a

valuable protein supplement to an unbalanced human diet. These "enriched" eggs could contribute approximately 368 mg n-3 / 60 g egg and 639 mg n-6 / 60 gram egg, depending on the lipid type and inclusion levels used during the manipulation of the dietary fatty acid profile (Yannakopoulos *et al*., 2005). The consumption of these "enriched" eggs would contribute to about 30% of the daily recommended dietary allowance for n-3 products of adults (Food & Nutrition Board, 2002). Additionally, supplying eggs enriched with these EFA as part of their daily diets to those individuals at risk of malnutrition or obesity could contribute significantly in the prevention of nutritional disorders.

Lately, another point of focus has been the influence of the Mediterranean diet on the occurrence and prevention of CVD. The Mediterranean diet is generally known as a high fat diet due to the customary inclusion of olive oil and whole nuts in human diets (Ros, 2008). As a result, these diets are rich in monounsaturated fatty acids (MUFAs) which help with reducing the risk related to CVD (Estruch *et al*., 2006; Ros, 2008; Lecerf, 2009). Monounsaturated fatty acids have the ability to lower the total- and low density lipoprotein (LDL) cholesterol levels while decreasing the plasma triglyceride levels and increasing the HDL cholesterol levels, which is one of the major means in decreasing the CVD risk (Kris-Etherton, 1999). This specific properties of MUFA have resulted that these fatty acids are considered as the "new important" group of omega-9 (n-9) fatty acids for humans.

Considering the beneficial effects of supplementing layer diets with supplemental lipids for fatty acid enrichment, one needs to be aware of the confounding documented results. Several authors (Baucells *et al*., 2000; Ansari *et al*., 2006; Turgut *et al*., 2006; Cachaldora *et al*., 2008) are in agreement regarding the alteration of egg yolk FAME according to the dietary fatty acid profile. However, they differ in their opinion regarding the effect of dietary fatty acid profile on specific egg components. Grobas *et al*. (1999b) concluded that lipid supplementation of layer diets resulted in heavier eggs and an increase in egg production, while other authors (Yannakopoulos *et al*., 1999; Huthail & Al-Yousef, 2010) reported that the usage of lipid sources for the alteration of dietary fatty acids had no effect on egg production or egg weight. A major concern associated with the feeding of long chain PUFAs in layer diets is the susceptibility of eggs to lipid oxidation and subsequent lower consumer acceptability due to a possible decrease in organoleptic properties (Hayat & Cherian, 2010). It further seems that lipid stability of n-3 enriched eggs are not only associated with the usage of fish oil, but that other vegetable oils such as flaxseed-, linseed- and rapeseed oil may also

cause a decrease in the lipid oxidative stability of eggs (Cherian *et al*., 2007; Hayat & Cherian, 2010; Dunn-Hurrocks *et al*., 2011). However, it seems that the decrease in organoleptic properties of "enriched" eggs is mainly confined to the usage of marine type n-3 lipid sources (Yannakopoulos *et al*., 2005) and that lipid oxidation of egg yolk fatty acids enriched with n-6 and n-9 occurs at a slower rate.

The current study was conducted in an attempt to improve scientific knowledge regarding the effects of dietary lipid saturation on nutrient digestibility, production efficiency of laying hens and egg quality characteristics associated with changes in the FAMEs of eggs. Since most documented studies focussed on the effect of different lipid sources and inclusion levels on egg production and egg quality characteristics over relatively short periods of time, the current study will focus on the degree of dietary lipid saturation, ranging from highly unsaturated to highly saturated diets over a longer period of time. Fatty acids that contain no carbon-carbon double bonds are commonly known as SFA (McDonald *et al*., 2002). On the other hand fatty acids with one double bond are known as MUFAs and those with two or more double bonds as PUFAs (McKee & McKee, 2003). Furthermore, McDonald *et al*. (2002) mentioned that unsaturated fatty acids (UFAs) are grouped into families based on their specific oleic (C18:1; n-9), linoleic (C18:2; n-6) and/or α-linolenic (C18:3; n-3) precursors. These families are also called omega-9, omega-6 and omega-3, referring to the positions of the double bonds nearest to the carbon atom in the fatty acid. During the present study, the inclusion of different lipid saturation sources in the diets was used to obtain different levels of dietary lipid saturation. To address the focus areas of this study, the aims of the study were as follow:

- Firstly, in Chapter 3 the effect of dietary lipid saturation on nutrient digestibility of layer diets was investigated.
- Secondly, in Chapter 4 the effect of dietary lipid saturation on production performance of laying hens during peak production (20 to 40 weeks of age) was evaluated.
- Lastly, in Chapter 5 the effect of lipid saturation on internal egg quality parameters, egg yolk FAMEs and -oxidative stability was investigated.

This dissertation consists of a general introduction (Chapter 1), a literature review (Chapter 2), three separate chapters of the conducted experiments (Chapters 3 − 5) and finishes with the general conclusions of the complete dissertation (Chapter 6). Although great care has been taken to avoid unnecessary repetition, some duplication is inevitable.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Eggs are nutritionally one of the most complete food sources for human consumption, yet the egg industry is being put under severe pressure (Basmacioglu *et al*., 2003) mainly due to the controversy regarding the cholesterol content of eggs. Human health issues continue to receive undue adverse publicity, although more recently, even medical professionals have questioned these views. There is little doubt that human diets high in saturated fatty acids (SFAs) are harmful in terms of accentuating arteriosclerotic type conditions in susceptible individuals, although the role of cholesterol in this issue has not been fully resolved. Regardless of absolute cholesterol levels, the undisputed fact is that eggs do contain cholesterol, provoking nutritional concerns in more affluent markets where food alternatives are available. It is interesting to note that cholesterol is rarely an issue in developing countries that are striving to meet human protein needs and where laying hens provides one of the most efficient means in converting feed sources, unsuited for human consumption, into one of the most balanced food protein sources known to mankind (Leeson & Summers, 1997).

In general, the public have become concerned about the relationship between dietary cholesterol and the development of coronary heart disease during the past few years (Basmacioglu *et al*., 2003), raising questions regarding egg consumption and its favourable and/or unfavourable health effects. However, dietary fat type and fatty acid profile of fat consumed seem to be more important than the quantity of cholesterol consumed (Leskanich & Noble, 1997; Simopoulos, 2000). Basmacioglu *et al*. (2003) confirmed that SFAs and *trans*-fatty acids cause negative effects on human health, but that the consumption of polyunsaturated fatty acids (PUFAs) has a positive effect on human health as to coronary heart disease.

The inclusion of lipid sources in layer diets is normally done to increase the energy levels and palatability thereof, acts as a source of essential fatty acids (EFAs) and as carriers of fat-soluble vitamins and their precursors within the body. However, lipids also act as energy storage in the form of a fat-pad within the body to supply energy during starvation (Mandal *et*

al., 2004) although it is considered that commercial layer hens would probably never have to utilize this energy resource. Jiang *et al*. (1991) indicated that the correct utilization of lipid sources in layer diets could aid in reducing the cholesterogenic effect of eggs by altering the fatty acid composition and incorporating more omega-3 (n-3) and -6 (n-6) type fatty acids into the egg yolk

The aim of this literature review is to evaluate available documented reports regarding the usage of different supplementary lipid sources used for the manipulation of dietary lipid saturation levels and the consequent effects of this practise on egg production and -quality characteristics.

2.2 Digestion of lipids in poultry

Since fats are insoluble in water, any factors that increase the miscibility of fat with water will facilitate fat digestion (Bondi, 1987). Pancreatic juice contains the enzymes trypsin, pancreatic amylase, pancreatic lipase, cholesterol esterase and maltase. The lipase enzyme is important in the sequential digestion of lipids since an occluded pancreatic duct resulted in the excretion of undigested dietary fat in the faeces (Perry, 1984) and a consequent dietary energy loss. Additionally, the liver produces yellowish-green alkaline bile, which aid in the digestion of fats and fatty acids by helping the actions of lipase enzyme (Gillespie, 2002) and assists in neutralising the acidic chyme coming from the gizzard into the small intestine (Bondi, 1987; Taylor & Field, 2004). Bile salts are synthesised from cholesterol by the hepatocytes in the liver and are conjugated with amino acids taurine or glycine, which increases the miscibility of fat (Drackley, 2000). Pancreatic lipase act at an oil-water interface created through the emulsification of lipids with bile salts. The lipase breaks down triglyceride into fatty acids, monoglycerides and glycerol while cholesterol esterase hydrolyses cholesterol fatty acid esters into cholesterol and free fatty acids (FFA). Leeson and Summer (2001) indicated that short chain fatty acids and glycerol are absorbed into the portal system while FFAs, monoglycerides and cholesterol are emulsified with bile salt, producing micelles and are solubilised in the aqueous phase of the intestinal tract (Larbier & Leclerq, 1994).

2.3 Absorption of lipids

Lipids are mainly absorbed from the gastro intestinal tract (GIT) in the jejunum area, but some fatty acid absorption does occur in the duodenum and the ileum as well. This could be

as a result of the anti-peristaltic movements found in the small intestine of poultry. Different end products of fat digestion, such as monoglycerides, glycerol, iso-lecithin, cholesterol and FFAs are organised with bile salts in a mixed micelle form (Mandal *et al*., 2004). Drackley (2000) indicated that the formation of these mixed micelles is necessary to move the non-polar lipids across the unstirred water layer present at the surface of the villi. The mixed micelle then travels to the villi brush border where it erupts (Mandal *et al*., 2004). All of the contents except the bile acids are absorbed into the intestinal epithelium while bile salts are not absorbed until they reach the terminal ileum (Drackley, 2000).

In the intestinal epithelium, the 2-monoglycerides, iso-lecithin, FFAs and cholesterol are further converted to triglycerides, phospholipids and cholesterol esters, which are organised and encapsulated by a thin layer of protein called portomicron, for absorption into the blood. These portomicrons are carried to the liver where they are further converted into very low density lipoproteins (VLDL). Free fatty acids consisting of a short chain lengths and glycerol are absorbed directly into the blood (Mandal *et al*., 2004) without the aid of bile salts or micelle formation.

Mandal *et al*. (2004) indicated that as a blood glucose source, glycerol plays an important role under starvation conditions as well as during the usage of carbohydrate free diets. Under these conditions, fatty acids are utilised for energy purposes through a series of chemical reactions called beta-oxidation (β-oxidation). Acetyl-CoA is produced as a result of the oxidation and is utilised as an energy source during the synthesis of fatty acids. Cholesterol is actively synthesised from acetyl-CoA in intestinal cells (Drackley, 2000).

2.4 Fatty acid transport in the body

Once fatty acids have been absorbed from the GIT they need to be transported to other parts of the body. Drackley (2000) reported that the transportation of fatty acids requires that highly non-polar lipids are packaged in such a manner that they are stable in the aqueous environment. Lipoproteins containing a high proportion of triglyceride (> 70%) are firstly synthesised by the intestine to transport dietary fatty acids to the rest of the body and secondly by the liver to transport triglycerides to the extra-hepatic tissues (Griffin & Hermier, 1988). The resulting lipoprotein that is produced within the intestine is classified as VLDL (Drackley, 2000), also referred to as portomicrons, emphasising their entry into the bloodstream. These portomicrons are secreted directly into the portal system, because the

lymphatic system is not fully developed in the fowl. The uptake of portomicrons by the liver is very slow and most protomicrons secreted into the portal system pass straight through the liver to the extra-hepatic tissues (Griffin & Hermier, 1988).

Lipids, with the exception of FFAs, which are bound to serum albumin, circulate as compounds of large lipoprotein particles. Lipoproteins consist of non-polar lipids, principally triglycerides and cholesterol esters, with phospholipids and cholesterol and specific apoproteins at the lipid/plasma interface (Griffin & Hermier, 1988). Gropper *et al*. (2005) indicated that lipoproteins are classified according to their buoyant density, which is determined by the ratio of lipids to proteins and the different proportion of lipid types i.e. triglycerides, cholesterol, cholesterol esters and phospholipids present. An increase in lipid content would result in a lower density, with the lowest to highest density order being; (i) portomicrons, (ii) VLDL, (iii) low density lipoprotein (LDL) and lastly (iv) high density lipoproteins (HDL) (Drackley, 2000). The protein component of lipoproteins, apolipoprotein (apo), not only stabilises the lipoprotein as it circulates in the aqueous environment, but also bestow specificity on lipoprotein complexes to be recognised by receptors on cell surfaces for further metabolism. Apolipoproteins also stimulate certain enzymatic reactions which regulates lipoprotein metabolic activity (Gropper *et al*., 2005).

In the liver VLDL is produced to facilitate the transportation of endogenous triglyceride in plasma (Drackley, 2000). After secretion from the liver, VLDL acquires apolipoprotein-CII (Apo-CII) from circulating HDL. Once transported to tissues, triglyceride contained in the VLDL needs to be hydrolysed, because only FFAs can pass through the tissue membranes (Leeson & Summers, 2001). Apolipoprotein-CII activates lipoprotein lipase (LPL) activity contained in the endothelial cell walls of the tissues (Sigmaaldrich, 2009). LPL hydrolyses the lipoprotein triglyceride to fatty acids and glycerol, enabling the fatty acid to enter the surrounding tissues by diffusion as either oxidised or re-esterified. Griffin and Hermier (1988) reported that LPL was identified in a wide range of tissues within chickens such as adipose tissue as well as muscle, heart and ovarian follicles. Hepatic VLDL is controlled by the supply of fatty acids, either from the diet, *de novo* lipogenesis in the liver or from other tissues (Griffin & Hermier, 1988).

2.5. Lipogenesis

The intestinal lymphatic system is poorly developed in fowls and lipoproteins are directly secreted into the portal vein (Hebbar, 2009), thus making the liver the main site of lipogenesis (Hood, 1984). The triglycerides present in the adipose tissue of laying hens is either synthesised in the liver from carbohydrates or derived from the diet (Griffin & Hermier, 1988). During egg production, the metabolic activity of the liver is increased to supply lipids to the growing oocytes. Butler (1975) suggested that the quantity of lipid synthesised during the laying period almost equals the body weight of the hen. Adipose tissue, skin (Hood, 1984) and even the skeleton (Nir *et al*., 1988) can make minor contributions to lipogenesis.

Acetyl-CoA is the building block for *de novo* synthesis of fatty acids in the liver (Hood, 1984; Drackley, 2000). It may derive from the (i) oxidative de-carboxylation of pyruvate, an end product of glucose metabolism; from the (ii) breakdown of exogenous or endogenous fatty acids or from (iii) catabolised amino acids, mainly glucogenic amino acids such as threonine, alanine and arginine (Nir *et al*., 1988). The acetyl-CoA is generated within the mitochondria whereas *de novo* synthesis occurs in the cytoplasm. Hood (1984) indicated that since acetyl-CoA does not freely diffuse through the mitochondrial membrane into the cytoplasm, it is converted by way of condensation with oxaloacetate to form citrate, which can then freely diffuse into the cytoplasm. In the cytoplasm, the citrate is cleaved to oxaloacetate and acetyl CoA by ATP-citrate lyase, making the acetyl-CoA available for lipogenesis. Acetyl-CoA carboxylase now produces malonyl-CoA from the acetyl-CoA, which is converted to SFAs by means of fatty acid synthetase enzyme action. Malonyl-CoA is the actual donor of acetyl units needed for the elongation process (Drackley, 2000).

Fatty acid synthetase is a multiple enzyme complex that requires NADPH (Hood, 1984) generated through metabolism of glucose in the pentose phosphate pathway and in the malic enzyme reaction (Drackley, 2000) to provide reducing equivalents. Smith (1994) indicated that this complex consists of two multifunctional polypeptide chains, each containing seven distinct enzyme activities necessary to elongate a growing fatty acid. Two polypeptide chains are arranged head-to-tail, resulting in two separate sites for synthesis of fatty acids enabling each enzyme complex to assemble two fatty acids simultaneously. Drackley (2000) illustrated the overall reaction for the synthesis of one molecule of palmitic acid as follow:

$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\ \text{NADPH} + 14\ H^+ \rightarrow \text{palmitic acid} + 7\ CO_2 + 8\ \text{CoA} + 14\ NADP^+ + 6\ H_2O$$

The synthesis of fatty acids is regulated by dietary factors and hormone levels that control enzyme synthesis in the hen (Hood, 1984). Ovarian hormone secretion at onset of puberty shows a significant increase in lipid synthesis during preparation for egg production (Butler, 1975). During increased physiological activity of the ovary towards sexual maturity, which is an oestrogen driven process, fat accumulation in the liver increases (Hebbar, 2009) in order to supply the fatty acids needed within the egg yolk. Subsequently, these specific fatty acids in egg yolk are needed to supply the developing embryo with constant levels of energy (Hood, 1984) during the incubation process and the first few hours after hatching.

2.6. Lipids

According to Mandal *et al*. (2004), lipids are a group of biomolecules which are insoluble in water but soluble in common organic solvents such as benzene, ether, hexane and chloroform. This group includes fats, oils, phospholipids, waxes, carotenoids, and sterols. Lipids can act as important structural components, stores of energy, chemical signals as well as transporters of fat soluble vitamins and pigments (McDonald *et al*., 2002). Some of the common factors that influence the body fat content of birds are age, sex, reproductive stage and nutritional status.

2.6.1 Classification of lipids

Fats and oils are constituents of both plants and animals and are a concentrated source of energy. The digestible energy values per unit of weight of fats and oils are generally much higher (2.25 times more) than that of glucose and other carbohydrate sources (Cheeke, 2005; Kleyn, 2006). The structure of both constituents is generally the same but they have different physical and chemical properties (McDonald *et al*., 2002) that will determine whether lipids are solid (fats) or liquid (oils) at room temperature (Leeson & Summers, 2001). Saturation of lipids determine the melting point thereof i.e. a lipid being more unsaturated have a lower melting point, thus being a determining factor to classification as either an oil or a fat (Cheeke, 2005).

The saturation level of lipids refers to the presence or absence of one or more double bonds in the fatty acid chain. Fatty acids that contain no carbon-carbon double bonds are SFAs and

are more commonly found in animals than in plants. Palmitic acid (C16:0) and stearic acid (C18:0) are examples of SFAs, which are solid at room temperature (McDonald *et al*., 2002). Fatty acids that contain one or more carbon double bonds are known as unsaturated fatty acids (UFAs) and are mostly found in plant materials. The double bonds in UFAs cause these fatty acids to occur in two isomeric forms namely the *cis*- and *trans*- form. In the *cis*-isomer, hydrogen atoms are on the same side of a double bond whereas in the *trans*- form, the hydrogen atoms appear on opposite sides of the double bond. The increased number of double bonds in UFAs makes them more susceptible to oxidative attack compared to SFAs. Fatty acids with one double bond are known as monounsaturated fatty acids (MUFAs) and those with two or more double bonds as PUFAs (McKee & McKee, 2003).

2.6.2 Essential fatty acids

Plants and bacteria have the ability to synthesise all needed fatty acids by themselves (McKee & McKee, 2003), but animals cannot synthesise any fatty acid of which the double bond is closer than nine carbons from the methyl group (Drackley, 2002; McDonald *et al*., 2002). These PUFAs are essential to animals for normal formation of cell membranes and as a source of eicosanoids. Polyunsaturated fatty acids are classified into two groups of fatty acids namely; n-3 and n-6, and since animals are unable to synthesise these fatty acids with double bonds in the n-3 and n-6 positions, they need to be supplied in the diet (McDonald *et al*., 2002). Linoleic acid and α-linolenic acid are of critical importance because from these fatty acids longer chain fatty acids, which are highly unsaturated, such as arachidonic acid (C20:4; n-6) are able to be synthesised. During elongation and desaturation of fatty acids, the final double bond is always fixed from the methyl group, hence eicosapentaenoic acid (C20:5; n-3) and docosahexaenoic acid (C22:6; n-3) cannot be synthesised from linoleic acid (C18:2) but only from α-linolenic acid (C18:3) (Drackley, 2002).

Cherian and Sim (1991) reported that hens fed flaxseed, a source of α-linolenic acid (short chain PUFA) to increase the n-3 fatty acids levels of eggs, eicosapentaenoic acid (EPA), docosapentaenoic acid (C22:5; DPA) and docosahexaenoic acid (DHA) levels increased significantly in the egg yolk. These findings clearly indicated that hens have the ability to convert α-linolenic acid to longer chain n-3 fatty acids and deposit it into the egg in an efficient manner.

2.6.3 Rancidity and antioxidants

Rancidity can drastically reduce the nutritional value of feed especially that of the fat and oil component, causing primarily a major decrease in energy value but also in the availability of fat soluble vitamins (Kleyn, 2006). Mandal *et al*. (2004) indicated that saturation of fats and oils plays a considerable role in the effect that rancidity has on these feed constituents and concluded that rancidity can either be categorized as hydrolytic or oxidative. Hydrolytic rancidity does not influence the nutritional value of the constituents and normally refers to the action of microorganisms on fats and oils, causing the simple hydrolysis of fat into fatty acids, diglycerides, monoglycerides and glycerols (Leeson & Summers, 2001).

Oxidative rancidity or lipid peroxidation decreases the nutritive value of fats and oils, a reaction catalysed through the presence of trace minerals in the existence of oxygen. At the site of lipid unsaturation, a hydrogen molecule is removed and replaced by an oxygen molecule, thereby producing a fatty acid peroxide free radical. An increase in the degree of lipid unsaturation would consequently result in a higher risk for lipid oxidation (Leeson & Summers, 2001). Once free radicals are formed they tend to attack other fatty acids, thereby readily creating an exponential reaction, which can be prevented by the supplementation of dietary antioxidants (McDonald *et al*., 2002). Antioxidants can prevent this action by providing a hydrogen molecule to the free radical, protecting the fatty acid from further oxidation (Surai & Sparks, 2002). Natural antioxidants that are generally used are different forms of vitamin A, D and E as well as carotenoids (Leeson & Summers, 2001).

2.6.4 Using lipids in diet formulation for poultry

In general, poultry diets must supply birds with their specific protein, energy, mineral and vitamin requirements to allow optimum production. The determining factor of feed consumption and the efficiency of utilization of such a diet is the energy density thereof (McDonald *et al*., 2002). Laying hens consume feed according to their energy requirements toward maintenance and egg production. Although the practise of lipid supplementation in layer diets is not as common as that in broiler diets, the usage of supplementary dietary lipid sources improve the efficiency of feed utilisation and energy supply in all poultry species (Leeson & Summers, 2001; McDonald *et al*., 2002; Kleyn, 2006).

Supplying diets with fats and oils have the beneficial effect of supplying EFAs which cannot be synthesised in the body itself. Vegetable and marine oils are normally preferred over fats

because of their UFA content (Palmquist, 2002). Fatty acids supplied by the diet are an invaluable source of fatty acids to yolk lipids. Furthermore, fats and oils also supply fat soluble vitamins, of which vitamin E is the most important. Additionally, a direct effect on the feed manufacturing and handling process of lipid supplemented diets is a decrease in dustiness and a consequent increase in the palatability thereof (Leeson & Summers, 2001; Mandal *et al*., 2004).

The energy value of fats and oils being used in poultry diets vary according to their saturation level, the absorbability from the GIT, FFA contamination and intrinsic animal characteristics. An increase in the UFA content of lipid sources results in a higher energy value thereof (Leeson & Summers, 2001; Kleyn, 2006). Medium chain fatty acids present in coconut oil have a considerable lower energy value than that of longer chain fatty acids due to their metabolic pathways (Gurr, 1984). Synergism exists between SFAs and UFAs when fed as a blend in the same diet as both Leeson and Summers (2001) and Kleyn (2006) reported that UFAs increases the energy value of SFAs resulting in a higher energy value for a blend of these fatty acids compared to when either fatty acid would be fed individually. This synergism is caused by the excellent emulsifying capacities of the UFAs (Ketels & De Groote, 1989). Taking into account that fatty acids are not excreted in the urine, their metabolisable energy (ME) value is a function of the absorbability of the fatty acids from the intestine (Leeson & Summer, 2001). Impurities and FFA contamination of the fats and oils being fed decrease the energy value of these products to such an extent that the fatty acids are not available for absorption in the intestines. The most important factor to consider when supplementing a diet with a fat or oil source is the type and age of the animals being fed. This is evident from the fact that younger birds do not utilise fat as efficiently as older birds (Kleyn, 2006). To further ensure that the benefits of supplying diets with fats and oils are maintained, it is of utmost importance that other nutrients are balanced according to the energy density of the diet. In the case where the energy density of the diet increases due to lipid supplementation, while other nutrients is not adjusted accordingly, nutrient deficiencies and/or malnutrition may occur (McDonald *et al*., 2002).

2.6.5 Sources of fats and oils used in poultry diets

Lipid sources commonly used in animal nutrition include fats and oils rendered from animals such as, lard, tallow, marine and/or fish oil as well that obtained from plants and oilseed crops such as, sunflower, linseed, soybean, cotton and rape seed oil. A short literature

summary regarding the fatty acid profiles of certain lipid sources used in layer diets are presented in Table 2.1, while the fatty acid ratios of these lipid sources are indicated in Table 2.2. Data of both tables clearly indicate differences between lipid sources regarding their degree of saturation. The decision upon the utilization of these lipid sources in layer diets is mainly determined by the purpose of production, the demand for specific "enriched" egg products and the financial cost implications of its usage. Fish oil is known for its high efficiency in depositing EPA and DHA in the end product and has been used extensively for producing eggs and meat enriched with these long chain n-3 fatty acids. Fish oil contains increased levels of EPA and DHA in comparison to α-linolenic acid and has a total n-3 fatty acid content of 30% on average (Tables 2.1 & 2.2). However, both source and level of marine oil influence the type and concentration of long chain n-3 fatty acid deposition into egg yolk (Herber-McNeill & Van Elswyk, 1996; Gonzalez-Esquerra & Leeson, 2000; Cachaldora *et al*., 2006).

Alternatively, vegetable oils high in n-3 fatty acids (linseed) could be used to provoke similar effects on the fatty acid profile of end-products. However, due to the use of vegetable oils for human consumption, it has become uneconomical to use them in animal nutrition. Linseed oil shows a nutritional enhancement of layer diets by supplying high levels (52 - 55%) of α-linolenic acid (n-3) as indicated in Table 2.1.

Oliveira *et al*. (2010) reported that a dietary inclusion of 3.4% linseed oil resulted in significantly higher levels of n-3 fatty acids in egg yolk as well as a limited incorporation of EPA and DHA into yolk. Hargis and Van Elswyk (1993) also found that birds consuming either canola or flaxseed experimental diets have a noticeably lower efficiency for converting α-linolenic acid into EPA and DHA compared to birds on fish oil diets. However, the main reason for this is the fact that vegetable oils such as linseed, canola and soybean oils contain different quantities of α-linolenic acid (n-3), whereas fish oils contain little α-linolenic acid acids and more EPA and DHA as demonstrated in Table 2.1. In another report, Hargis and Van Elswyk (1993) concluded that an important consideration to account for during the decision whether to feed fish oil or plant oils in layer diets, other than the sensory acceptance of the end products, are the efficiency and ability of vertebrates to convert α-linolenic acid to long chain fatty acids (i.e. C20 & C22 fatty acids).

Table 2.1 Literature regarding certain of the major fatty acid methyl esters (%) of different lipid sources used in poultry diets.

		Myristic acid (C14:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	α-Linolenic acid (C18:3)	EPA[1] (C20:5)	DPA[2] (C22:5)	DHA[3] (C22:6)
Linseed oil	Crespo & Esteve-Garcia (2002)	0.1	5.9	4.0	18.6	14.7	55.4			
	Cachaldora *et al*. (2008)	-	5.5	3.1	19.2	16.8	47.7			
	Oliveira *et al*. (2010)	0.06	4.42	5.3	20.4	14.4	52.7			
Sunflower oil	Codex (1999)	ND-0.2	5 - 7.6	2.7-6.5	14.0-39.4	48.3-74	ND-0.3			
	Crespo & Esteve-Garcia (2002)	0.1	6.8	4.5	25.6	62.2	0.1			
	Bozkurt *et al*. (2008)	-	6.4	3.2	22.7	66.4	0.1			
	Oliveira *et al*. (2010)	0.07	6.1	3.6	33.6	54.7	0.2			
High oleic sunflower oil	Codex (1999)	-	2.6-5	2.9-6.2	75-90.7	2.1-17	ND-0.3			
	Ortiz *et al*. (2006)	-	4.3	4.3	73	16.3	-			
Fish oil	Cachaldora *et al*. (2006)	7.4	13.8	1.2	7.7	3.5	1.8	8.3	0.7	8.2
	Cachaldora *et al*. (2008)	-	17.9	5.3	16	1.6	0.6	7.5	1.7	21
	O'Fallon *et al*. (2007)	6.2	13.4	2.6	8.6	2.0	0.7	12.8	1.3	7.19
	Basmacıoglu-Malayoglu (2009)	8.9	20.2	4.4	17.1	1.9	2.2	9.9	1.6	17.9
Tallow	Codex (1999)	2.0 – 6.0	20.0 - 30.0	15.0 – 30.0	30.0 – 45.0	1.0 – 6.0	<1.5			
	Grobas *et al*. (2001)	3.59	26.7	20.6	37.7	4.0	2.5			
	Crespo & Esteve-Garcia (2002)	3.3	27.2	21.4	37.7	4.2	0.5			
	Bozkurt *et al*. (2008)	12.7	25.3	8.4	25.7	15.6	4.5			

[1] Eicosapentaenoic acid; [2] Docosapentaenoic acid; [3] Docosahexaenoic acid

Table 2.2 Literature regarding the total fatty acid concentration (%) and ratios of different lipid sources used in poultry diets.

		SFA[1]	MUFA[2]	PUFA[3]	n-3	n-6	n-6 / n-3[4]
Linseed oil	Crespo & Esteve-Garcia (2002)	10.3	18.9	70.9	56.1	14.8	0.26
	Cachaldora *et al*. (2008)	13.8	19.4	-	47.8	16.8	0.35
	Oliveira *et al*. (2010)	12.3	20.6	67.1	52.7	14.2	0.27
Sunflower oil	Crespo & Esteve-Garcia (2002)	11.7	25.9	62.4	0.1	62.4	672
	Bozkurt *et al*. (2008)	9.6	-	-	0.1	66.4	603
	Oliveira *et al*. (2010)	11.2	33.7	55.1	0.24	54.7	-
High oleic sunflower oil	Ortiz *et al*. (2006)	10.1	73.2	16.7	-	-	-
Fish oil	Cachaldora *et al*. (2006)	22.5	44.3	-	24.6	5.7	0.23
	Cachaldora *et al*. (2008)	28	25.6	-	30.8	4.9	0.16
	Basmacıoglu-Malayoglu (2009)	33.5	25	36.2	33.7	2.5	0.07
Tallow	Grobas *et al*. (2001)	52.0	41.2	6.5	2.5		
	Crespo & Esteve-Garcia (2002)	52.6	42.5	4.9	0.5	4.4	8.7
	Bozkurt *et al*. (2008)	47.3	-	-	4.7	16.6	3.5

[1] Total saturated fatty acids (%).
[2] Total monounsaturated fatty acids (%).
[3] Total polyunsaturated fatty acids (%).
[4] n-3 to n-6 ratio

2.7. Manipulation of egg fatty acid content

The fatty acid methyl esters (FAME) of egg yolk can be influenced by the fatty acid profile of the diet offered to the birds (Gao & Charter, 2000). Enriching the diets of laying hens with α-linolenic acid has been reported to increase the concentration of the α-linolenic acid in the egg yolk and also, although to a limited extent but significantly, increased the concentration of C20 fatty acids (Caston & Leeson, 1990; Leskanich & Noble, 1997; Baucells *et al*., 2000). Koutsos (2007) concluded that the FAMEs of eggs can be enriched between five- to thirty folds, without any negative effect on egg quality and/or production, depending on the type of fatty acids fed to hens.

The addition of vegetable oil to layer diets decreased ($P < 0.05$) the concentration of oleic acid in the egg yolk (Mazalli *et al*., 2004; Oliveira *et al*., 2010), which could be due to the

fact that these fatty acids act as precursors of n-3 and n-6 fatty acids. Oliveira *et al*. (2010) further reported that the concentration of linoleic acid and α-linolenic acid in egg yolk were proportional to their levels in the diets, irrespective of the lipid source used. The highest level of n-3 fatty acids in egg yolk were found for the linseed treatment, resulting in an improvement of the omega-6 to omega-3 (n-6 / n-3) ratio when compared with sunflower- and soybean oil (linseed oil, 2.09 / 1 *vs.* sunflower oil, 23.46 / 1 *vs.* soybean oil, 11.82 / 1). Kralic *et al*. (2008) reported a very favourable n-6 / n-3 ratio of 2.93 / 1 for a diet containing fish and linseed oil at a 50 / 50 ratio. Farrell (2011) concluded that an important factor that needs consideration during the enrichment of eggs with n-3 type fatty acids is the n-6 / n-3 ratio since it is regarded that a dietary ratio of 4:1 and lower is as appropriate to benefit human health (Simopoulos, 2006). Wang and Huo (2010) also concluded that dietary n-6 / n-3 ratio had an impact on the n-3 deposition in the egg yolk.

Various authors (Baucells *et al*., 2000; Cachaldora *et al*., 2006; Cachaldora *et al*., 2008) reported that although the fatty acid profile of the egg yolk resembled that of the diet, differences between specific fatty acid concentrations was observed. Baucells *et al*. (2000) found that birds show a tendency to keep the degree of saturation to monounsaturation (SFA / MUFA) of the egg yolk fatty acids within narrow margins. However, Mazalli *et al*. (2004) and Oliveira *et al*. (2010) concluded that the use of dietary lipid sources with different SFA content resulted in a variation in the SFA profile of egg yolk. When replacing dietary fish oil with sunflower oil, linseed oil or tallow, Baucells *et al*. (2000) observed a decrease ($P < 0.01$) in the quantity of DHA and EPA in egg yolk. They concluded that between 78% and 85% of these fatty acids (DHA & EPA) present in the egg yolk can be contributed to their proportion in the diet fed to the birds.

It further seems that the usage of dietary lipid sources in altering the dietary fatty acid profile also influences the fat content of egg yolk, although results are controversial. Cachaldora *et al*. (2008) found no effect ($P < 0.05$) on the total lipid content of the egg yolk with an increase in the dietary inclusion of fish oil. In contrast, Cachaldora *et al*. (2006) reported a significant ($P < 0.05$) increase in total yolk fat content if fish oil sources high in EPA and DHA were used. Conversely, Cherian *et al*. (2007) found that by adding both fish oil and CLA in combination to the yellow grease, the total lipid content of egg yolk was decreased ($P < 0.05$) by 5.4%.

2.7.1 Omega-3 enriched eggs

Omega-3 PUFAs is essential for normal growth and development and play an important role in the prevention and treatment of coronary heart disease (Burr *et al.*, 1989), hypertension (Morris *et al.*, 1994), inflammation and autoimmune disorders (Meluzzi *et al*., 1997) and even cancer (Lewis *et al*., 2000; Simpoulos, 2000). Furthermore n-3 fatty acids are important to human health in the prevention and management of type-2 diabetes (Connor *et al.*, 1993), renal disease (Donadio, *et al.*, 1994), rheumatoid arthritis (Kremer, 1996) and ulcerative colitis (Stenson *et al.*, 1992). It's also found (Kirubakaran *et al*., 2011) that n-3 fatty acids plays an important role in the development of brain and nervous tissues of foetuses and infants.

As mentioned before, these fatty acids can be incorporated into egg yolk by altering the fatty acid profile of the diet. Commercially produced table eggs normally contain a high portion of n-6 fatty acids but are a poor source of n-3 fatty acids (Surai, 2002). Omega-3 fatty acids (α-linolenic acid, DHA, EPA) are normally not present in the egg since the hen's diet is usually devoid in them. The main sources of PUFAs for enriching poultry eggs are fish oil, flaxseed, linseed, and marine algae. A major limitation in supplying fish oil to enrich the egg content with long chain n-3 fatty acids is the fishy taint and high susceptibility to lipid oxidation associated with these fatty acids (Leeson, 1999). However, the replacement of fish oil with vegetable oils in diets in an attempt to overcome this organoleptic problem has been done with great success. Various authors (Scheideler & Froning, 1996; Caston & Leeson, 1990; Ceylan *et al*., 2004) reported a significant increase in the levels of n-3 fatty acids, especially α-linolenic acid, in egg yolk of laying hens fed diets containing between 1.5% and 15% flaxseed. Goncuglu and Ergun (2004) reported that dietary flaxseed levels up to 10% were associated with an increase ($P < 0.05$) in yolk n-3 fatty acids. Carillo-Dominguez *et al*. (2005) concluded that the dietary inclusion of red crab meal (RCM) also resulted in a significant increase in n-3 fatty acids of egg yolk, but that the inclusion levels (3 & 6%) of RCM had no effect on the α-linolenic acid content of egg yolk. This is mainly ascribed to the fact that RCM is a source of n-3 fatty acids from marine origin, which is generally lower in α-linolenic fatty acids than plant sources. The Bio-omega-3™ egg produced by Yannakopoulos *et al*. (2005) using a diet supplemented with flaxseed, a herbal mixture, vitamin E, selenium, iodine, and folic acid were found to contain less SFAs and more PUFAs

compared to the regular eggs. Bio-omega-3™ eggs were also found to contain higher levels of n-3 fatty acids, particularly of the DHA type (120 mg/egg), compared to ordinary eggs (0 mg/egg).

In studying varying ratios of n-6 / n-3 fatty acids, Baird *et al*. (2008) used diets containing maize oil as n-6 source and flaxseed oil as n-3 source. As the inclusion of maize oil decreased and that of the flaxseed oil increased, α-linolenic acid and DHA increased incrementally and the dietary ratio of n-6 / n-3 decreased. Although they noted the same response in the n-6 / n-3 ratios of egg yolk, no significant effects on production parameters could be determined. It is believed that the dietary α-linolenic acid levels is responsible for the reduction in the n-6 / n-3 ratios of the egg yolk, because α-linolenic acid shares the same enzymatic pathway whereby arachidonic acid is synthesised from linoleic acid (Brenner, 1981) and thereby inhibits the action of the n-6 desaturase enzyme, reducing the conversion of linoleic acid to arachidonic acid (Garg *et al*., 1988).

Chickens have the ability to convert α-linolenic acid to DHA and to a lesser amount EPA, given that there is sufficient α-linolenic acid supplied in the hen's diet. Farrell (2011) reported that by supplying layer diets with 10% flax seeds amounts to approximately 2% α-linolenic acid and resulted to an increase of 600 mg n-3 / 100 g of egg, of which approximately 30% would be of the EPA and DHA type fatty acids. The capacity to convert α-linolenic acid to EPA and DHA is limited though, which may be related to the desaturase enzyme activity responsible for n-3 fatty acid metabolism (Grobas *et al*., 2001; Cachaldora *et al*., 2008).

The benefits of α-linolenic acid in human health are limited until they are converted to longer chain n-3 fatty acids, since these fatty acids (DHA and EPA) have stronger ties to health benefits compared to the shorter chain length n-3 PUFAs (α-linolenic acid) (Farrell, 2000; Renema *et al*., 2010). However, the efficient conversion of shorter chain n-3 fatty acids to longer chain n-3 fatty acids is compromised due to the competition between n-3, n-6 and n-9 fatty acids for desaturation and elongation enzymes (Simopoulos, 1991) as well as the fact that high levels of linoleic acid could decrease the rate of α-linolenic acid conversion to EPA and DHA (Sinclair, 1991).

2.8 Influences of the chemical manipulation of eggs on production parameters

2.8.1 Egg production

Aymond and Van Elswyk (1995) as well as Ansari *et al*. (2006) concluded that although diets containing up to 15% flaxseed resulted in a decreased ($P < 0.05$) egg production, an increase ($P < 0.05$) in the total UFA content of egg yolk were recorded. Cachaldora *et al*. (2006) studied the effect of four dietary inclusion levels (15, 30, 45, 60 g/kg) of various fish oil sources and concluded that hen-day egg production decreased with an increase in dietary inclusion level of the respective fish oil sources. In another finding, Criste *et al*. (2009) reported that a 5% dietary inclusion of linseed oil produced eggs enriched with α-linolenic acid and DHA without affecting egg production negatively. In support to these findings, Novak and Scheideler (2001) showed minimal differences ($P > 0.05$) in egg production between diets of different flaxseed inclusion levels.

Both Jiang *et al*. (1991) who used a 15% inclusion level of flaxseed and Yannokopoulos *et al*. (1999) who used 5 to 15% flaxseed inclusion did not find any significant effect on egg production of layers. Wang and Huo (2010) also concluded that the long-term feeding of 15% flaxseed to layers had no significant effect on egg production. The usage of various lipid sources such as flaxseed (Bean & Leeson, 2003), fish oil Hargis and Van Elswyk (1991), linseed oil, sunflower oil (Baucells *et al*., 2000), tallow, soybean oil, lard (Cachaldora *et al*., 2008) and red crab meal (Carillo-Dominguez *et al*., 2005) at different inclusion levels ranging from 0.4% to 15% resulted in no effect on egg production.

In contrast with these findings, Yannakopoulos *et al*. (2005) and Scheideler and Froning (1996) showed that egg production increased ($P < 0.05$) when feeding flaxseed at dietary inclusion levels of 5% to 15% to layers. Celebi and Macit (2008) also concluded that the supplementation of dietary fat (tallow, flaxseed oil or sunflower oil) to a basal diet resulted in an increase ($P < 0.05$) in egg production. On the other hand, Huthail and Al-Yousef (2010) concluded that feeding either 5% or 10% roasted flaxseed had a positive effect on egg production but that 15% flaxseed inclusion resulted in impaired egg production. Basmacioglu *et al*. (2003) also found fish oil had no effect ($P > 0.05$) on egg production but that the dietary inclusion of 4.3% flaxseed increased egg production ($P < 0.01$). Much of the controversy in literature regarding the effect of various lipid sources on egg production

performances could be ascribed to variation in inclusion levels as well as differences in the apparent metabolisable energy (AME) content of the diets.

2.8.2 Egg weight

Egg weight could be influenced by several factors, including the dietary energy content and the fatty acid composition of the diet. Grobas *et al*. (1999b) stated that the usage of fat in layer diets increases the energy content of the diet and results in a confounding effect on egg weight. It is believed that an increase in dietary energy resulted in a heavier body weight and consequently an increase in egg weight (Grobas *et al*., 1999b). Various authors (Grobas *et al*., 1999b; Novak & Scheideler, 2001; Yannakopoulos *et al*., 2005) found that egg weight increased significantly by feeding supplemental dietary lipid sources, even if these sources varies in their saturation profile.

In contrast, other authors (Leeson *et al*., 1998; Yannakopoulos *et al*., 1999; Bean & Leeson, 2003; Huthail & Al-Yousef, 2010; Wang & Huo, 2010) reported that diets containing between 4% and 20% flaxseed had no effect ($P > 0.05$) on egg weight. Accordingly Baucells *et al*. (2000), Basmacoiglu *et al*. (2003), Cachaldora *et al*. (2006) and Cachaldora *et al*. (2008) reported that the dietary inclusion of fish oil, linseed oil, sunflower oil, soybean oil, tallow and lard had no effect ($P > 0.05$) on egg weight. Carillo-Dominguez *et al*. (2005) also found no difference ($P > 0.05$) in egg weight when feeding hens with 3% and 6% red crab meal.

However, in a study done by Scheideler and Froning (1996) it was found that dietary flaxseed inclusion (5%, 10%, 15%) resulted in a decrease ($P < 0.01$) of egg weight. The decrease in egg weight was associated with a decrease in feed intake due to the higher energy density of the diets with high levels (10% & 15%) of flaxseed. From literature, it seems that the detrimental effects of dietary lipid inclusion on egg weight are limited and that variable results occurred.

2.8.3 Egg yolk colour

Egg yolk colour is an important factor in egg marketing due to consumer preferences that differs between geographical areas. Yannakopoulos *et al.* (1999) reported egg colour values of 10 in Bio-omega-3™ eggs and 12 in regular commercial table eggs using the DSM Roche

colour fan™ system. The authors ascribed the lower value of yolk colour in Bio-omega-3™ eggs to a lower natural source of lutein and zeaxanthin which are present in the feeds used during diet formulation and/or in the herbal mix.

Cachaldora *et al*. (2006) reported that fish oil sources high in EPA or DHA resulted in a decrease ($P < 0.0001$) in yolk colour , especially at higher dietary inclusion levels (15, 30, 45 or 60 g/kg) . In another report, Cachaldora *et al*. (2008) found that an increase in dietary fish oil supplementation (0 to 30 g/kg) resulted in a decreased ($P < 0.01$) yolk colour, whereas a dietary increase in soybean oil, linseed oil as well as lard resulted in a distinct increase ($P < 0.0001$) in yolk colour. In yet another opinion, Huthail and Al-Yousef (2010) concluded that neither flaxseed inclusion (0%, 5%, 10%, 15% and 20%) nor flaxseed treatment (roasted or unroasted) have any effect ($P > 0.05$) on egg yolk colour of any of the dietary treatments. Although egg yolk colour is no indicator of nutrient composition of eggs, it remains an important aspect for consumers in their purchasing decisions and a low colour score (< 3) could counteract the beneficial aspects of n-3 enriched eggs.

2.8.4 Weight of egg components and egg shell quality

An increase in hen age is associated with an increase in yolk and albumen weight. Proportionally, most of the increase is due to an increase in yolk weight, and the associated increase in yolk to albumen ratio (yolk / albumen) with age (Grobas *et al*., 1999a). Results indicated that the ratio of yolk / albumen in Bio-omega-3™ eggs increased ($P < 0.05$) when compared to eggs from hens consuming a commercial diet (Yannakopoulos *et al*., 1999). However, the greater yolk / albumen ratios observed in Bio-omega-3™ eggs was associated with a lower concentration of solids (e.g. fatty acids) in the yolk.

Cachaldora *et al*. (2006) reported no significant difference in Haugh units by feeding four levels (15, 30, 45, 60 g/kg) of fish oil sources differing in their concentration of EPA and DHA. They reported that shell thickness decreased ($P < 0.003$) with an increase in fish oil inclusion. Usayran *et al*. (2001) and Kirubakaran *et al*. (2011) found that feeding a diet enriched with either soybean oil, flaxseed or sardines have no effect ($P > 0.05$) on Haugh units or shell thickness. In agreement with these findings, Cedro *et al*. (2009) found no differences ($P > 0.05$) in Haugh units or yolk index by using diets enriched with marine algae or fish oil. As expected, they reported that an increase in storage temperature (25^{o}C *vs.* 5^{o}C)

resulted in a deterioration ($P < 0.05$) of both Haugh units and yolk index, irrespective of dietary treatment.

Basmacioglu *et al*. (2003) observed that neither flaxseed (43.2 g/kg) nor fish oil (15 g/kg) have an effect ($P < 0.05$) on yolk ratio (25.33% *vs.* 25.23%), albumen ratio (64.99% *vs.* 64.65%), shell weight (6.07 g *vs.* 6.32 g), shell ratio (9.68% *vs.* 10.12%) or shell thickness (396 μm *vs*. 416 μm). Bean and Leeson (2003) as well as Wang and Huo (2010) also concluded that neither long-term feeding or inclusion levels of flaxseed in layer diets had any effect ($P < 0.05$) on egg shell thickness, shell weight, albumen height and yolk ratio. However, Bean and Leeson (2003) reported a significant decrease in yolk weight with an increase in flaxseed inclusion levels. This finding is in agreement with that of Scheideler and Froning (1996) who also found that feeding flaxseed (5%, 10% and 15%) and fish oil (1.5%) reduced the percentage egg yolk compared to a control diet containing 3.22% tallow and 0.6% maize oil. In yet another study, Jia *et al*. (2008) concluded that canola seed caused lower ($P < 0.05$) eggshell quality.

2.8.5 Feed intake

Novak and Scheideler (2001) reported that feed consumption was higher ($P < 0.05$) at a 10% dietary inclusion of flaxseed compared to that of the soybean control diet. However, Basmacioglu *et al*. (2003) reported that feed intake was not affected ($P > 0.05$) by fish oil (1.5%) and flaxseed (4.32%) inclusion. Baucells *et al*. (2000), Yannakopoulos *et al*. (2005), Cachaldora *et al*. (2006) and Cachaldora *et al*. (2008) also reported no significant differences in feed consumption using different dietary inclusion levels and sources of fish oil, linseed oil, sunflower oil, tallow, flaxseed as well as a herbal mixture containing selenium, α-tocopherol and folic acid.

In contrast Grobas *et al*. (1999b) reported that an increase in diet density due to fat inclusion resulted in a lower ($P < 0.05$) feed intake (116 g/bird/day *vs.* 122 g/bird/day). By using soybean oil as dietary lipid supplement, Usayran *et al*. (2001) also reported a decrease in feed intake of hens when compared to a control diet (0% lipid inclusion). Huthail and Al-Yousef (2010) concluded that feeding higher levels (5% to 20%) of flaxseed resulted in a decrease ($P < 0.05$) of feed intake of hens.

2.8.6 Feed efficiency

Supplying 4% soybean oil to a layer diet resulted in an improved ($P < 0.05$) feed efficiency as opposed to birds being fed the control diet (0% soy oil diet) (Usayran *et al*., 2001). A similar conclusion was made by Grobas *et al*. (1999b), Celebi and Macit (2008) and Safaa *et al*. (2008) who reported an improvement ($P < 0.001$) in feed efficiency with the dietary inclusion of supplemental fats (tallow, fish oil, flaxseed oil, sunflower oil) in layer diets.

However, Basmacioglu *et al*. (2003) found no differences ($P > 0.05$) in feed conversion efficiency when comparing a control diet (no added lipid source) with diets containing either a fish oil (15 g/kg) or flaxseed oil (43.2 g/kg). Baucells *et al*. (2000) also reported similar feed efficient coefficients ($P > 0.05$) irrespective of the dietary lipid source or level of inclusion.

2.8.7 Factors affecting fat digestion

Many factors influence the digestibility of fats and oils in birds and that could deteriorate the nutritional value of lipid sources when fed to birds. Wiseman (2003) showed that saturation of the fatty acids being fed to animals plays an important role in the digestibility of the fatty acids. The number of double bonds present in the fatty acids determines the digestion efficiency of the fatty acids since UFAs are more readily digested than SFAs. However, when both these fatty acid types are fed in combination, a synergism exists where UFA increase the digestibility of SFA (Wiseman, 1990; Wiseman *et al*., 1991; Leeson & Summers, 2001; Kleyn, 2006). According to Pesti *et al*. (2002) UFA have the ability to readily form mixed micelles with bile salts in which saturated fatty acid are then solubilised and absorbed. Furthermore, fats and oils, being in triglyceride form, have a higher digestibility than lipid sources high in FFA levels since the FFA cannot be absorbed directly from the intestine but requires monoacylglycerides to assist in its absorption (Leeson & Summers, 2001). It would appear that an increasing proportion of FFAs in the diet would result in a linear reduction of fat digestibility. Wiseman (1990) indicated that a reduction in AME associated with an increase in FFA content is more pronounced with increasing degree of saturation.

The spatial formation of UFA results in fatty acids that are either being in the *cis*- or *trans*-configuration (McDonald *et al*., 2002). The *trans*-fatty acid are commonly found in hydrogenated fats and oils and have a lower digestibility than that of the *cis*- configuration.

Kleyn (2006) noted that poultry does not have a problem utilizing *trans*-fatty acids, with AME values of these fatty acids comparing favourable with that of vegetable oils.

A well documented fact is that young birds cannot digest fat as efficient as older birds (Renner & Hill, 1960; Young, 1961; Wiseman, 1990) with a more pronounced negative effect when using SFAs, suggesting that fat saturation is an indispensable characterization to consider when using specific supplementary lipid sources. Wiseman and Salvador (1989) concluded that prior to seven-and-a-half weeks of age, no significant difference was seen for the utilisation of fats being fed, hence concluding that two ages (being 1½ and 7½ weeks) being adequate for the nutritional evaluation of fats. The main reason for this is that young birds are poorly developed in their capacity to recycle bile salts, resulting in a lower efficiency in utilisation of fats (Leeson & Summers, 2001). However, it seems that bird age is not the only criteria to consider but genetic differences within a population may also play an important role in the efficient digestion of lipids. Renema *et al*. (2010) conducted a study on the efficiency of n-3 fatty acid utilisation by laying hens and observed that hens with higher energy efficiencies had longer and wider villi inside their GIT. They also concluded that a higher ratio of villi length to crypt depth was present in the more energy efficient strains of birds.

Moisture, impurities and unsaponifiable (M.I.U.) are non-fat materials that do not contribute to the energy value of fatty acids, thereby acting as diluents. Moisture and minerals are the most common pollutants of fats and oils and can lead to an increase in lipid oxidation. Fats and oils containing M.I.U. must be adjusted for their digestibility values (Leeson & Summers, 2001; Kleyn, 2006). Minerals can influence the digestion of fats and oils, due to the association that exists by means of soap formation with FFA in the small intestine. This reaction could result in both fatty acids and minerals becoming unavailable to birds. Although it is believed that soap formation is of a lesser problem to older birds, this phenomenon is of importance when feeding layers, due to their high dietary levels of calcium (Freeman, 1984). Leeson and Summers (2001) indicated that another factor for consideration is the digesta pH since acidic conditions decrease the micellar solubilisation of fatty acids in mixed bile salts.

High levels of indigestible fibre affect fat digestion since the bile salts and fibre form a complex, lowering the available bile for normal fat digestion (Leeson & Summers, 2001). The rate of passage influences not only the total digestibility of the diet, but also that of dietary lipid sources as well. A delay in the rate of passage allows the digesta to be in contact with dietary enzymes, carriers and absorption sites for a longer time, thus increasing digestibility and absorption. Dietary addition of fat may thus lead to an increase in the digestion of non-fat components as suggested by Leeson and Summers (2001). Nonetheless fats and oils are utilised more efficiently at lower intake levels (Wiseman *et al*., 1986), a situation which is more pronounced at a poor nutritional value of the specific lipid source (Wiseman, 1990).

Due to the reduction of *de novo* fatty acid synthesis in poultry, body fat deposition mainly depends on the direct uptake of preformed fatty acids. For this reason, the fatty acids composition of body lipids is highly correlated with that of the fatty acid intake of the bird itself (Doreau & Chilliard, 1997).

2.8.8 Organoleptic properties of enriched eggs

A concern when feeding long chain UFA, such as α-linolenic acid, is the typical fishy or "paint-like" aroma associated with these fatty acids which can cause a certain degree of "off-flavours" in eggs (Leeson *et al*., 1998). Zentek (2003) reported that this is a result of either an increase in fat intake or endogenous production of smelling substances, resulting in the transfer of flavour pigments into egg yolk. It further seems that trimethylamine (TMA) are associated with the occurrence of egg flavour taints (Zentek, 2003). TMA oxidase activity within the hen is of crucial importance and Pearson *et al*. (1979) illustrated that brown hens with a low TMA oxidase activity are likely to produce flavour tainted eggs. In contrast, no flavour tainted eggs were detected by a sensory panel when White Leghorn hens were fed diets containing rapeseed, choline or fishmeal (Horiguchi *et al*., 1998), demonstrating that white hens normally have a higher capability of dealing with problematic feed ingredients that might result in organoleptic problems (Zentek, 2003).

Leeson *et al*. (1998) concluded that although panellists found that off-flavour does exist in the eggs, aroma, flavour and overall acceptability was unaffected by both the level of vitamin E and flaxseed inclusion and that the eggs were generally still acceptable to panellists

(Leeson *et al*., 1998). Jiang *et al*. (1992) reported increases ($P < 0.05$) in α-linolenic acid content of eggs when feeding flaxseed associated with a moderate incidence (36%) of "off-flavour" as observed by sensory panellists.

2.8.9 Effect of egg enrichment on lipid oxidation

Shahryar *et al*. (2010) conducted a study to determine the effect of supplementing fish oil and sunflower oil diets with α-tocopheryl acetate (α-TA) and vitamin A on lipid oxidation in eggs. Supplying α-TA to the diet had a positive effect on egg quality throughout the 60 day storage period of eggs and promoted lipid stability within egg yolk. Fish oil diets had higher ($P < 0.01$) thiobarbituric acid values (TBARS) than the sunflower oil diet for fresh eggs (D_0) as well as eggs stored for either 30 (D_{30}) or 60 (D_{60}) days. In another study, Hayat *et al*. (2010) concluded that both antioxidant types (α-tocopherol and butylated hydroxytoluene) were efficient ($P < 0.05$) in decreasing the TBARS in flaxseed containing diets over a 60 day storage period. Cherian *et al*. (2007) suggested that the onset of lipid oxidation is enhanced by supplying dietary long chain UFAs. By increasing the level of dietary n-3 fatty acid, the UFA profile of egg increases, which can lead to an increase of lipid oxidation and reducing overall egg quality. Further storage of these eggs has a negative effect (Ahn *et al*., 1995) on lipid oxidation of egg yolk. To maintain egg quality and fatty acid stability during prolonged periods of storage, lipid oxidation should be kept to a minimum (Hayat *et al*., 2010). However, the addition of antioxidants (α-tocopherol and butylated hydroxytoluene) to diets containing 10% flaxseed resulted in lower ($P < 0.0001$) n-6 / n-3 ratio compared to the control diet without an antioxidant. These results clearly suggested that antioxidants modulate the desaturase and elongase pathway in a favourable way (Hayat *et al*., 2010) and that their usage in diets containing UFAs is indispensable.

2.9 Conclusions

From the available literature, it is evident that numerous possibilities exist to improve the nutritional properties of eggs as human food source by means of dietary intervention. These strategies would not only ensure that demands for improved, "healthier" food products by wealthier consumers are addressed, but might also aid in improving the nutritional status of many malnourished poor people worldwide. However, a vast number of contradictory findings in literature regarding the beneficial and/or detrimental effects of dietary lipid profile manipulation on production performance and egg quality characteristics occurred. Therefore,

the contradictory findings in literature need to be addressed and clarified by means of further research, which is specially focused on the effects of dietary saturation in terms of its fatty acid profile.

CHAPTER 3
EFFECTS OF LIPID SATURATION ON THE DIGESTIBILITY OF LAYER DIETS

3.1 Introduction

Changing the fatty acid composition of egg yolk through the alteration of the dietary fatty acids profile is a well recorded technique used in layer nutrition. Several researchers (Jiang *et al*., 1991; Scheideler *et al*., 1994; Baucells *et al*., 2000; Bean & Leeson, 2003; Cherian *et al*., 2007; Cachaldora *et al*., 2008) reported that the fatty acid composition of egg yolk directly relates to that of the diet consumed. Supplementary lipids not only change the fatty acids of the diet but also influence the palatability, energy density and subsequently digestibility thereof (Mateos & Sell 1980; McDonald *et al*., 2002).

During diet formulation, a combination of feed ingredients are used according to their potential nutritional value derived from chemical analysis, to calculate their contribution to dietary amino acids, energy, fibre, minerals and vitamins. Although supplementary dietary lipid sources generally increases energy utilisation of the basal diet (Mateos & Sell, 1980), they do differ in terms of their digestibility efficiencies. Leeson and Summers (2001) indicated that the digestibility of lipid sources differ significantly, varying from 70% for tallow to 96% for soybean- and menhaden fish oil, depending on chick age and the fatty acid profile of the respective lipid source. Disparities in the digestibility of individual fatty acids vary significantly from 2% for palmitic acid up to 95% for linoleic acid, depending on the age of the birds (Leeson & Summers, 2001). Other authors (Fernandez *et al.*, 1994; Zollitsch *et al*., 1997; Honda *et al.*, 2009) reported that the digestibility of lipid sources such as tallow, lard, cottonseed oil and soybean oil differ significantly, especially at higher inclusion levels ($\geq$ 6%) and concluded that a decrease in fatty acid saturation resulted in improved fatty acid digestibility. Similarly, Leeson and Summers (2001) and Kleyn (2006) are of opinion that an increase in the unsaturated fatty acid (UFA) content of lipid sources resulted in a higher energy value thereof. Viveros *et al*. (2009) showed that diets supplied with high oleic acid (HO) sunflower oil (high in monounsaturated fatty acids) have a higher total fat digestibility (81% *vs.* 78%) than diets supplied with palm oil (high in saturated fatty acids). In agreement, Jia *et al*. (2008) reported no difference ($P > 0.05$) in total fat digestibility by using diets

containing flaxseed or canola seed, both lipid sources being high in polyunsaturated fatty acids (PUFA).

However, nutrient digestibility is not only influenced by dietary lipid saturation, but also by dietary lipid inclusion levels. Excessive fat inclusion levels (8 to 10%) have a suppressing effect on protein and carbohydrate digestion in the ileum of chickens (Honda *et al*., 2009; Salarmoini & Golian, 2009) due to its suppressive effects on pancreatic enzyme secretion such as protease and amylase. Another important aspect in layer nutrition is the interaction between dietary minerals and fatty acids. The formation of Ca-soaps (Freeman, 1984; Kleyn, 2006) not only have a serious effect on eggshell quality through less available Ca^{2+} for absorption, but also impairs the apparent metabolisable energy (AME) supplied by the supplementary lipid source. Salarmoini and Golian (2009) reported that diets high in calcium (≥ 3.5% Ca) resulted in a decreased (14.8 *vs.* 14.5 MJ/kg DM) true metabolisable energy (TME) when supplemented with lipid sources. In yet another finding, Atteh and Leeson (1985) reported that in spite of an increase in calcium soap formation and losses *via* the excreta, no significant differences were observed in eggshell quality when supplementary lipids were used.

From literature available, it is seems that lipid inclusion levels and the fatty acid profile of dietary lipid sources have a combined effect on nutrient digestibility. However, literature regarding the comprehensible effects of dietary lipid saturation on nutrient digestibility and the subsequent production response in layer diets is scarce and inconsistent due to various dietary constraints. Most of the variation in documented studies could be ascribed to diets that are not *isoenergetic*, differences in lipid inclusion levels as well as differing Ca and fibre levels. Considering the renewed interest in the fatty acids enrichment of eggs, as well as the inadequate information on nutrient digestibility, the need arises to determine the digestibility of *isoenergetic* diets, differing in lipid saturation.

The aim of the present study was to determine the effect of dietary lipid saturation on nutrient digestibility of laying hens at 42 weeks of age.

3.2 Materials and Methods

3.2.1 Birds and husbandry

Two hundred (n=200) Hy-Line Silver Brown point-of-lay pullets (17 weeks of age) were obtained from a commercial pullet producer. Hens were housed individually (Figure 3.1) in metabolic birdcages (1600 cm^3), each fitted with a wooden perch, individual drinker nipple and a feed tray, within a natural ventilated building. A step-up photoperiod schedule (Hy-Line, 2009) was followed as recommended by breed guidelines, whereby birds received a total of 14½ hours daylight at 18 weeks, 15 hours daylight at 21 weeks and 15½ hours daylight from 22 to 42 weeks of age. From arrival of birds (17 weeks of age) until onset of the experimental procedures (20 weeks of age) a commercial pre-layer mash were fed to all birds. At 20 weeks of age, birds were randomly divided into five dietary treatments each consisting of 40 individual animals per treatment (n = 40 replicates/treatment). The experimental diets were fed for a total period of 22 weeks before the study finished with the execution of a digestibility trial at 42 weeks of age. Feed and water was provided on an *ad libitum* basis throughout the experimental period while egg collection was conducted daily each morning before 07:00 am.

Figure 3.1 Birds housed individually in metabolic birdcages.

3.2.2 Experimental diets

Five experimental diets (Table 3.4) were formulated using different supplementary lipid sources at a constant 30 g/kg inclusion level to increase the dietary concentration of specific

omega-type fatty acids. The number of double bonds (i.e. none, one, two or three and more) on the carbon atom as described in Chapter 1 was used as reference in defining dietary lipid saturation namely; an increase in the number of double bonds are associated with an higher lipid unsaturation and *vice versa*. Furthermore, unsaturated fatty acids were grouped into three families based on their oleic- (n-9), linoleic- (n-6) and α-linolenic acid (n-3) precursors, thereby referring to the position of the double bonds nearest to the omega carbon atom as illustrated by McDonald *et al*. (2002). The control n-3 diet were formulated using a blend of linseed- and fish oil (50 / 50) to increase the dietary levels of shorter chain omega-3 (n-3) fatty acids such as α-linoleic acid. For the second experimental diet (n-3 treatment), refined, deodorised fish oil were used to increase the dietary concentration of longer chain n-3 fatty acids such as eicosapentaenoic- (EPA), docosapentaenoic- (DPA) and docosahexaenoic acid (DHA). Sunflower oil was used to increase the dietary omega-6 (n-6) fatty acids such as linoleic acid, while high oleic acid (HO) sunflower oil was used to increase omega-9 (n-9) fatty acids such as oleic acid in the third (n-6 treatment) and fourth (n-9 treatment) experimental diets respectively. Lastly, tallow was used as supplementary lipid source in the saturated diet (SFA treatment) to increase the levels of highly saturated fatty acids such as palmitic- and stearic acid. These definite omega-type fatty acids were not exclusive to a specific diet, but the high concentration thereof within a respective diet was used for defining the treatment. Additionally, the total concentration (%) of monounsaturated fatty acids (MUFAs) and PUFAs were combined and defined as total unsaturated fatty acids (UFAs). The total dietary concentration of unsaturated and saturated fatty acids was further used as indication of the specific dietary saturation (Table 3.5), irrespective of the omega-type fatty acids. The choice of the control (n-3) diet used during the present study was based upon the fact that similar oil blends of vegetable and marine origin are currently being used for the production of “n-3 enriched eggs” in South Africa. Since the control n-3 diet is higher in short chain (C18:3c9) n-3 fatty acids compared to the n-3 treatment where pure fish oil was used (Table 3.4), it would also serve as a good indicator regarding the effects of fatty acid chain length (irrespective of saturation) on diet digestibility as well as production performance and egg quality parameters of layers.

The objective was to formulate five *isoenergetic* and *isonitrogenous* diets, with a constant inclusion level of the various supplementary lipid sources, differing only in their fatty acid profile. To ensure the formulation of *isoenergetic* diets, the AME content of each dietary lipid source was predetermined. The AME value of the respective lipid sources were

calculated based upon the free fatty acid (FFA) and the unsaturated to saturated fatty acid ratio (U/S), using the following prediction equation described by Wiseman (1990).

$$AME\ (kcal/kg) = 239[A + (B \times FFA) + (C \times e^{(D \times U/S)})]$$

Where: FFA is the free fatty acids in g/kg
U/S is the unsaturated to saturated ratio

Constants to be used for birds older than 21 days of age are as follow:
A = 39.025
B = -0.006
C = -8.505
D = -0.403

The fatty acid methyl esters (FAME) of the experimental dietary lipid sources were determined by extracting the fat content thereof using the method firstly described by Folch *et al*. (1957). Extraction of the total lipid content from the respective dietary lipid sources was performed quantitatively using chloroform and methanol in a ratio of 2:1. Butylated hydroxytoluene was added at a concentration of 0.001% to the chloroform : methanol mixture as an antioxidant. The extracts were dried under vacuum in a rotary evaporator and further dried overnight in a vacuum oven at 50^{o}C with phosphorus pentoxide as moisture absorbent. The total extractable fat content was determined by weighing and is expressed as percentage (%) fat (w/w) per 100 g lipid source.

Approximately 10 mg of total lipid extracted during the Folch extraction process was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acid methyl esters were prepared for gas chromatography by methylation of the extracted fat, using methanol–BF_3 (Slover & Lanza, 1979; Diaz *et al*., 2005, Hur *et al*., 2004). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness). Column temperature was 40^{o} – 230°C (hold for 2 minutes; 4°C/minute; hold for 10 minutes). Fatty acid methyl esters in hexane (1 μL.) were injected into the column using a Varian 8200 CX Auto sampler with a split ratio of 100:1. The

injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software (VSCS) recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were expressed as the relative percentage of individual fatty acids as a percentage of the total fatty acids present in the sample. The following fatty acid combinations and ratios were calculated using the fatty acid data: (i) total SFAs, (ii) total MUFAs, (iii) total PUFAs, (iv) total UFAs, (v) total n-6 fatty acids, (vi) total n-3 fatty acids, (vii) ratio of PUFAs to SFAs, (viii) ratio of MUFAs to SFAs, (ix) ratio of UFAs to SFAs, and (x) omega-6 to omega-3 (n-6 / n-3) ratio. Free fatty acid concentration of lipid sources was determined according to method number 940.28 for refined oils (AOAC, 2000).

Data of the FFA, U/S ratio as well as the predicted AME values for the five lipid sources used during the experimental period is presented in Table 3.1. These predicted AME values were then used to formulate five *isoenergetic* (12.6 MJ/kg DM) and *isonitrogenous* (170 g CP/kg DM) experimental diets as illustrated in Tables 3.2 and 3.3 by means of the Format feed formulation program.

Table 3.1 Mean free fatty acid (g/kg), unsaturated to saturated ratio (g/g) and calculated apparent metabolisable energy (MJ/kg DM) of the different lipid sources used in the experimental diets.

	Control[1]	Fish oil	Sunflower oil	HO[2] sunflower oil	Tallow
FFA[3]	0.11	0.27	0.17	0.51	1.83
U/S[4]	4.4	2.2	7.7	9.6	0.6
AME[5]	37.52	35.51	38.63	38.82	32.15

1 A blend of 50% fish oil and 50% linseed oil
2 High oleic acid sunflower oil
3 Free fatty acids in g/kg
4 Unsaturated to saturated ratio as g/g
5 Apparent metabolisable energy in MJ/kg DM (Wiseman, 1990)

Due to a total dietary fat content of approximately 61 g fat/kg DM, as well as the duration of the experimental period (± 6 months), three batches of feed were mixed on a two monthly interval in attempt to limit the oxidation of feed lipids and its subsequent effects on animal production performances. The fat content of each batch of feed were extracted (Folch *et al*., 1957) and analysed (AOAC, 2000) for peroxide value (PV) (AOAC no. 965.33) and FFA

concentration (AOAC no. 940.28) in an attempt to determine the lipid oxidative quality of feed. In a further attempt to delay the rate of lipid oxidation within the respective diets, the premix used during the study were specially formulated with considerably higher vitamin E levels (100 000 IU/ton feed) as well as the addition of 150 g Sel-Plex™/ton feed.

After calculating the predicted AME of the respective supplementary lipid sources, diets (Table 3.2 & 3.3) were formulated according to the nutritional recommendations of a commercial feed supplier for laying hens during their peak egg production period. Accordingly, their specific nutrient composition for particular feed ingredients, generated over a prolonged period of time was used. During diet formulation, the inclusion of full-fat soya as well as poultry-by-product is avoided while other feed ingredients such as sunflower oilcake (10%) were kept constant across treatments. This was done as a preventative measurement to ensure that the contribution of fatty acids from feed ingredients doesn't interfere with those supplied by the respective supplementary lipid sources.

3.2.3 Digestibility study

At 42 weeks of age, six hens per treatment (n = 6/treatment) were randomly selected to partake in a digestibility study over a 7 day collection period. Diet digestibility was done by total collection of excreta voided and the accurate measurement of food intake over a period of seven days as described by Larbier and Leclercq (1994). Birds were weighed to the closest gram and fasted for 24 hours prior to onset of the study. The subsequent day (1st day of experiment) pans for excreta collection (Figure 3.2) were placed underneath the birdcages and birds received their respective experimental diets. Feed were weighed out accurately to the closest gram on a daily basis. Excreta voided as well as feed refusals were collected daily in the morning where-after it was weighed (closest gram) and dried in an oven at 70°C. On the morning of the 7th day of the experimental period (last day) feed trays were removed and the hens were fasted for 24 hours. The final faeces voided after the 24h fasting period were collected and birds were weighed once again.

Table 3.2 Mean physical composition (%) of the experimental diets fed to the layers from 20 to 42 weeks of age (as is basis).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
Yellow maize	54.72	55.67	54.03	54.03	56.71
Wheat bran	5.57	4.40	6.37	6.37	3.17
Soybean oilcake (48%)	14.30	14.50	14.20	14.20	14.67
Sunflower oilcake (38%)	10.00	10.00	10.00	10.00	10.00
Limestone – coarse[1]	2.00	2.00	2.00	2.00	2.00
Limestone – fine[2]	8.57	8.57	8.57	8.57	8.57
Monocalcium phosphate	1.03	1.05	1.02	1.02	1.07
Salt	0.43	0.43	0.43	0.43	0.43
Linseed oil	1.50	-	-	-	-
Sunflower oil	-	-	3.00	-	-
HO[3] sunflower oil	-	-	-	3.00	-
Fish oil	1.50	3.00	-	-	-
Tallow	-	-	-	-	3.00
Sodium bicarbonate	0.06	0.06	0.06	0.06	0.06
Choline powder[4]	0.01	0.01	0.01	0.01	0.01
DL-Methionine[5]	0.02	0.02	0.02	0.02	0.02
Premix[6]	0.30	0.30	0.30	0.30	0.30

1 Coarse limestone with particle sizes: ≥ 2.0 mm = 70%; > 1.0 mm = 25% & < 1 mm = 5%.
2 Fine limestone with particle sizes: ≥ 2.0 mm = 34%; >1.0 mm = 30% & < 1 mm = 36%.
3 High oleic acid sunflower oil.
4 Contains a minimum of 60% choline chloride.
5 DL-methionine powder with a purity of 99%.
6 Commercial mineral, vitamin and phytase enzyme premix containing 100 000 IU Vit. E/ton and 150 g Sel-Plex™/ton feed (other nutrients and inclusion levels are confidential).

Table 3.3 Mean calculated chemical composition (g/kg DM) of layer diets fed during the experimental period (20 to 42 weeks of age).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
AME[1] (MJ/kg DM)	12.6	12.6	12.6	12.6	12.6
Crude protein	171.8	172.0	171.8	171.8	172.1
Fat	61.1	61.4	60.9	60.9	60.9
Ash	157.5	157.4	157.5	157.5	157.3
Crude fibre	45.4	44.4	46.1	46.1	43.4
NDF[2]	122.3	118.5	124.9	124.9	114.5
ADF[3]	59.6	58.4	60.4	60.4	57.2
Calcium	39.4	39.5	39.4	39.4	39.5
Phosphorus	6.1	6.1	6.2	6.2	6.1
Magnesium	2.8	2.9	2.8	2.8	2.7
Potassium	6.7	6.6	6.7	6.7	6.5
Chloride	3.3	3.3	3.3	3.3	3.3
Sodium	2.0	2.0	2.0	2.0	2.0
AvP[4]	3.2	3.2	3.2	3.2	3.2
Ca:AvP[5]	13.6	13.6	13.6	13.6	13.6
Arginine[6]	11.4	11.4	11.4	11.4	11.4
Isoleucine[6]	7.0	7.0	7.0	7.0	7.0
Lysine[6]	7.9	7.9	7.9	7.9	7.9
Methionine[6]	3.4	3.4	3.4	3.4	3.4
Threonine[6]	6.1	6.1	6.1	6.1	6.1
Tryptophan[6]	2.0	2.0	2.0	2.0	2.0
Methionine and Cystine[6]	6.8	6.8	6.8	6.8	6.7

[1] Apparent metabolisable energy in MJ AME/kg DM.
[2] Neutral detergent fibre.
[3] Acid detergent fibre.
[4] Available phosphorus.
[5] Ratio of calcium to available phosphorus.
[6] Total amino acid concentration

Figure 3.2 Pans used for the collection of excreta during the digestibility study.

3.2.4 Chemical analysis

To ensure a homogenous mixture, all dried feed, feed refusal and excreta samples were milled to pass through a sieve size of 1 mm (Dozier *et al*., 2008). Represented samples were taken by using the quadratic method for further laboratory analyses.

3.2.4.1 Dry matter

Dry matter (DM) content of all feed, feed refusal and excreta samples were determined by drying a 2 g sample in an oven at 97°C for 12 hours to a constant weight according to procedure number 934.01 (AOAC, 2000). After drying, samples were placed in desiccators and allowed to cool until room temperature, before the samples were weighed. The weight of individual crucibles was deducted to determine the weight loss on drying and the weight of the sample, due to weight difference between crucibles. The following equations were used to calculate the moisture (%) and dry matter (%) content of feed, feed refusal and excreta samples.

Moisture (%) = weight loss (g) / sample weight (g) x 100

Dry matter (%) = 100 – moisture (%)

3.2.4.2 Gross energy

Gross energy (GE) content of feed, feed refusal and the excreta samples were determined using a LECO AC500 Isoperibol Calorimeter (Leco Corp., St. Joseph, MI) following procedure number D5865 of the American Society for Testing and Materials (ASTM, 2009) as described by Cantrell *et al*. (2010). Approximately 0.2 g (on a DM-basis) of each sample (feed, feed refusals and excreta) was weighed accurately to the 4^{th} decimal and placed in a steel crucible. A platinum wire (5 cm) was connected to the electrodes of the bomb and the steel crucible containing the sample were carefully placed inside the bomb vessel before filling it with oxygen to a pressure of 3000 kPa. Special attention was given to ensure that the platinum wire were in direct contact with the sample but not in contact with the steel crucible itself. Sample weight was entered into a computer, the vessel was placed into a water bath and the electrodes were connected to it. A Windows operating program recorded the temperature every six seconds accurately to 0.0001°C using an electronic thermometer. The gross energy was expressed as mega joule per kilogram dry matter (MJ/kg DM).

3.2.4.3 Crude protein

Nitrogen content of the feed-, feed refusal- and excreta samples were determined according to the nitrogen combustion method (method no. 990.03), using a LECO (Model: FP-528) nitrogen analyser (AOAC, 2000). The principle of the combustion method is that the nitrogen freed by combustion at high temperature ($950^{o}C$) in oxygen (O_2) with a purity of 99.9%, is detected and converted to an equivalent protein basis. The nitrogen content of the diet was converted to crude protein by using the numerical factor of 6.25 (McDonald *et al*., 2002).

Approximately 0.12 g of each sample (on a DM-basis) was accurately weighed into aluminium foil cups that was sealed and placed on the automated carousel of the LECO instrument, ensuring the uninterrupted analysis of samples. Nitrogen values were recorded on a computer connected to an electronic scale (4^{th} decimal) as well as the LECO FP-528 analysing instrument. The protein equivalent was calculated by a Windows operating program using the numerical factor as described above.

3.2.4.4 Crude fat

The fat content of the feed-, feed refusal- and excreta samples was determined by means of Soxhet extraction (method number 920.39), using petroleum ether (40 - 65^{o}C boiling point) as a solvent (AOAC, 2000). A 2 g sample (on DM-basis) was closed inside a pre-weighed Schleicher and Schull Nr. 589/2 filter paper were carefully placed within the extraction thimble. The thimble were then closed properly with a clean cotton wool plug and placed inside the glass extractor that was connected with the pre-weighed glass flask before pouring the 150 mL solvent (petroleum ether) gently into the thimble. The extractor unit were placed into the individual heating units and connected with the water cooling unit. The heating was set to ensure a droplet speed of approximately 5 to 6 drops of solvent per second and the samples were boiled for 6 hours. After the extraction process, glass flasks were removed from the heating units and all remaining solvent were damped off over a warm water bath before drying overnight in an oven at 60^{o}C. After drying, glass flask containing the lipid fraction were placed inside a desiccator until reaching room temperature and weighted accurate to the 4th decimal.

3.2.4.5 Ash and organic matter

Ash content of the feed-, feed refusal- and excreta samples were measured in a muffle furnace according to procedure number 942.05 (AOAC, 2000). A 2 g DM sample was weighed into a pre-weighted porcelain crucible and placed into a muffle furnace. The muffle furnace was then heated to 600°C and kept constant at this temperature for four hours. After incineration, samples were transferred to a desiccator for cooling before weighing. The weight of individual crucibles was deducted to determine the weight of the ash and that of the sample, due to weight difference between crucibles. The ash content and organic matter (OM) was calculated as follows:

Ash (%) = weight of ash (g) / sample weight x 100

Organic matter (%) = 100% - Ash

3.2.5 Calculations

Feed intake and excreta weights were used for the calculation of gross energy intake and excretion to equate the AME of the diets. The AME value was corrected for nitrogen equilibrium by assuming that excreta nitrogen, as a consequence of tissue nitrogen

catabolism, has an energy value of 34.4 kJ/g and using the following formulae (Hill & Anderson, 1958; Kim & Patterson, 2000; Leeson & Summers, 2001; Lopez & Leeson, 2008).

$$AME_n\ (MJ/kg) = \frac{GE_{intake} - GE_{excreta} - 34.4(N_{input} - N_{output})}{\text{Feed intake (kg)}}$$

Where: GE_{intake} = gross energy of the food ingested (kJ/g)

$GE_{excreta}$ = gross energy of the excreta (kJ/g)

N_{input} = nitrogen content of the feed ingested (g/g)

N_{output} = nitrogen content of the excreta (g/g)

The following formulas as described by Dhanoa *et al*. (2008) were used to determine apparent diet digestibility:

$$I = f_{off} - f_{ref}$$

Where: I = feed intake (g DM/day)

f_{off} = feed offered (g DM/day)

f_{ref} = feed refused (g DM/day).

DM digested (g DM/day) = I – F

Where: F = total faecal output (g DM/day).

$$\text{Digestibility of the diet (g/g)} = \frac{I - F}{I}$$

The apparent digestibility of the specific feed components (D_x) was calculated according to the formulae of Dryden (2008):

$$D_x = \frac{[(F_{feed} \times C_{feed}) - (F_{ref} \times C_{ref})] - (Fa_{output} \times C_{faeces})}{[(F_{feed} \times C_{feed}) - (F_{ref} \times C_{ref})]}$$

Where: F_{feed} = quantity of feed offered (g DM/day)

F_{ref} = quantity of food refused (g DM/day)

C_{feed} = concentration of the component in the diet

C_{ref} = concentration of the component in the refusals

Fa_{output} = quantity of faeces voided (g DM/day)

C_{faeces} = concentration of the component in the faeces

3.2.6 Statistical analysis

The effect of dietary lipid saturation on the apparent digestibility of diets and dietary components was analyzed using a fully randomized one way ANOVA design. The PROC ANOVA procedures of the SAS program (SAS, 1999) were used to test for significant differences between treatments. When significant ($P \leq 0.05$) differences were found, further multiple comparisons, using Tukey's honest significant difference (HSD) test, was used to identify differences between treatment means.

3.3 Results and Discussions

3.3.1 Dietary fatty acid methyl esters and lipid oxidation

Results regarding the effect of dietary lipid saturation on the FAME, as well as the total fatty acid concentration and fatty acid ratios are presented in Table 3.4 and Table 3.5 respectively. It must be noted that the respective FAME presented in Table 3.4 are not the only fatty acids recorded in the diets, but that only individual fatty acids with a dietary concentration of more than 0.04%, as well as those used for identification of dietary treatments were included in the table. Furthermore, it is important to note that the total fatty acid concentration and fatty acid ratios as indicated in Table 3.5 were not calculated on the individual fatty acid concentrations of Table 3.4, but on the total dietary fatty acid profile, irrespective of individual fatty acid concentration. This was done to ensure that the total dietary UFA / SFA ratio could be calculated correctly, irrespective of dietary fatty acids low in concentration, since the energy contribution of individual fatty acids could only be evaluated with the consideration of the total dietary UFA / SFA levels (Wiseman, 1990).

Although statistical comparisons were not possible on the experimental diets, the following observations are of interest. As expected, the inclusions of dietary lipid sources differing in their fatty acid saturation were not only successful in altering the dietary FAME as noticeable in Table 3.4, but also in manipulating the total dietary saturation and/or unsaturation as

illustrated in Table 3.5. It is clearly evident (Table 3.5) that all the poly- and monounsaturated treatments had a higher concentration of total dietary UFAs (on average 80.9%) and a lower total SFAs concentration (on average 19.09%). In contrast, the total dietary UFAs of the SFA treatment were 59.07% while its total SFAs concentration was 40.74%. Accordingly the lipid saturation of the PUFA (n-3 & n-6) and MUFA (n-9) diets varied (total SFA 28.2 to 13.5% and total UFA 71.8 to 86.6%). Furthermore, when considering the total omega type (n-3, n-6 & n-9) fatty acid concentrations as used for defining the various experimental dietary treatments, it is also clearly evident that both the control n-3 (21.42%) and the polyunsaturated n-3 diet (12.53%) had the highest total n-3 concentrations, while the total n-6 concentration of the polyunsaturated n-6 (55.27%) and the total n-9 concentration of the monounsaturated n-9 (55.90%) diets were respectively the highest. It must be emphasised that the marginally lower total UFA concentration of the control n-3 (80.26%) and polyunsaturated n-3 (71.83%) treatments are mainly ascribed to using fish oil which is generally high in saturated fatty acids (Table 2.1). such as myristic-, palmitic- and stearic acid. Since fish oil are regarded as a polyunsaturated n-3 source from animal origin, the concentration of long chain PUFAs such as EPA, DPA and DHA is not sufficient to "off-set" its high concentration of saturated fatty acids. Furthermore, it must be remembered that the basal diet (approximately 3% fat on a dry matter basis) would always contribute significantly to the n-6 type polyunsaturated fatty acids due to the inclusion of maize meal ($\geq$ 50%).

By considering concentrations of specific individual fatty acids within the respective treatments, the higher concentration (15.38%) of α-linolenic acid (C18:3c9,12,15) in the control n-3 diet could be clearly recognised and is mainly ascribed to the elevated levels of linseed. Similarly in the polyunsaturated n-3 treatment, the usage of fish oil resulted in lower α-linolenic acid (1.13%) concentration and an increased EPA (8.17%) and DHA (2.57%) concentration. Accordingly, the effect of using alternative lipid sources in altering the dietary FAME profile can clearly be seen (Table 3.4) with the highest concentration of linoleic acid (C18:2c9,12) in the polyunsaturated n-6 diet (using sunflower oil) and the highest levels of oleic acid (C18:1c9) in the monounsaturated n-9 diet (using HO sunflower oil).

Table 3.4 The effect (mean) of dietary lipid saturation on the fatty acid methyl esters (FAME) of layer diets.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
FAME (Fatty acids as % of total fatty acids)					
Saturated fatty acids:					
Myristic acid (C14:0)	2.39	5.22	0.05	-	2.01
Palmitic acid (C16:0)	13.03	18.18	9.14	7.56	19.99
Margaric acid (C17:0)	0.15	0.25	0.06	0.04	0.77
Stearic acid (C18:0)	3.36	3.47	4.56	4.47	17.11
Monounsaturated fatty acids (n-9):					
Palmitoleic acid (C16:1c9)	2.82	5.96	0.08	0.07	1.39
Oleic acid (C18:1c9)	23.19	21.32	27.82	54.86	30.24
Eicosenoic acid (C20:1c11)	0.65	1.12	0.20	0.21	0.18
Polyunsaturated fatty acids (n-6):					
Linoleic acid (C18:2c9,12)	29.96	27.09	55.27	29.88	24.04
Eicosadienoic acid (C20:2c11,14)	0.04	0.08	-	-	-
Arachidonic acid (C20:4c5,8,11,14)	0.20	0.39	-	-	-
Polyunsaturated fatty acids (n-3):					
α-Linolenic acid (C18:3c9,12,15)	15.38	1.13	0.84	0.77	0.95
Eicosapentaenoic acid (C20:5c5,8,11,14,17)	4.26	8.17	-	-	-
Docosapentaenoic acid (C22:5c7,10,13,17)	0.41	0.66	-	-	-
Docosahexaenoic acid (C22:6c4,7,10,13,16,19)	1.38	2.57	-	-	-

Table 3.5 The effect (mean) of dietary lipid saturation on the total fatty acid concentration (%) and fatty acid ratios of layer diets.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
Fatty acid concentration (%):					
Total SFA[1]	19.75	28.17	14.97	13.45	40.74
Total MUFA[2] (Total n-9)	28.49	31.55	28.91	55.90	34.27
Total PUFA[3] (Total n-6 & n-3)	51.77	40.28	56.11	30.65	25.00
Total UFA[4] (Total n-9, n-6 & n-3)	80.26	71.83	85.02	86.55	59.07
Total n-6	30.35	27.75	55.27	29.88	24.04
Total n-3	21.42	12.53	0.84	0.77	0.95
Fatty acid ratios:					
MUFA / SFA[5]	1.44	1.12	1.93	4.16	0.84
PUFA / SFA[6]	2.64	1.46	3.76	2.28	0.61
UFA / SFA[7]	4.06	2.55	5.68	6.43	1.45
n-6 / n-3[8]	1.44	2.49	65.99	38.67	25.35

[1] Saturated fatty acids.
[2] Monounsaturated fatty acids.
[3] Polyunsaturated fatty acids.
[4] Unsaturated fatty acids (monounsaturated and polyunsaturated fatty acids).
[5] The ratio of monounsaturated to saturated fatty acids.
[6] The ratio of polyunsaturated to saturated fatty acids.
[7] The ratio of unsaturated to saturated fatty acids.
[8] The ratio of omega-6 to omega-3 fatty acids.

Leeson and Summers (2001) and Verleyen (2010) indicated that the energy value of supplementary lipid sources as well as diet digestibility and absorption are related to their oxidative quality. Verleyen (2010) further indicated that the usage of PV (primary oxidation products) and thiobarbituric acid value (TBA, secondary oxidation products) is mostly suited to determine the oxidative stability of pure lipid sources. The effects of dietary saturation on lipid oxidation stability of the diets as reflected by the, PV and FFA concentration are presented in Table 3.6. Thiobarbituric acid value of feed samples were not determined during the present study and it was decided upon using the FFA concentration, which is more commonly used in South Africa, as indication of lipid peroxidation in feed. Feed samples that were taken on the day of blending the oil with the basal diets were regarded as "fresh", whereas samples taken after two months of feeding the diets were regarded as "stored". Since three batches of experimental diets were mixed during the progress of the study (see paragraph 3.2.2), mean values of the "fresh" and "stored" diets are presented in Table 3.6.

Table 3.6 The effect of dietary lipid saturation on the peroxide- (milliequivalent peroxide / kg fat) and free fatty acid (%) content of both fresh and stored diets (Means).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
Peroxide value (millieq. peroxide/kg fat)					
Fresh	44.60	47.17	47.03	26.88	11.84
Stored	48.74	47.81	101.74	26.16	11.38
Free fatty acid (%)					
Fresh	23.19	21.19	25.14	26.53	21.25
Stored	40.35	42.29	48.24	43.18	46.47

Statistical comparisons were not possible due to a lack of replications. However, it is interesting to note that the PV of the feed remains relatively similar between the fresh and stored feed samples, except in the case of the polyunsaturated n-6 treatment where a 116% increase in PV were noted. These results are very high in both PV and FFA values of the stored and fresh diets. A possible explanation for these results could be the fact that there was a time-lap of at least six months duration from sampling of feed to analyses thereof. Although the samples were frozen at –18°C they were not vacuum packed to prevent any possible further oxidation. The oxidative status of the oils and individual raw materials were not determined before every mixture and could also have played a huge role the resultant higher oxidation of the final mixed diets. However, irrespective to the higher PV and FFA as recorded in Table 3.6, McGill *et al.* (2011) reported that high dietary peroxide values of 75

to 150 milliequivalent peroxide / kg fat had no negative effect on feed intake and production performances of birds, provided that an antioxidant (125 mg / kg) is included in the diet. These results support Verleyen (2010) who reported that the occurrence of peroxidation in pure lipid sources is not related to their specific fatty acid profile.

By using the PV category classification system of Verleyen (2010) for pure lipid sources as reference, it is evident that all experimental treatments, with the exception of the saturated (SFA) treatment, were severely oxidized (> 20 millieq. peroxide/kg fat) when freshly mixed. Since there was no increase in the PV of experimental diets during the two months storage period, except in the case of the polyunsaturated n-6 treatment, it could be speculated that secondary oxidation products (TBA) were formed during the storage period, rather than a continuation of primary oxidation. The increase in FFA concentration from the fresh to the stored diets could further support the suggestion that secondary oxidation had occurred due to the increase in free radicals, although it was not quantified by means of TBA analysis. Supportive to the thought that the diets were already subjected to primary peroxidation, are the high FFA concentration (23.5% on average) of the freshly mixed diets. Free fatty acids at such high levels suggest that the metabolisable energy content of the diet might be influenced, because Leeson and Summers (2001) indicated that any diet with a FFA content of more than 20% would result in suppressed growth due to a shortage of energy. Leeson and Summers (2001) concluded that glucose cannot be formed due to the absence of glycerol moiety at high levels of dietary FFA, resulting in a decrease of blood glucose levels.

3.3.2 Nutrient digestibility

Nutrients as illustrated in Table 3.3 were not all physically analysed. The actual chemical composition (g/kg DM) of the different experimental diets is presented in Table 3.7. The diets analysed on average about 8% higher for CP as well as 6.8% and 8% lower for fat and ash respectively, when compared to that of the calculated chemical composition (Table 3.3).

Although the chemical composition of the experimental diets differs in some instances, it did not influence the effect of lipid saturation investigated in this study. Variation in analytic procedures, chemical composition of feed ingredients and diet mixing as well as sampling could have contributed to the differences between analysed (Table 3.7) and formulated diets (Table 3.3). The ideal would be to chemically determine the nutrient composition of each feed ingredient that will be used during the manufacturing of diets and used those specific

nutrient values during the diet formulation process, instead of "average" feed matrices. After mixing the experimental diets, it needs to be analysed once again to determine if it is comparable with the formulated diet before feeding it to the birds. However, due to practical and time limitations, the experimental diets used during the present study were mixed after formulation and then fed to hens while the chemical analysis was done at a later stage.

Table 3.7 Mean dry matter chemical analysis (%) of the different experimental diets.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)
Dry matter	95.34	95.29	95.36	96.03	95.60
Gross energy (MJ/kg)	16.57	16.69	16.45	16.64	16.68
Crude protein	19.42	18.10	18.80	17.75	18.96
Fat	5.63	5.80	5.75	5.77	5.76
Ash	14.35	14.26	14.76	14.41	14.59

Data regarding the effect of dietary lipid saturation on dry matter feed intake and apparent digestibility of nutrients is shown in Table 3.8. Dietary lipid saturation level had no effect ($P = 0.60$) on feed intake of hens. From the results in Table 3.8 it further seems that dietary lipid saturation had generally no effect on the apparent digestibility of dry matter ($P = 0.18$), organic matter ($P = 0.12$), ash ($P = 0.84$) and energy ($P = 0.30$). However, the apparent digestibility of CP ($P < 0.05$) and fat ($P < 0.0001$) were influenced by dietary treatment. The monounsaturated n-9 diet resulted in the highest ($P < 0.05$) CP digestibility (58.4%), which differs statistically only with that of the polyunsaturated n-6 diet (55.0%), but not with any of the other dietary treatments. Therefore, no clear influence of dietary lipid saturation on apparent digestibility of CP could be detected. Furthermore, dietary lipid saturation had a highly significant effect ($P < 0.0001$) on fat digestibility of the experimental diets. The UFA diets had a higher fat digestibility (94.2 to 95.6%) than the SFA diet (90.4%).

It is further evident from Table 3.8 that the PUFA n-3 diet resulted in both the highest ($P < 0.01$) AME (13.29 MJ/kg DM) and AME_n (12.74 MJ/kg DM) content. In contrast, the polyunsaturated n-6 diet resulted in the lowest AME (12.92 MJ/kg DM) and together with the control n-3 diet the lowest AME_n (12.36 MJ/kg DM) content. However, despite the significant differences in AME and AME_n as mentioned, no clear influence of dietary lipid saturation on apparent metabolisable energy could be observed.

Table 3.8 Mean (± s.d.) effects of dietary saturation on dry matter feed intake, apparent digestibility coefficients and the apparent metabolisable energy content of diets fed to laying hens during the digestibility study.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (*P*)	CV[1] (%)
Feed intake (g/bird/day)	100.24 ± 5.53	100.50 ± 10.37	101.78 ± 5.23	105.38 ± 2.74	102.40 ± 3.04	0.60	5.92
Apparent digestibility coefficients (%)							
Dry matter	72.0 ± 0.8	73.1 ± 0.8	72.3 ± 0.7	72.4 ± 0.9	72.7 ± 0.7	0.18	1.09
Organic matter	74.7 ± 1.3	75.8 ± 0.6	74.8 ± 0.6	74.8 ± 1.0	75.6 ± 0.8	0.12	1.17
Crude protein	56.5^{ab} ± 2.7	56.3^{ab} ± 1.7	55.0^{b} ± 1.8	58.4^{a} ± 1.3	57.9^{ab} ± 1.6	< 0.05	3.3
Fat	94.5^{a} ± 1.3	94.2^{a} ± 2.0	95.6^{a} ± 1.2	94.8^{a} ± 0.7	90.4^{b} ± 1.8	< 0.0001	1.59
Ash	55.3 ± 2.7	56.1 ± 4.7	57.1 ± 2.0	57.2 ± 3.2	56.0 ± 2.7	0.84	5.68
Gross energy	77.6 ± 1.3	78.8 ± 1.0	77.7 ± 0.3	78.1 ± 1.1	78.1 ± 0.9	0.30	1.26
Apparent metabolisable energy (MJ/kg DM)							
AME[2]	12.96^{b} ± 0.21	13.29^{a} ± 0.21	12.92^{b} ± 0.07	13.18^{ab} ± 0.22	13.10^{ab} ± 0.12	< 0.01	1.34
AME_n[3]	12.36^{b} ± 0.20	12.74^{a} ± 0.21	12.36^{b} ± 0.06	12.61^{ab} ± 0.21	12.50^{ab} ± 0.11	< 0.01	1.34

[a,b,c] Row means with different superscripts differ significantly at $P < 0.05$.

[1] Coefficient of variance (%).

[2] Apparent metabolisable energy in MJ/kg.

[3] Apparent metabolisable energy corrected for nitrogen retention in MJ/kg.

The lower dietary AME values (control n-3 & polyunsaturated n-6 treatments) were probably related to marginally lower dietary gross energy concentrations (Table 3.7) and apparent digestibility of gross energy (Table 3.8). The observed (Table 3.6) increase in PV value of the polyunsaturated n-6 diet during storage could have contributed to the lower AME value of this diet. However, this theory is highly debatable, since it is doubtful that the marginal increase in PV of the control n-3 diet would resulted in its significant lower AME and AMEn values. Furthermore, data in Table 3.8 suggested that the lower fat digestibility (90.4%) of the saturated fatty acid treatment was not associated with a decrease in the AME (13.10 MJ AME/kg DM) content of this specific dietary treatment.

Results regarding fat digestibility are in agreement with that of Smink *et al.* (2008) who reported that UFAs are known for their higher digestibility coefficients and absorption efficiencies compared to that of SFAs. One of the explanations for these findings are the fact that monoglycerides and long chain UFAs have the ability to link up with conjugated bile salts to form micelles which increases their ability to be digested, whereas SFAs have an inferior ability to form micelles because of their characteristic low polarity (Freeman, 1984; Wiseman & Lessire, 1987). Viveros *et al.* (2009) also reported that a diet high in monounsaturated fatty acids (HO sunflower oil) resulted in an increased ($P < 0.001$) fat digestibility compared to a saturated fatty acid diet containing palm oil which are characteristically high (± 45%) in palmitic acid (C16:0). The results of the present study are also in agreement with other authors (Jia *et al*., 2008) who reported no significant differences in fat digestibility between various polyunsaturated n-3 and n-6 dietary lipid sources by using canola-, rapeseed-, flaxseed, soybean oil and sunflower oil as dietary lipid supplements. Zollitsch *et al*. (1997) further reported that diets high in saturated fatty acids, due to the usage of supplementary lipid sources such as lard, tallow and palm oil, which are all relatively high in palmitic (C16:0) and stearic (C18:0) acid, resulted in a lower ($P < 0.001$) fat digestibility compared to diets high in UFAs.

3.4 Conclusions

Results of the present study indicate that dietary lipid saturation have no significant effect on feed intake of hens as well as the apparent digestibility coefficients of most analysed nutrients, except in the case of crude protein ($P < 0.05$) and fat ($P < 0.0001$). Diets high in saturated fatty acids (3% tallow inclusion) resulted in a lower ($P < 0.0001$) fat digestibility. However, the decrease in fat digestibility of the highly saturated diet was not associated with

a decrease in either the AME or AME_n, content thereof. The failure to establish a clear trend between dietary lipid saturation and the recorded differences in CP and fat digestibility as well as AME and AME_n content between treatments, suggest that the dietary fatty acid profile is of lesser importance in establishing digestibility coefficients for nutrients, compared to other factors such as constant lipid inclusion levels as well as *isoenergetic* and *isonotrogenous* levels of diets.

CHAPTER 4
EFFECT OF LIPID SATURATION ON PRODUCTION PERFORMANCES OF LAYERS

4.1 Introduction

The use of supplementary lipid sources in poultry diets are mostly done to improve the energy density and/or to improve feed acceptability and handling properties thereof. Lately, an additional role for the usage of dietary lipid sources is the modification of the fatty acid profile of food products such as eggs (Cachaldora *et al*, 2008; Wang & Huo, 2010). For these purposes, vegetable and marine type oils are normally preferred over animal fats due to their unsaturated fatty acid (UFA) profile and higher apparent metabolisable energy (AME) content (Palmquist, 2002; Pesti *et al*., 2002). However, many other factors such as bird age (Wiseman, 1990), rancidity and oxidation (Mandal *et al*., 2004), dietary mineral levels (Freeman, 1984) and dietary fibre content (Leeson & Summers, 2001) might influence the type of lipid source used in poultry diets.

Some reports (Grobas *et al*., 1999a,b; Safaa *et al*., 2008) indicated that the inclusion of UFA lipid sources into layer diets have a beneficial ($P < 0.05$) effect on egg size. However, much controversy still exist regarding the effects of dietary fatty acid saturation on production performances of layers in terms of feed intake, egg production, egg weight and -output, with equal numbers of reports find either a beneficial or detrimental effect on the mentioned parameters while the remainder (almost 50% of reports) recorded no effect at all. These differences in literature could mainly be ascribed to genotype of birds (Bean & Leeson 2003), age and phase of production (Grobas *et al*., 1999b) as well as lipid sources (Whitehead *et al*., 1993; Caston *et al*., 1994; Scheideler & Froning, 1996) and inclusion levels (Novak & Scheideler, 2001; Yannakopoulos *et al*., 2005; Ansari *et al*., 2006).

From a nutritional point of view, dietary inclusion of fats or oils could result in heavier eggs being produced as well as the possible interference of saturated fatty acids (SFAs) with calcium metabolism (Leeson & Summers, 2001) and the consequent formation of Ca-soaps. However, data regarding the effect of dietary fatty acids on eggshell quality is just as contradictory as that of production performance parameters. While some authors (Cachaldora *et al*., 2006; Jia *et al*., 2008) reported that dietary lipid sources (irrespective of

degree of fatty acid saturation) have a detrimental effect on eggshell quality, others (Basmacioglu *et al*., 2003; Bean & Leeson, 2003; Wang & Huo, 2010) reported no effects ($P > 0.05$) on eggshell quality. These conflicting results regarding the effect of dietary lipid saturation on production parameters and eggshell quality characteristics makes it difficult for unambiguous formulation decisions, suggesting that research is still needed in an attempt to provide nutritionists with more comprehensive information. Furthermore, since most of the documented studies focused on either the usage of specific lipid sources or the effects of inclusion levels, to alter the fatty acid methyl esters (FAME) of eggs, very few studies focused on the effects of diets differing in their degree of lipid saturation. This study was therefore an attempt to address some of the shortfalls in literature by using dietary lipid saturation, as defined by the number of double bonds (e.a. none, one, two, three) on the carbon atom, as an evaluative measurement on egg production performances and eggshell quality characteristics.

The aim of the present study was to determine the effects of dietary lipid saturation on production performance of laying hens during peak production (20 to 40 weeks of age).

4.2 Materials and Methods

4.2.1 Birds and husbandry

The same birds and animal husbandry practises as comprehensively discussed in paragraph 3.2.1 (Chapter 3) was used during the present study. At 20 weeks of age, birds were randomly allocated into five dietary treatments, each consisting of 40 individual replicates (n = 40 replicates/treatment). Feed and water was provided *ad libitum*, while egg collection was conducted daily each morning before 07:00 am. Daily minimum and maximum temperature was recorded at five different locations within the house at 07:00 am for the entire experimental period. Production data was recorded during weeks 24, 28, 32, 36 and 40 of age and is considered as the peak production period of the hens. Data of all the eggs produced during the respective collection weeks were individually recorded and summarized before pooled for statistical analysis. Individual body weights (Figure 4.1) of hens were also recorded during the mentioned weeks.

Figure 4.1 Recording body weights at 24, 28, 32, 36 and 40 weeks of age.

4.2.2 Diets

Diets as well as lipid sources used during the present study are comprehensively described in paragraph 3.2.2 (Chapter 3), while the physical composition (Table 3.2), calculated chemical composition (Table 3.3), fatty acid methyl esters (Table 3.4), total fatty acid concentration and ratios (Table 3.5), dietary oxidative stability (Table 3.6) and analysed chemical composition (Table 3.7) of the diets are illustrated in the mentioned tables. The five *isoenergetic* (12.6 MJ AME/kg DM) and *isonitrogenous* (170 g CP/kg DM) diets were formulated using various lipid sources at a constant 30 g/kg inclusion level. Diets were defined according to their degree of lipid saturation as comprehensively explained in paragraph 3.2.2 (Chapter 3). Briefly, the control n-3 diet were formulated by using a blend of linseed- and fish oil (50 / 50) to increase the dietary levels of omega-3 (n-3) fatty acids such as α-linolenic acid. For the second experimental diet (n-3 treatment), refined, deodorised fish oil were used to increase the dietary concentration of n-3 fatty acids such as eicosapentaenoic- (EPA), docosapentaenoic- (DPA) and docosahexaenoic acid (DHA). Sunflower oil was used to increase the dietary omega-6 (n-6) fatty acids such as linoleic acid, while high oleic acid (HO) sunflower oil was used to increase the omega-9 (n-9) fatty acids such as oleic acid in the third (n-6 treatment) and fourth (n-9 treatment) experimental diets respectively. Lastly, tallow was used as supplementary lipid source in the saturated diet (SFA treatment) to increase the levels of highly saturated fatty acids such as palmitic- and stearic acid.

4.2.3 Feed intake

Hens were individually fed (± 110 – 115 g/bird/day) from a pre-weighed feed bucket between 07:00 and 08:00 am every day. To ensure an *ad libitum* supply of feed, feed trays were inspected at 15:00 pm and only topped-up with feed if needed. Feed intake was determined on a weekly basis and any remaining feed refusals in the feed trays were weighed and brought into consideration during the determination of weekly feed intake (g/bird/week). The weekly feed intake was then used to calculate the average daily feed intake (g/bird/day) for the specific period of time. Feed efficiency (FE) was determined during weeks 24, 28, 32, 36 and 40 of age, respectively, by dividing the weekly egg output by the weekly feed intake and expressed as a coefficient (g egg / g feed consumed) as described by Palic *et al*. (2009).

4.2.4 Production parameters

Eggs were collected daily before 07:00 am and individually recorded. Hen-day egg production was calculated according to Ahmad & Balander (2003) where the number of eggs produced was divided by the number of live birds in each replicate. Hen-day production was summarised on a weekly basis and is then expressed as a percentage (%) weekly egg production per individual hen. Eggs that were either cracked, had soft shells or those without a shell (shell-less) was recorded as egg production and the sum thereof was then subtracted from the number of eggs laid to give a total number of intact sellable eggs which was used for the calculation of percentage sellable eggs produced.

Individual egg weights (g) were recorded by accurately weighing each egg to the closest 0.0001 gram on a Shimadzu (Model: AY 220) electronic scale (Figure 4.2). The total weekly egg weight (g) was used to calculate mean egg weight for the collection weeks. Egg output (g) for the specific collection weeks was determined according to Rose (1997) by multiplying the number of eggs produced by an individual hen with her weekly mean egg weight (g).

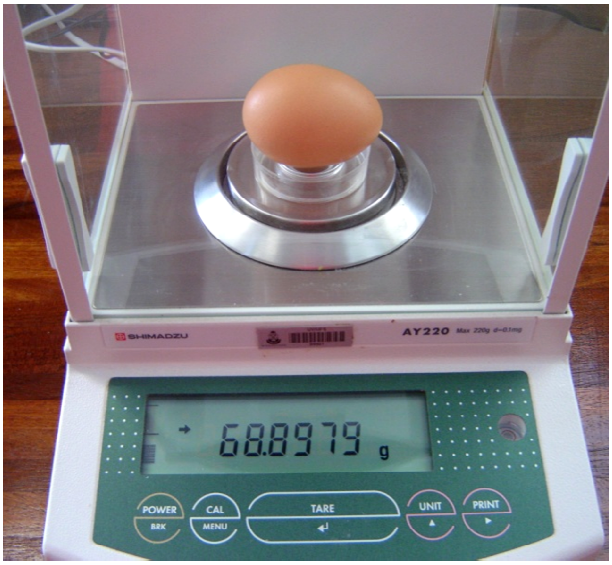

Figure 4.2 Individual weighing of eggs for each hen during the calculation of egg output.

4.2.5 Eggshell quality

At 24, 28, 32, 36 and 40 weeks of age, 20 eggs per treatment were randomly selected on a daily basis (n = 140/treatment/collection week) for the determination of eggshell quality parameters. After recording egg weight, the individually marked eggs were broken at the equator into two halves (Figure 4.3). The procedures described by Strong (1989) as well as Kul and Seker (2004) were used for washing of eggshells under slow flowing water to remove the adhering albumen. The shells were then allowed to dry for an hour at room temperature before shell thickness measurements were conducted (Figure 4.4). An AMES (Model: BG 3602-0-04) shell thickness meter, accurate to 0.001 mm, was used for three shell thickness measurements per measurement point; namely the blunt end (BE), equator (EQ) and sharp end (SE) of individual eggshells (De Ketelaere *et al*., 2002; Ehtesham & Chowdhury, 2002; Ahmad & Balander, 2003; Kul & Seker, 2004). In total, nine shell thickness (ST) readings were recorded per individual eggshell. Shell thickness data of each hen at a specific collection period was then pooled to calculate the mean ST at the BE, EQ and SE as well as an average shell thickness of the entire egg.

Figure 4.3 Eggshell halves left to dry after being rinsed to remove adhering albumen.

Figure 4.4 Recording eggshell thickness with an AMES micrometer.

After recording eggshell thickness, all shells were stored individually for the determination of dry shell weight (g) (Figure 4.5) and shell ash content (g) according to the procedures of Clunies *et al*. (1992). Individually marked crucibles were dried overnight at 100^{o}C and weighed after cooling to room temperature in a desiccator. Individual eggshells (shell and adhering membranes) were carefully crushed into a pre-weighed crucible and dried to a constant weight for 12 hours at 100^{o}C according to procedure no. 934.01 (AOAC, 2000).

After recording dry eggshell weight, shells were incinerated in a muffle furnace at 550^{o}C for 16 hours to determine the eggshell ash content.

Figure 4.5 Recording of eggshell weight.

Eggshell quality variables such as egg surface area (ESA), shell weight per unit surface area (SWUSA), percentage eggshell and calculated eggshell Ca content (g) were determined by using the following formulae:

Egg surface area (ESA) = $3.9782W^{0.7056}$ (Carter, 1975)
Where: W = egg weight (g)

Shell weight per unit surface area (SWUSA) (mg/cm^2) = SW/ESA (Wells, 1967)
Where: SW = shell weight (g DM)
ESA = egg surface area

Percentage eggshell (%) = (SW/EW) x 100 (Orban & Roland, 1990)
Where: SW = shell weight (g DM)
EW = egg weight (g)

Eggshell Ca content (g) = SW (g) x 0.373 (Simons, 1986)
Where: SW = shell weight (g DM)

4.2.6 Statistical analysis

The effect of dietary lipid saturation on egg production and eggshell quality parameters were analyzed using a fully randomized one way ANOVA design. The PROC ANOVA procedures of the SAS program (SAS, 1999) were used to test for significant differences between treatments. When significant differences were found ($P \leq 0.05$) a further multiple comparison test, Tukey's honest significant difference (HSD) test, was used to identify these differences. Fisher's least significant difference (LSD) test was used to explain statistically significant differences with low magnitude that could not be explained by Tukey's studentized range test (HSD) in Table 4.2.

4.3 Results and Discussions

4.3.1 Temperature

The average maximum and minimum house temperatures as recorded during the experimental period are presented in Figure 4.6. The clear decreasing trend in temperature is characteristics of the time period (January to May) for the Bloemfontein area where this study was conducted. Decreases in the maximum temperature that do not coincide with decreases in minimum temperature within specific weeks (week 22 and 27 of age) are ascribed to above normal rainfall which resulted in cloud coverage and lower maximum temperatures, without a consequential lowering in minimum temperatures. The average daily temperature for the experimental period was 20.6^{o}C, while the mean maximum and minimum temperatures for the same period were 26.9^{o}C and 13.3^{o}C respectively.

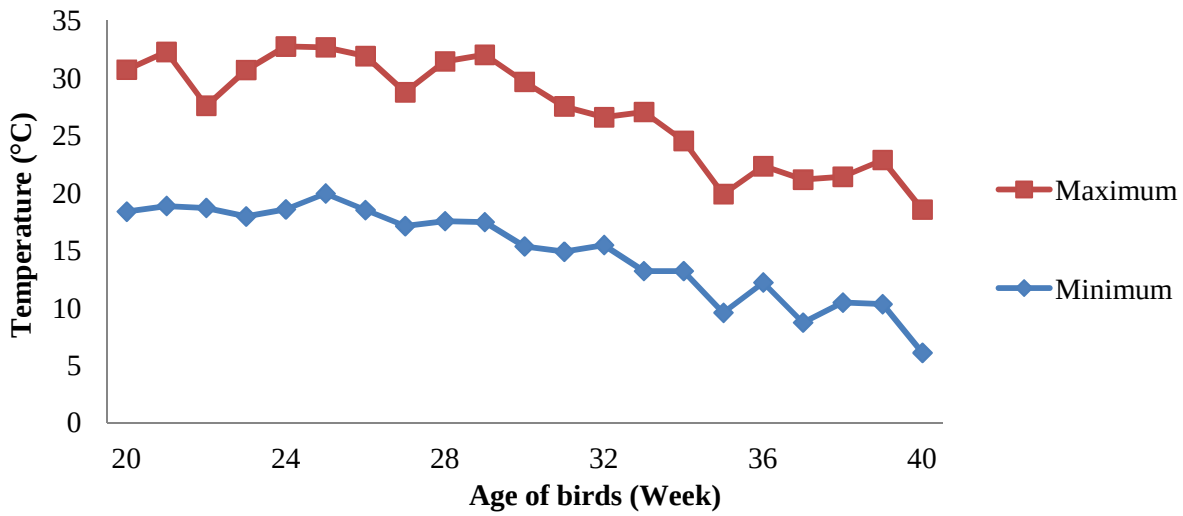

Figure 4.6 Average minimum and maximum temperatures from 20 to 40 weeks of age.

Environmental temperature plays an important role in feed intake, and essentially energy intake, of laying hens. Kleyn (2006) indicated that an environmental temperature of 19^{o}C is regarded as the lower critical temperature (LCT), while the upper critical temperature (UCT) is regarded as 27^{o}C. Any deviation from these temperatures would therefore result in changes in metabolic heat production and consequently energy/feed consumption. An increase in environmental temperature above the UCT is associated with an increase in energy demand to enable birds to dissipate body heat by means of sensible and evaporative heat loss, while environmental temperatures that lower than the LCT also requires more energy to ensure sufficient heat generation. Usayran *et al*. (2001) concluded that the exposure of hens to constant high environmental temperatures resulted in a lower feed intake and subsequently lower egg production. They further concluded that the addition of 4% supplementary lipids to the diet resulted in heavier eggs and a better feed efficiency. Although the mean minimum temperature (13.3^{o}C) was lower than the LCT and the mean maximum temperature (26.9^{o}C) was equal to the UCT during the present study, the average daily temperature (20.6^{o}C) were within the comfort zone of birds. Furthermore, the effect of environmental temperature on animal production was constant across dietary treatments for the duration of the experimental period.

4.3.2 Production

The effects of dietary saturation on the respective production performance parameters are presented in Table 4.1. Dietary lipid saturation had no effect on feed intake (P = 0.36), egg production (P = 0.32), egg weight (P = 0.40), egg output (P = 0.14) and body weight (P = 0.43) of the hens during the peak production period. The SFA diet, using tallow as supplementary lipid source, resulted in the lowest (P < 0.015) percentage sellable eggs (89.17%). This was a direct result of the higher percentage (10.3%) cracked eggs produced by hens in the SFA treatment compared to that of the other treatments (between 1.86% and 3.86%). The remainder (0.53%) of egg deformities for the saturated treatment constitute of broken, shell-less and soft-shelled eggs. No difference (P > 0.05) was observed when comparing percentage sellable eggs of the UFA treatments (poly- and monounsaturated dietary treatments) with each other. Results from the present study is in agreement with various researchers (Jiang *et al*., 1991 & 1992; Novak & Scheideler, 2001; Basmacioglu *et al*., 2003; Bean & Leeson, 2003; Cachaldora *et al*., 2006 & 2008; EL-Husseiny *et al*., 2008) who reported no differences (P > 0.05) in egg production, feed intake or egg weight by using different supplementary lipid sources differing in their FAME from highly unsaturated to

highly saturated. In contrast with the general non-significant effects of dietary lipid saturation on production performances during the present study (Table 4.1), Kirubakaran *et al*. (2011) concluded that an increase in dietary n-3 fatty acids resulted in a lower ($P < 0.05$) feed intake and an increase ($P < 0.05$) in egg weight. Other authors (Scheideler & Froning, 1996; Gonzalez-Esquerra & Leeson, 2000) reported that the dietary inclusion of unsaturated fatty acids from lipid sources such as fish- and flaxseed oil resulted in a higher ($P < 0.05$) egg production than that of saturated fatty acids as a result of tallow supplementation. Turgut *et al*. (2006) reported that a dietary inclusion of saturated lipid sources (tallow) resulted in a higher ($P < 0.05$) egg production than that of unsaturated lipids (sunflower oil). They also found that by increasing the dietary inclusion level to 5% for both lipid sources (tallow and sunflower oil), egg production decreased ($P < 0.007$) while egg weight increased ($P < 0.08$) for the tallow treatment. Scheideler and Froning (1996) and Sari *et al*. (2002) found that egg weight decreased ($P < 0.05$) when using polyunsaturated n-3 enriched diets. Celebi and Macit (2008) recorded a higher ($P < 0.01$) feed intake by using a saturated diet (tallow as supplementary lipid source) compared to that of highly unsaturated diets (flaxseed- and sunflower oil).

Bean and Leeson (2003) founded that body weights of hens fed flaxseed diets were lower ($P < 0.05$) than that of the control diets containing no supplementary lipids, but ascribed their findings to the negative effects of anti-nutritional factors, also reported by Gonzalez-Esquerra and Leeson (2000) and Rodriquez *et al*. (2001), rather than to a direct lipid treatment effect. These discrepancies between results of the present study and those previously mentioned could be ascribed to the fact that some authors used highly unsaturated and saturated lipid sources at the same inclusion levels, without correcting the AME content of the basal diet according to the metabolisable energy yield of the respective lipid source used (Wiseman, 1990).

From Table 4.1 it further seems that the monounsaturated n-9 treatment (supplemented with HO sunflower oil) resulted in the lowest ($P < 0.001$) feed efficiency (0.471), while that of the control n-3 (0.494) and saturated (0.491) treatments were respectively the best. The poor FE of the monounsaturated n-9 treatment could partly be explained by the collective effects of respectively the highest feed intake (108.86 g/b/d) and the lowest egg output (357.52 g/b), although these results were not statistically significant ($P > 0.05$).

Table 4.1 The mean (± s.d.) effects of dietary lipid saturation on layer performance during peak production (20 - 40 weeks of age).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated(n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (*P*)	CV[1] (%)
Feed intake (g/bird/day)	106.36 ± 6.39	107.43 ± 8.12	108.26 ± 7.90	108.86 ± 7.12	106.02 ± 6.92	0.36	6.82
Hen-day egg production[#] (%)	95.43	94.14	95.93	94.57	96.14	0.32	5.03
Sellable eggs[#] (%)	95.82[ab]	97.92[a]	96.49[ab]	97.09[a]	89.17[b]	< 0.015	13.08
Egg weight (g)	55.77 ± 1.79	54.88 ± 2.94	56.10 ± 2.36	54.76 ± 2.31	55.21 ± 3.10	0.40	4.60
Egg output (g)	366.60 ± 19.85	358.06 ± 23.25	368.54 ± 23.83	357.52 ± 23.38	364.37 ± 28.08	0.14	6.56
Feed efficiency (g/g)	0.494[a] ± 0.031	0.478[ab] ± 0.028	0.487[ab] ± 0.024	0.471[b] ± 0.024	0.491[a] ± 0.027	< 0.001	5.52
Body weight (g)	1706 ± 105	1691 ± 120	1732 ± 110	1711 ± 99	1729 ± 109	0.43	6.36

[#] Mean values for hen-day egg production (%) and sellable eggs (%).
[a,b] Row means with different superscripts differ significantly at $P < 0.05$.
[1] Coefficient of variance (%).

Since the feed intake of the monounsaturated n-9 treatment (108.86 g/b/d) is only marginally higher than that of the control n-3 (106.36 g/b/d) and the saturated treatment (106.02 g/b/d), it could be suggested that feed intake alone was not the cause for poor FE recorded in the monounsaturated n-9 treatment. Since the chemical composition (Table 3.7) and nutrient digestibility (Table 3.8) of the monounsaturated n-9 treatment indicate that the diets were correctly formulated in terms of its energy contribution, the marginally lower egg weight and egg output of the monounsaturated n-9 treatment could not be attributed to energy and/or protein deficiencies. Furthermore, it also seems that the statistically lower AME and AME_n content of the control n-3 and polyunsaturated n-6 treatments (Table 3.8) had no statistical significant negative effects on any of the production parameters, included FE, for these two treatments.

The results of Kirubakaran *et al*. (2011), indicating that a lower ($P < 0.05$) feed intake and a higher ($P < 0.05$) egg weight of n-3 enriched diets resulted in a subsequent improvement ($P < 0.01$) of FE, is only partly supported by the results of the control n-3 treatment during the present study (Table 4.1). However, since the SFA treatment illustrated similar patterns than the control n-3 treatment regarding feed intake of hens and egg weights during the present study, it is highly doubtful that the improvement in FE as recorded by Kirubakaran *et al*. (2011) could only be attributed to the dietary n-3 concentration during the present study. In another finding, Turgut *et al*. (2006) reported that polyunsaturated sunflower oil resulted in a lower ($P < 0.02$) feed conversion ratio (FCR) compared to saturated tallow (1.87 *vs.* 1.91) at a 2.5% dietary inclusion level. However, these results were not supported by the present study (Table 4.1) because the FE of both the polyunsaturated n-6 (0.487) and the saturated (0.491) treatments did not differ statistically ($P > 0.05$). According to Turgut *et al*. (2006) dietary fatty acid profile influenced feed intake ($P < 0.01$) of birds. This was however not observed during the present study. Turgut *et al*. (2006) also ascribed the differences recorded in FCR to a higher feed intake of the saturated tallow treatment (126.1 g/b/d) and not to an increase in egg production or egg weight.

Most of the variation observed in certain production parameters between the present and former studies could be ascribed to genetic differences between the birds (Hy-Line Silver; Lohmann Brown; ISA Brown; Bovans White), age of birds (ranging from 19 to 44 weeks of age), lipid sources (tallow, fish-, linseed-, rapeseed-, soybean-, olive-, sunflower- and palm

oil), lipid inclusion levels (ranging from 1.2% to 5%) and dietary AME values (ranging from 12.3 to 13.6 MJ AME/kg DM).

4.3.3. Eggshell quality

Data representing the effect of dietary lipid saturation on egg properties and eggshell quality measurements are indicated in Table 4.2. Supplying laying hens with diets differing in their fatty acid profile had no effect ($P > 0.05$) on eggshell properties in general. Dietary lipid saturation only affected ($P < 0.05$) the percentage eggshell (Table 4.2), whereby the polyunsaturated n-3 treatment produced the highest (9.64%) and the polyunsaturated n-6 treatment the lowest (9.39%) proportion of eggshell. However, it must be noted that Tukey's (HSD) test was not able to identify differences between these treatment means and therefore, the Fisher (LSD) test which is less strict than the Tukey's (HSD) test, was used to identify these differences. Since the calculation of percentage eggshell (Orban & Roland, 1990) depends on both eggshell weight and egg weight, a combination of a light shell and heavy egg weights would result in a lower percentage eggshell, while the opposite scenario whereby a heavy eggshell weight and a light egg weight would result in a higher percentage eggshell is also true.

Considering the data for egg and eggshell weights during the present study (Tables 4.1 & 4.2), it is evident that the polyunsaturated n-3 treatment have the highest ($P > 0.05$) eggshell weight (5.31 g) as well as the second lowest ($P > 0.05$) egg weight, thereby contributing to the highest ($P < 0.05$) percentage eggshell, whereas the opposite scenario is true for the polyunsaturated n-6 treatment. Various authors (Jiang *et al*., 1992; Basmacioglu *et al*., 2003; Bean & Leeson, 2003; Turgut *et al*., 2006; Celebi & Macit, 2008; EL-Husseiny *et al*., 2008) reported that dietary lipid source, varying from highly saturated to highly unsaturated, had no negative effects ($P > 0.05$) on shell weight, shell thickness and shell breaking strength and percentage eggshell, to which the current study attests to. The only differences found between results of the present and that of the mentioned authors are the effects of lipid inclusion on percentage eggshell. Since most of these authors didn't report any significant effects on eggshell- and egg weights, as well as the fact that these parameters differ only marginally in their studies, it is assumed to be the reason why they didn't reported significant differences for percentage eggshell.

Table 4.2 The effect (mean ± s.d.) of dietary lipid saturation on eggshell quality measurements during peak production (20 - 40 weeks of age).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (*P*)	CV[1] (%)
Eggshell properties							
Shell weight (g DM)	5.25 ± 0.20	5.31 ± 0.28	5.25 ± 0.26	5.19 ± 0.19	5.28 ± 0.23	0.61	4.50
Shell ash content (g/g)	0.94 ± 0.040	0.95 ± 0.003	0.95 ± 0.003	0.95 ± 0.003	0.95 ± 0.003	0.45	1.76
Shell thickness							
Blunt end (μm)	352 ± 15	357 ± 12	351 ± 14	352 ± 14	358 ± 12	0.38	3.83
Sharp end (μm)	368 ± 13	373 ± 15	370 ± 15	372 ± 15	377 ± 14	0.45	3.93
Equator (μm)	364 ± 11	371 ± 12	364 ± 14	364 ± 10	369 ± 12	0.13	3.23
Average eggshell thickness (μm)	361 ± 11	367 ± 11	362 ± 13	362 ± 11	368 ± 11	0.21	3.10
Eggshell quality variables							
ESA[2] (cm^2)	67.80 ± 1.18	67.32 ± 2.31	67.98 ± 1.63	67.26 ± 1.62	67.24 ± 2.12	0.58	2.69
SWUSA[3] (mg/cm^2)	77.35 ± 2.32	78.77 ± 2.14	77.15 ± 3.05	77.20 ± 2.46	78.49 ± 2.28	0.10	3.17
Percentage eggshell[#] (%)	9.43^{bc} ± 0.28	9.64^{a} ± 0.24	9.39^{c} ± 0.36	9.44^{bc} ± 0.33	9.61^{ab} ± 0.31	< 0.05	3.21
Eggshell calcium content (g)	1.96 ± 0.07	1.98 ± 0.11	1.96 ± 0.10	1.94 ± 0.07	1.97 ± 0.09	0.61	4.50

[#] Differences between treatments means were identified using the Fisher least significant difference test (LSD).
[a,b,c] Row means with different superscripts differ significantly at $P < 0.05$.
[1] Coefficient of variance.
[2] Eggshell surface area.
[3] Shell weight per unit surface area.

During the present study (Table 4.1), the SFA treatment resulted in a lower ($P < 0.015$) number of sellable eggs due to an increase in eggshell cracks and breakages. However, eggshell weight (5.28 g), eggshell thickness (368 µm), percentage eggshell (9.61%) and eggshell Ca-content (1.97 g) of the SFA treatment were relatively high compared to the other treatments and are definitely not a reflection of the high number of cracked/broken eggshells as recorded for this treatment. However, it is generally known that percentage eggshell as well as shell thickness is related to eggshell strength. Roberts (2004) concluded that shell strength is not only determined by the quantity of eggshell that is present but also by the quality of eggshell construction. This researcher found that when eggshell weight, shell thickness and percentage eggshell are categorised as good quality, but the breaking strength is relatively poor, the explanation most probably lies in the ultra structure of the eggshell and/or how the shell was constructed in terms of calcium salt deposition on the external shell membrane. Considering the explanation of Roberts (2004), it might be possible that although none of the eggshell properties were negatively influenced by the SFA treatment, a poor macrostructure of the shells might have been responsible for the increase in cracked/broken eggs associated with this treatment. It would be the ideal to quantify the pore sizes between Ca-crystals deposited onto the external eggshell membranes by means of electron microscopy in an attempt to ascribe the high incidence of eggshell breakages of the SFA treatment. Another possibility in the assessment of high eggshell breakages is the determination of Ca-soaps within the excreta of birds. Atteh and Leeson (1984 & 1985) suggested that Ca-soaps formed by free fatty acids from saturated fat sources are not absorbed by the birds. Although the calculated Ca-content of eggshells (1.97 g) for the SFA treatment was similar to that of other treatments, the formation of Ca-soaps in the excreta is not known. Since the birds were in their peak production period, it might be speculated that they were still able to maintain their shell Ca-content by means of medullar bone mobilisation to the uterus. However, this scenario would not be sustainable for a prolonged period of time, before the Ca-reserved in medullar bone becomes depleted.

While specific supplementary lipid sources were used for the modification of the dietary FAME during the present study, the usage of other feed ingredients with a high fat content would result in similar FAME alterations, although at a lower magnitude. However, the rationale behind the changes in FAME would remain analogous with comparable production and eggshell quality outcomes, given that the diets are properly formulated.

4.4 Conclusions

Results from the present study suggested that dietary lipid saturation have in general no effect ($P > 0.05$) on most of the production performances and eggshell quality characteristics evaluated. The few significant effects observed during the study namely, total sellable eggs ($P < 0.015$), feed efficiency ($P < 0.001$) and percentage eggshell ($P < 0.05$), indicates no clear trend and could not be directly related to the degree of dietary lipid saturation or to specific nutrient digestibility results in Chapter 3. In general, it could be concluded that the degree of dietary lipid saturation will have no detrimental effect on egg production and eggshell quality parameters, with the exclusion of highly saturated diets whereby the percentage of eggs available for sale is drastically influenced, given the fact that diets are *isonitrogenous* and *isoenergetic* and that the total dietary fat content remains within acceptable norms for layers.

CHAPTER 5
THE EFFECT OF LIPID SATURATION ON INTERNAL EGG QUALITY

5.1 Introduction

Wealthier modern consumers changed their discernment towards food by shifting their focus from the financial costs to a combination of health benefits and associated risks with the consumption thereof. Dietary manipulation with supplementary lipids is a well known technique used to increase essential fatty acid concentration in animal proteins such as poultry meat and eggs (Scheideler & Froning, 1996; Farrell, 1998; Yannakopoulos *et al*., 2004; Hayat *et al*., 2010; Singh *et al*., 2012). However, this technique is not without any flaws and the successful essential fatty acid enrichment depends on various biological aspects such as lipid sources used, type of animal and the oxidative stability of enriched products. Furthermore, the consumption of these enriched products should be sufficiently to experience the true benefits thereof for humans.

Commercially produced table eggs are normally poor sources of omega 3 (n-3) type fatty acids and contain a high portion of omega 6 (n-6) fatty acids (Surai *et al*., 2000; Hayat *et al*., 2010). Since n-3 polyunsaturated fatty acids (PUFAs) have numerous beneficial functions in humans, such as decreasing blood pressure, decreasing the risk of cardiovascular diseases, inhibiting growth of cancer cells, reducing blood viscosity, assist in early neural development and delaying losses in immunological functions (Ansari *et al*., 2006), it is understandable that most of the dietary manipulation programs focus on the improvement of this n-3 type fatty acids in food sources. Additionally, Jiang *et al*. (1991) concluded that a favourable dietary fatty acid profile, high in PUFAs and monounsaturated fatty acids (MUFAs), could reduce the cholesterogenic effect of eggs by altering their fatty acid composition by incorporating n-3, n-6 and omega-9 (n-9) fatty acids into egg lipids.

The positive response in altering the fatty acid profile of eggs through the manipulation of the hen's diet is possible due to the ability of fowls to utilise and deposit fatty acids directly into adipose tissue and egg yolk without significant modification (Ansari *et al*., 2006; Celebi & Macit, 2008). However, increasing the PUFA content of eggs increases their susceptibility to lipid oxidation, which could deteriorate egg quality, mainly due to a decrease in organoleptic

properties of eggs, thereby lowering consumer acceptability toward these “enriched” products (Hargis and Van Elswyk, 1991). Since fish oil have a relatively high concentration of longer chain n-3 PUFA such as eicosapentaenoic- (EPA), docosapentaenoic- (DPA) and docosahexaenoic acid (DHA) (Farrell, 1998; Hayat *et al*., 2010) it is normally used in combination with vegetable type lipid sources (linseed-, flaxseed- or rapeseed oil), which is higher in shorter chain n-3 PUFA (α-linolenic acid). However, the dietary usage of PUFAs, especially longer chain n-3 type fatty acids, result in an increased lipid oxidation of egg yolk (Leeson *et al*., 1998; Grashorn, 2005; Hayat *et al*., 2010) that becomes more severe over an extended egg storage period (± 40 days), even at refrigerated temperatures (Cherian *et al*., 2007). Dunn-Hurrocks *et al*. (2011) further reported that dietary supplementation with fish- or flaxseed oil (high in n-3 PUFAs) have a detrimental effect ($P < 0.05$) on the vitelline membrane strength of egg yolk, thereby reducing the Haugh units of enriched eggs, as a result of the increase in yolk diameter. In contrast, other authors (Meluzzi *et al*., 2000; Celebi & Macit, 2008; Cedro *et al*., 2009) reported no differences ($P > 0.05$) on internal egg quality characteristics such as albumen- and yolk ratio, yolk weight and Haugh units between conventional (maize, soybean type diets) and n-3 type diets (fish oil, marine algae substrate, linseed oil). Although literature regarding the effects of supplemental lipid sources as well as lipid inclusion levels in layer diets is in abundance, variation in conclusions between studies complicates the comprehensive interpretation and application of information. Furthermore, the lack of literature regarding the specific effects of dietary lipid saturation, varying n-3, n-6, n-9 fatty acid to highly saturated fatty acids (SFAs) are of major concern and suggest the need for further scientific exploration.

The aim of this study was to determine the effects of dietary lipid saturation on internal egg quality parameters, egg yolk fatty acid composition as well as lipid oxidation stability of egg yolk during the early laying period.

5.2 Material and Methods

5.2.1 Birds and housing

The same birds and husbandry practises as comprehensively discussed in paragraph 3.2.1 (Chapter 3) were used for the determination of egg quality parameters. Internal egg quality was evaluated during 24, 28, 32, 36 and 40 weeks of age and data collected during this period were pooled to represent the peak production period, as explained in paragraph 4.2.1 (Chapter 4). Fatty acid methyl esters (FAME), thiobarbituric acid reactive substances (TBARS) and

peroxide values (PV) of eggs were determined at 30 weeks of age which was regarded as the "mid-point" during the 20 week production study (20 – 40 weeks of age).

5.2.2 Diets

Diets used during this study are described in detail within paragraph 3.2.2 (Chapter 3), while the physical composition (Table 3.2), calculated chemical composition (Table 3.3), fatty acid methyl esters (Table 3.4), total fatty acid concentration and ratios (Table 3.5), dietary oxidative stability (Table 3.6) and analysed chemical composition (Table 3.7) of the diets are illustrated in the mentioned tables. All birds received these diets for the duration of the experiment (20 weeks) as discussed in paragraph 4.2.3 (Chapter 4) and production parameters were recorded (paragraph 4.2.4) and comprehensively discussed (paragraphs 4.3.2) in Chapter 4.

5.2.3 Internal egg quality

Internal egg quality was evaluated to determine the effect of dietary lipid saturation on parameters such as yolk and albumen indices, Haugh units, the ratios of albumen- and yolk weight to egg weight (albumen / egg weight and yolk weight / egg weight) and yolk colour. The mentioned parameters were evaluated at the respective collection weeks (24, 28, 32, 36 & 40 weeks of age). To limit the effect of environmental temperature, all internal egg quality measurements were conducted in an enclosed laboratory at a temperature of 22^{o}C throughout the study.

5.2.3.1 Egg weight

Individual egg weights (g) of all eggs produced were recorded as described in paragraph 4.2.4. After recording egg weight, twenty eggs per treatment (n = 20/trt) were randomly selected for the further analyses of internal egg quality properties. These eggs were broken in halve on an egg break-out stand (Figure 5.1) equipped with a level glass surface (Keener *et al*., 2006; Suma *et al*., 2007) and a mirror angled at 45^{o}C, which allowed the accurate measurements of yolk and albumen properties. After egg breaking, shells were weighed ("as is") to the closest 0.0001 g and the shell weight was used for further calculations.

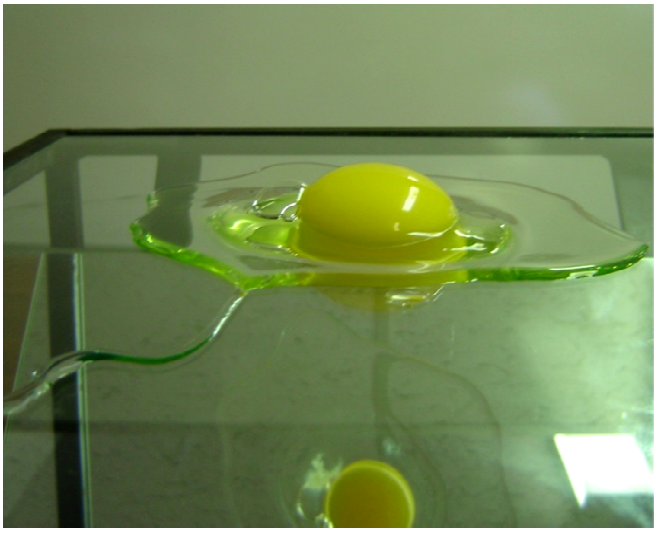

Figure 5.1 Inner egg content on a glass surface of an egg break-out table.

5.2.3.2 Albumen and yolk measurements

Egg yolk diameter (Figure 5.2) was measured accurately to 0.01 mm at two different locations according to the cross-over technique, using a digital vernier calliper (Omni-Tech® model no. SHA 1890). For improved accuracy, this technique allowed two yolk diameter readings, each made on a 90^{o} intersect with another, in order to calculate an average yolk diameter as described by Berardinelli *et al*. (2008). Accordingly, albumen diameter (Figure 5.3) was also measured accurately to 0.01 mm both longitudinal and equatorial (Berardinelli *et al*., 2008) to allow the calculation of an average albumen diameter (Kul & Seker, 2004).

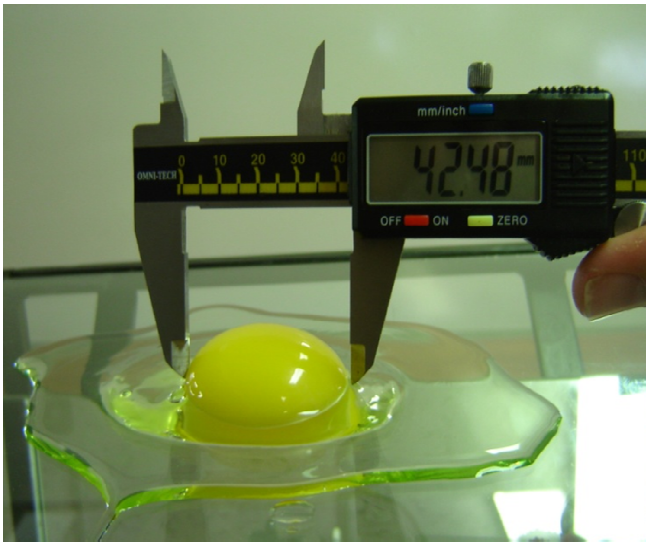

Figure 5.2 Measuring of yolk diameter.

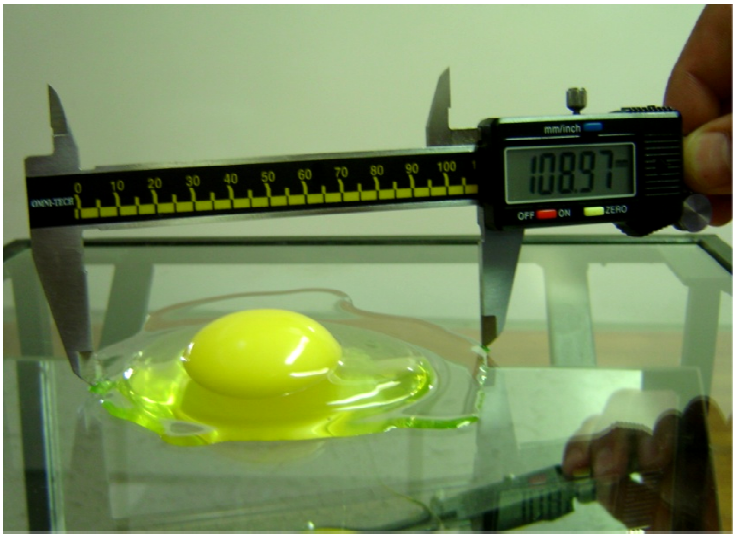

Figure 5.3 Measuring albumen diameter.

A digital tripod micrometer (Ames model no. BG 3112-0-04) accurate to 0.001 mm was used for the measurement of yolk and albumen height. Yolk height (Figure 5.4) was measured at the centre of the yolk without separating the yolk from the albumen according to the technique firstly described by Funk (1948) as well as Keener *et al*. (2006) and Berardinelli *et al*. (2008).

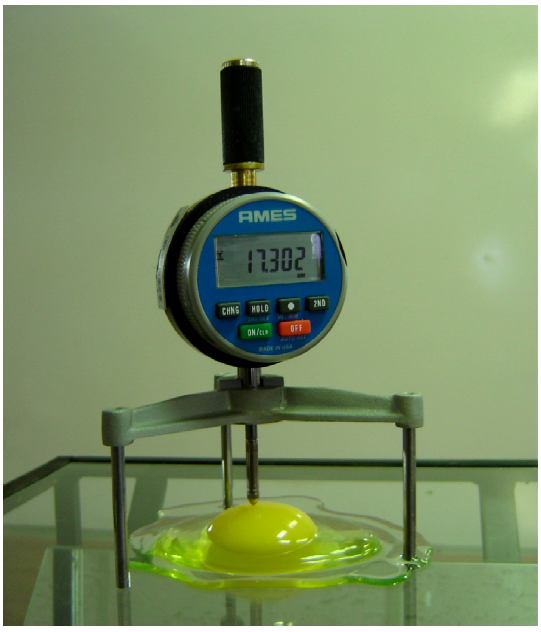

Figure 5.4 Measuring yolk height with an Ames tripod micrometer.

Albumen height measurements (Figure 5.5) were done on the thick albumen according to the techniques described by Keener *et al*. (2006) and Suma *et al*. (2007), without touching the

yolk and avoiding measurements on the chalazae. Micrometer placement for albumen measurements were done at two points on the thick albumen, one approximately 6 mm from the edge of the yolk while the other measurement point was about 6 mm from the edge of the thick and thin albumen. Measurements obtained during this process were used for the calculation of different internal egg quality parameters.

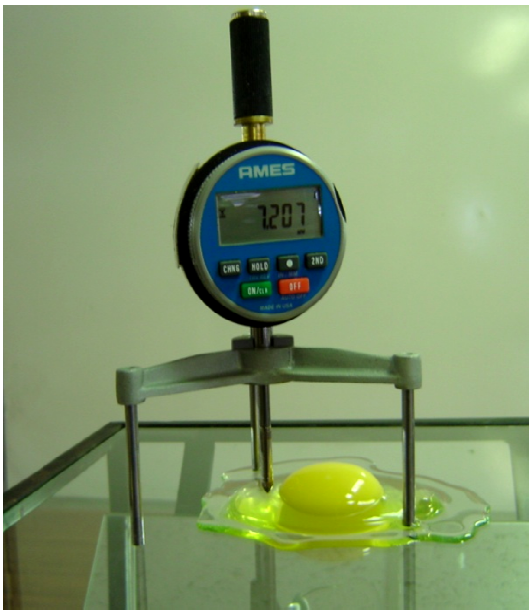

Figure 5.5 Measuring albumen height using an AMES tripod micrometer.

5.2.3.3 Yolk colour

Yolk colour was determined by using a DSM Roche® colour fan according to the technique described by Vuilleumier (1969). The person responsible for the evaluation of yolk colour was pre-tested for colour perception to ensure objective results and was solely in charge of yolk colour measurements throughout the experimental period. The egg break-out stand was fitted with a white fluorescent lamp and yolk colour evaluation was conducted against a white background to eliminate adjacent colours. Yolk colour was evaluated by holding the blades of the colour fan directly above the yolk (Figure 5.6) and vertically observing the blades to match the yolk colour.

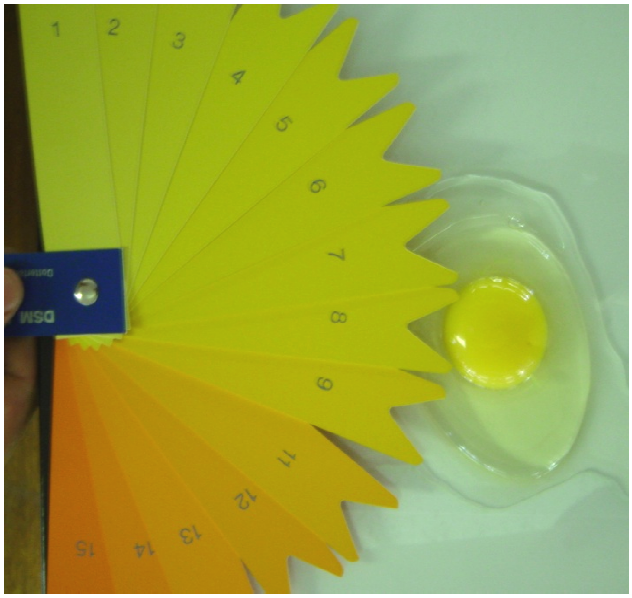

Figure 5.6 Determining egg yolk colour using a DSM Roche® colour fan.

5.2.3.4 Yolk weight

After recording all mentioned measurements on the egg break-out table, the inner egg content was carefully placed into a yolk separator to separate the yolk from the albumen (Figure 5.7). The yolk was then rolled (Figure 5.8) on a damp paper towel as described by Hussein *et al*. (1992) and Grobas *et al*. (1999b) to remove adhering albumen and chalazae (Figure 5.9) prior to the weighing thereof. Yolk weight was recorded on a Shimadzu (Model: AY 220) electronic scale accurate to 0.0001 gram and used for the calculation of albumen weight.

Figure 5.7 Separating egg yolk and albumen.

Figure 5.8 Removing adhering albumen from egg yolk on a damp paper towel.

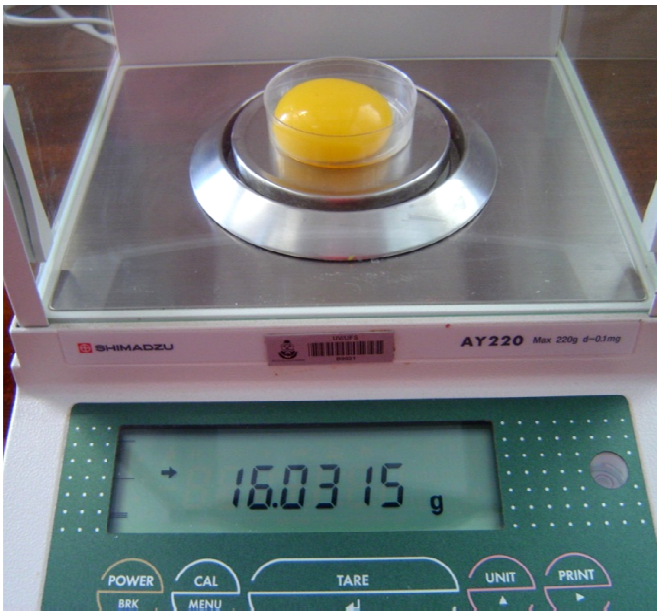

Figure 5.9 Recording yolk weight after removing albumen.

5.2.3.5 Calculations

Haugh unit (HU) of eggs were determined according to the procedures described by Haugh, (1937) using the egg break-out table and a standard tripod micrometer as described in paragraph 5.2.3.1 (determination of egg weight) as well as paragraph 5.2.3.2 (albumen and yolk measurements). By using the albumen height in millimetres (H), along with egg weight (W) and the gravitational constant (G) of 32.2, HU were calculated with the following formula (Keener *et al*., 2006):

$HU = 100 \log \{H – [\sqrt{G}(30W^{0.37} -100)/100] + 1.9\}$

The albumen index is defined as the height (mm) of the albumen divided by the width (mm) of the albumen (Heiman & Carver, 1936; Kul & Seker, 2004) and was calculated by using the following formula:

Albumen index (%) = (Albumen height / Albumen diameter) x 100

The calculation of the yolk index is similar to that of the albumen index. It was determined by using the yolk height (mm) and yolk diameter (mm) to calculate the index by using the following formula (Kul & Seker, 2004; Keener, *et al*., 2006):

Yolk index (%) = (Yolk height / Yolk diameter) x 100

Albumen weight (g) was calculated as the difference between total egg weight (g) and the weight (g) of the eggshell (g) plus the yolk (g) (Grobas *et al*., 1999b):

Albumen weight (g) = Egg weight – (Shell weight + Yolk weight)

The albumen and yolk ratios were calculated as a percentage of the component weight (g) compared to the weight (g) of the egg. The formulas described by Kul and Seker (2004) were used for the calculation thereof:

Albumen ratio (%) = (Albumen weight / Egg weight) x 100

Yolk ratio (%) = (Yolk weight / Egg weight) x 100

5.2.4 Egg fatty acid methyl esters

At 30 weeks of age, 24 hens per treatment (n = 24/treatment) which had laid an egg before 07:00 am were randomly selected to participate in the determination of egg yolk FAME and lipid oxidation. On the day of egg collection, 12 eggs per treatment were analysed as fresh (D_0) while the other 12 eggs were stored for a period of 28 days (D_{28}) at 4°C in a still air refrigerator according to the procedures described by Hayat *et al*. (2010). The length of the

storage period (28 days) was chosen to simulate the "best-before" date of packed eggs currently used in South Africa (Red Barn, 2010).

Fresh eggs (D_0) were analysed for FAME, TBARS, and peroxide value (PV), while the stored eggs (D_{28}) were only analysed for TBARS and PV. All eggs were individually weighed (Figure 4.2) as described in paragraph 4.2.4 (Chapter 4), where after the egg was broken into an egg separator to enable the separation of yolk from albumen and the recording of yolk weight (see paragraph 5.2.3.2). After recording yolk weight, the vitteline membrane was carefully removed from the yolk before an aliquot (± 5 g) of the yolk was taken for chemical analysis of the mentioned parameters.

5.2.4.1 Lipid extraction

The total lipid content of the yolk samples were extracted according to the method described by Folch *et al*. (1957) and comprehensively explained in paragraph 3.2.2 (Chapter3).

5.2.4.2 Analysis of fatty acid methyl esters

The same methods used for determination of FAME in lipid sources and feed samples (paragraph 3.2.2; Chapter 3) were used for the determination of the yolk FAME. Fatty acids identified were expressed as the relative percentage of individual fatty acids as a percentage of the total fatty acids present in the sample (% FAME). Total extractable fat content was determined by weighing and expressed as % fat (w/w) per 100 g egg yolk. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for fat extraction (Folch *et al*., 1957) after drying it according to procedure number 934.01 of the AOAC (AOAC, 2000). By determining the difference in weight, the FFDM was then expressed as % FFDM (w/w) per 100 g yolk. The moisture content of the yolk was determined by means of subtraction (100 - % lipid - % FFDM) and expressed as % moisture (w/w) per 100 g yolk

By using the concentration of individual fatty acids, the following fatty acid combination and ratios for egg yolk were calculated namely: (i) total SFAs, (ii) total MUFAs, (iii) total PUFAs, (iv) total unsaturated fatty acids (UFAs), (v) total n-6 fatty acids, (vi) total n-3 fatty acids, (vii) ratio of PUFAs to SFAs (PUFA / SFA), (viii) ratio of MUFAs to SFAs (MUFA / SFA), (ix) ratio of UFAs to SFAs (UFA / SFA) and the (ix) omega-6 to omega-3 (n-6 / n-3) ratio. Furthermore, levels of n-6 and n-3 fatty acids were respectively expressed as either mg

n-6 or mg n-3 per g egg (shell included) by considering the egg weight and the % lipid within the egg yolk.

5.2.4.3. Thiobarbituric acid reactive substances

For the determination of lipid oxidation in fresh (D_0) and stored (D_{28}) egg yolk, approximately 5 g of the yolk sample were used for analysis of TBARS according to the aqueous acid extraction method described by Raharjo *et al*. (1992).

5.2.4.4 Peroxide value

The PV of fresh (D_0) and stored (D_{28}) egg yolk were determine according to technique number 965.33 of the AOAC (2000) as formerly described in paragraph 3.2.2 (Chapter 3) for feed samples.

5.2.5 Statistical analyses

Data collected during the specific collection weeks (i.e. 24, 28, 32, 36 & 40 weeks of age) were pooled for statistical analysis of the specific tested parameters and were regarded as the early laying period. The effect of dietary lipid saturation on internal egg quality characteristics were analyzed using a fully randomized one way ANOVA design. The PROC ANOVA procedures of the SAS program (SAS, 1999) were used to test for significant differences between treatments. When significant differences were found ($P < 0.05$), further multiple comparison tests, using Tukey's honest significant difference (HSD) test, was used to identify differences between treatment means.

5.3 Results and Discussions

5.3.1 Internal egg quality

Results regarding the effect of dietary lipid saturation on internal egg quality parameters are presented in Table 5.1. Dietary lipid saturation in general had no significant ($P > 0.05$) effect on the tested parameters, except in the case of yolk weight ($P < 0.05$), -height ($P < 0.05$) and -colour score ($P < 0.0001$). Yolk weight (13.31 g) of the layers fed the PUFA n-3 diet was lower than that of the PUFA n-6 diet. Accordingly this diet resulted in a lower yolk height (18.24 mm) compared to the control n-3 diet (18.76 mm). Yolk colour was the highest ($P < 0.0001$) for the SFA treatment and it was the only dietary treatment to score significantly higher than any of the UFA treatments. The lowest ($P < 0.0001$) yolk colour was recorded for the PUFA n-3 treatments. The general limited response of dietary saturation on internal

egg quality parameters as illustrated in Table 5.1 is however in agreement with the production performance results as reported in Chapter 4 (Tables 4.1 & 4.2).

The results of the present study is not in agreement with Kehui *et al*. (2004) who reported that the dietary inclusion of 5% fish oil (PUFAs of n-3 type) resulted in heavier ($P < 0.05$) yolk weights compared to that of palm oil (high in SFAs). However, they found no significant differences in yolk weights between the dietary inclusion of PUFA n-3 (fish oil) and PUFA n-6 (soybean oil) type dietary lipid sources. Hargis and Van Elswyk (1991), Ferrier *et al.* (1995) and Meluzzi *et al.* (2000) reported that dietary supplementation of flaxseed, (n-3 PUFA), soybean oil (n-6 PUFA), palm oil and lard (high in SFAs) had no effect ($P > 0.05$) on egg and albumen weights, as was observed in the present study. However, other authors (Whitehead *et al.*, 1993; Scheideler *et al.*, 1994; Van Elswyk *et al.*, 1994) concluded that both egg and yolk weights were decreased by the inclusion of dietary lipid sources high in both n-3 and n-6 types polyunsaturated fatty acids. Van Elswyk *et al.* (1994) suggest that the hypolipodemic effect of certain lipid sources such as fish oil might reduce the hepatic lipogenisis and lipid transport from the blood into the ova, thereby affecting egg- and yolk weights negatively. Conversely, yet another group of authors (Baucells *et al.*, 2000; Meluzzi *et al*, 2000; Chen & Hsu, 2003) concluded that at a constant lipid inclusion level as well as the usage of *isocaloric* and *isonitrogenous* diets, as implemented in the present study, resulted in the lack of significant differences in egg properties such as egg and yolk weights Yolk colour results from the present study supported Hammershoj (1995) who reported that yolk colour was lower ($P < 0.05$) in eggs produced from diets containing 3% fish oil (high in n-3 PUFAs) compared to a diet with 3% animal fat inclusion (high in SFAs). These results were also confirmed by a study conducted by Cachaldora *et al*. (2008) who found that increasing dietary inclusion levels of fish oil resulted in a lower ($P = 0.01$) egg yolk colour. Contrary to the findings of the present study, Jiang *et al*. (1992) concluded that egg yolk colour was higher ($P < 0.05$) by feeding whole oilseeds such as flaxseed (n-3 PUFAs type) compared to both sunflower seed (PUFAs of n-6 type) and HO sunflower seed (MUFAs of n-9 type).

Table 5.1 Mean (± s.d.) effects of dietary lipid saturation on internal egg quality parameters (20 to 40 weeks of age).

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (P)
Egg weight (g)	55.77 ± 1.79	54.88 ± 2.94	56.10 ± 2.36	54.76 ± 2.31	55.21 ± 3.10	0.40
Shell weight (g)	5.61 ± 0.31	5.66 ± 0.32	5.66 ± 0.34	5.55 ± 0.26	5.70 ± 0.29	0.57
Yolk weight (g)	13.80^{ab} ± 0.66	13.31^{b} ± 0.64	13.96^{a} ± 0.94	13.73^{ab} ± 0.73	13.63^{ab} ± 0.66	≤ 0.05
Yolk diameter (mm)	39.63 ± 1.51	39.02 ± 0.66	39.31 ± 0.80	39.49 ± 1.16	39.24 ± 0.98	0.53
Yolk height (mm)	18.76^{a} ± 0.47	18.24^{b} ± 0.50	18.66^{ab} ± 0.54	18.63^{ab} ± 0.56	18.59^{ab} ± 0.60	≤ 0.05
Yolk ratio (%)	24.52 ± 1.33	24.24 ± 0.88	24.86 ± 1.19	25.02 ± 0.89	24.73 ± 1.10	0.19
Yolk index (%)	47.66 ± 1.18	46.72 ± 1.44	47.28 ± 1.26	47.46 ± 1.70	47.62 ± 1.58	0.24
Albumen weight (mm)	36.51 ± 1.60	35.91 ± 2.27	36.47 ± 1.60	35.50 ± 1.71	35.87 ± 2.53	0.44
Albumen diameter (mm)	112.94 ± 4.78	113.43 ± 6.48	112.58 ± 6.92	112.62 ± 5.04	112.32 ± 6.70	0.98
Albumen height (mm)	8.06 ± 0.79	7.55 ± 0.73	7.72 ± 0.97	7.92 ± 0.76	7.72 ± 0.88	0.33
Albumen ratio (%)	65.41 ± 1.34	65.44 ± 1.08	65.05 ± 1.20	64.83 ± 0.98	64.92 ± 1.21	0.33
Albumen index (%)	7.23 ± 0.86	6.78 ± 0.83	6.98 ± 1.12	7.12 ± 0.81	6.99 ± 0.98	0.61
Haugh units	90.56 ± 3.97	87.96 ± 4.20	88.44 ± 5.64	90.04 ± 4.18	88.85 ± 4.46	0.33
Colour	5.28^{d} ± 0.23	5.45^{cd} ± 0.28	5.64^{bc} ± 0.26	5.83^{b} ± 0.23	6.14^{a} ± 0.27	< 0.0001

^{a,b,c,d} Row means with different superscripts differ significantly at $P \leq 0.05$.

Cachaldora *et al*. (2008) suggested that high levels (> 45 g/kg) of dietary PUFAs may interact with the deposition of oxicartenoids due to the increase in total yolk fat concentration (Grobas *et al.*, 2001), resulting in an increased peroxidation of cartenoid pigments and its retention in egg yolk. This explanation supports the yolk colour results of the present study, as discussed later (Table 5.4), where the control, PUFA n-3 and n-6 treatments were more susceptible to dietary (Table 3.5) and yolk lipid oxidation when compared to the MUFA n-9 and SFA treatments.

5.3.2 Yolk fatty acid methyl esters

The effect of dietary lipid saturation on yolk properties such as percentage yolk fat, FFDM (%), moisture content (%), FAME of egg yolk as well as the total concentration and fatty acid ratios are indicated in Tables 5.2 and 5.3 respectively. It must be noted that the respective FAME presented in Table 5.2 are not the solitary fatty acids recorded within egg yolk, but only those specific individual fatty acids that were also recorded for the diets (Table 3.4). Furthermore, it is important to note that the total fatty acid concentration and fatty acid ratios as indicated in Table 5.3 were not calculated on the fatty acid concentrations of the single mentioned fatty acids in Table 5.2, but on the total dietary fatty acid profile, irrespective of individual fatty acid concentration. Dietary lipid saturation had no significant effect on the fat (P = 0.24), FFDM (P = 0.17) and the moisture (P = 0.66) content of egg yolk. As expected, differences in dietary lipid saturation (Tables 3.4 & 3.5) resulted in a highly significant (P < 0.0001) alteration of egg yolk FAME in response to the specific dietary treatment. The control n-3 diet (50 / 50 linseed- / fish oil) resulted in the highest (P < 0.0001) concentration of α-linolenic acid (PUFA n-3) (2.42%) as well as the second highest concentration of total n-3 (9.42%) and second lowest n-6 to n-3 (n-6 / n-3) ratio (1.51) in egg yolk. In agreement to the dietary FAME, the polyunsaturated n-3 diet resulted in the highest (P < 0.001) concentration of n-3 type PUFAs such as EPA (0.95%); DPA (0.90%) and DHA (8.33%). Additionally, the polyunsaturated n-3 treatment also resulted in the highest (P < 0.0001) concentration of total n-3 fatty acids (10.53%) as well the lowest n-6 / n-3 ratio (1.16) and the highest concentration of n-3 (7.78 mg n-3/g whole egg).

The polyunsaturated n-6 treatment resulted in the highest (P < 0.0001) concentration of linoleic acid (PUFA n-6) (20.41%), total polyunsaturated fatty acids (25.50%) and total n-6 (24.49%) within egg yolk. Subsequently, the PUFA / SFA ratio (0.67) as well as the n-6 / n-3 ratio (24.60) was the highest (P < 0.0001) for the polyunsaturated n-6 treatment. The

monounsaturated n-9 treatment resulted in the highest ($P < 0.0001$) concentration of oleic acid (MUFA n-9) (44.80%), total MUFAs (49.23%) and total UFAs (65.36%) in egg yolk. The MUFA / SFA ratio (1.43) as well as the UFA / SFA ratio (1.90) of the monounsaturated n-9 treatment were also the highest ($P < 0.0001$). Interesting to note that the usage of tallow as a dietary lipid source in the SFA treatment, resulted in no distinct elevated levels of individual fatty acids, total fatty acid concentration or fatty acid ratios, except for the individual highest ($P < 0.0001$) concentration of margaric acid (SFA) (0.32%) in the egg yolk. It is further interesting to note that although individual fatty acids, as well as total fatty acid concentration and ratios are distinctively recognisable in the experimental diets (Tables 3.4 & 3.5) according to the dietary lipid sources used, the magnitude thereof are less noteworthy in the FAME of yolk itself (Tables 5.2 & 5.3). Results of the present study suggests that the extent in which the FAME of egg yolk is altered, differs between the SFA and UFAs (control n-3, polyunsaturated n-3, polyunsaturated n-6 & monounsaturated n-9) treatments, whereby a more pronounced effect in the alteration of egg yolk FAME are recorded with the usage of UFAs. Although dietary lipid saturation resulted in differences ($P < 0.0001$) of egg yolk FAME between treatments for individual SFAs (for example: myristic-, palmitic-, margaric- and stearic acid), these differences were not nearly as profound as those observed between treatments for UFAs (for example: oleic-, linoleic-, α-linolenic-, eicosopentaenoic-, docosapentaenoic- and docosahexanoic acid). These results of the present study agrees with that of Cachaldora *et al.* (2008) who found that diets varying from 15 g/kg to 60 g/kg in various types of fish oils have no significant effect on the yolk concentration of myristic-, palmitic-, margaric- and stearic acid (SFAs). They further concluded that the endogenous synthesis of SFAs within birds might be responsible for the lack of significant differences in yolk FAME for these fatty acids.

Important is the fact that although the different lipid sources used for alteration of the dietary saturation had an effect ($P < 0.0001$) on chancing the FAME of yolk, it had no effect ($P > 0.05$) on yolk properties as indicated in Table 5.2. These results attest to, Jiang *et al*. (1991) and Cachaldora *et al*. (2008) who reported that neither dietary lipid source nor dietary lipid saturation had any effect ($P > 0.05$) on the total lipid content of egg yolk.

Table 5.2 The mean (± s.d.) effect of dietary lipid saturation on egg yolk properties and fatty acid methyl esters (FAME) at 30 weeks of age.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (P)
Yolk properties (%):						
Yolk fat	31.28 ± 0.70	30.51 ± 1.70	30.35 ± 2.13	31.45 ± 0.82	30.98 ± 0.98	0.24
Yolk fat free dry matter	19.51 ± 2.17	20.31 ± 0.89	21.19 ± 2.93	20.24 ± 0.50	20.84 ± 0.60	0.17
Yolk moisture	49.21 ± 2.33	49.18 ± 1.85	48.46 ± 3.58	48.31 ± 0.79	48.18 ± 1.18	0.66
FAME (% of total fatty acids)						
Saturated fatty acids:						
Myristic acid (C14:0)	0.35^{b} ± 0.04	0.51^{a} ± 0.06	0.23^{c} ± 0.05	0.28^{c} ± 0.05	0.39^{b} ± 0.06	< 0.0001
Palmitic acid (C16:0)	26.66^{b} ± 1.54	30.00^{a} ± 0.97	26.67^{b} ± 0.87	25.59^{b} ± 0.97	26.52^{b} ± 1.16	< 0.0001
Margaric acid (C17:0)	0.17^{c} ± 0.03	0.21^{b} ± 0.01	0.16^{cd} ± 0.02	0.13^{d} ± 0.02	0.32^{a} ± 0.04	< 0.0001
Stearic acid (C18:0)	10.16^{ab} ± 1.23	9.51^{bc} ± 0.94	11.29^{a} ± 1.30	8.61^{c} ± 1.53	10.04^{ab} ± 1.15	< 0.0001
Monounsaturated fatty acids (n-9):						
Palmitoleic acid (C16:1c9)	2.45^{a} ± 0.49	2.79^{a} ± 0.48	1.63^{b} ± 0.24	2.44^{a} ± 0.60	2.74^{a} ± 0.55	< 0.0001
Oleic acid (C18:1c9)	34.64^{c} ± 2.85	31.91^{c} ± 1.93	33.06^{c} ± 1.44	44.80^{a} ± 3.07	41.82^{b} ± 2.78	< 0.0001
Eicosenoic acid (C20:1c11)	0.21^{ab} ± 0.04	0.24^{a} ± 0.03	0.18^{b} ± 0.02	0.23^{a} ± 0.02	0.21^{ab} ± 0.03	< 0.001
Polyunsaturated fatty acids (n-6):						
Linoleic acid (C18:2c9,12)	12.71^{b} ± 0.77	11.03^{d} ± 0.52	20.41^{a} ± 1.42	12.25^{bc} ± 0.80	11.58^{cd} ± 0.85	< 0.0001
Eicosadienoic acid (C20:2c11,14)	0.10^{c} ± 0.02	0.08^{c} ± 0.01	0.26^{a} ± 0.03	0.13^{b} ± 0.03	0.10^{bc} ± 0.02	< 0.0001
Arachidonic acid (C20:4c5,8,11,14)	1.23^{c} ± 0.31	1.00^{c} ± 0.17	3.71^{a} ± 0.84	2.77^{b} ± 0.82	2.75^{b} ± 0.73	< 0.0001
Polyunsaturated fatty acids (n-3):						
α-Linolenic acid (C18:3c9,12,15)	2.42^{a} ± 0.67	0.22^{b} ± 0.05	0.14^{b} ± 0.05	0.12^{b} ± 0.04	0.16^{b} ± 0.04	< 0.0001
EPA[1] (C20:5c5,8,11,14,17)	0.54^{b} ± 0.11	0.95^{a} ± 0.14	-	-	-	< 0.0001
DPA[2] (C22:5c7,10,13,16,19)	0.56^{b} ± 0.16	0.90^{a} ± 0.12	-	-	0.08^{c} ± 0.02	< 0.0001
DHA[3] (C22:6c4,7,10,13,16,19)	5.76^{b} ± 1.50	8.33^{a} ± 0.90	0.66^{c} ± 0.15	0.65^{c} ± 0.24	0.88^{c} ± 0.26	< 0.0001

[a,b,c,d] Row means with different superscripts differ significantly at $P < 0.05$; [1] Eicosopentaenoic acid; [2] Docosapentaenoic acid; [3] Docosahexanoic acid.

Table 5.3 Mean (± s.d.) effect of dietary saturation on total fatty acid concentration (%) and fatty acid ratios of egg yolk.

	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (P)
Total fatty acid concentration (%)						
Total SFA[1]	$37.40^{b} \pm 2.33$	$40.33^{a} \pm 1.14$	$38.35^{ab} \pm 1.58$	$34.64^{c} \pm 2.04$	$37.43^{b} \pm 1.93$	< 0.0001
Total MUFA[2] (Total n-9)	$39.13^{b} \pm 3.48$	$37.03^{b} \pm 2.21$	$36.15^{b} \pm 1.67$	$49.23^{a} \pm 3.55$	$46.83^{a} \pm 3.30$	< 0.0001
Total PUFA[3] (Total n-6 & n-3)	$23.47^{b} \pm 1.60$	$22.64^{b} \pm 1.24$	$25.50^{a} \pm 0.92$	$16.14^{c} \pm 1.73$	$15.74^{c} \pm 1.69$	< 0.0001
Total UFA[4] (Total n-9, n-6 & n-3)	$62.60^{b} \pm 2.32$	$59.67^{c} \pm 1.14$	$61.65^{bc} \pm 1.58$	$65.36^{a} \pm 2.04$	$62.57^{b} \pm 1.93$	< 0.0001
Total n-6	$14.05^{b} \pm 0.80$	$12.11^{c} \pm 0.57$	$24.49^{a} \pm 0.89$	$15.20^{b} \pm 1.49$	$14.47^{b} \pm 1.41$	< 0.0001
Total n-3	$9.42^{b} \pm 1.18$	$10.53^{a} \pm 1.00$	$1.02^{c} \pm 0.14$	$0.93^{c} \pm 0.26$	$1.28^{c} \pm 0.31$	< 0.0001
Fatty acid ratios						
MUFA / SFA[5]	$1.06^{c} \pm 0.17$	$0.95^{c} \pm 0.08$	$0.95^{c} \pm 0.08$	$1.43^{a} \pm 0.19$	$1.26^{b} \pm 0.16$	< 0.0001
PUFA / SFA[6]	$0.63^{a} \pm 0.04$	$0.56^{b} \pm 0.02$	$0.67^{a} \pm 0.04$	$0.47^{c} \pm 0.03$	$0.42^{d} \pm 0.04$	< 0.0001
UFA / SFA[7]	$1.68^{b} \pm 0.18$	$1.48^{c} \pm 0.07$	$1.61^{bc} \pm 0.11$	$1.90^{a} \pm 0.18$	$1.68^{b} \pm 0.15$	< 0.0001
n-6 / n-3[8]	$1.51^{d} \pm 0.19$	$1.16^{d} \pm 0.12$	$24.60^{a} \pm 4.09$	$17.22^{b} \pm 4.12$	$11.90^{c} \pm 2.72$	< 0.0001
mg n-3 / g whole egg[9]	$7.26^{a} \pm 0.80$	$7.78^{a} \pm 0.99$	$0.73^{b} \pm 0.10$	$0.74^{b} \pm 0.21$	$0.95^{b} \pm 0.24$	< 0.0001
mg n-6 / g whole egg[10]	$10.87^{bc} \pm 0.96$	$8.92^{d} \pm 0.57$	$17.69^{a} \pm 1.58$	$12.10^{b} \pm 1.40$	$10.70^{c} \pm 1.19$	< 0.0001

[a,b,c,d] Row means with different superscripts differ significantly at $P < 0.05$.

1 Saturated fatty acids.

2 Monounsaturated fatty acids.

3 Polyunsaturated fatty acids.

4 Unsaturated fatty acids.

5 The ratio of monounsaturated to saturated fatty acids.

6 The ratio of polyunsaturated to saturated fatty acids.

7 The ratio of unsaturated to saturated fatty acids.

8 The ratio of omega-6 to omega-3 fatty acids.

9 The n-3 content per gram of whole egg (mg / g).

10 The n-6 content per gram of whole egg (mg / g).

Alvarez *et al*. (2004; 2005) also reported that dietary supplementation with fish- and HO sunflower oil in attempt to alter the dietary lipid saturation had no effect ($P > 0.05$) on the percentage moisture (%) of egg yolk. However, they reported that an increased inclusion level (from 1 to 5 g/kg) of conjugated linoleic acid (CLA) resulted in an increased yolk moisture content, which they ascribed to the associated decrease in yolk fat content. Contrary to the results of the present study, Cherian *et al*. (2007) indicated that by adding both fish oil and CLA at 0.25% in combination to yellow grease (high in SFAs), a decrease ($P < 0.05$) in total lipid content of egg yolk of approximately 5.4% was recorded, which they ascribed to the decreased fat synthesis within the liver associated with supplementary CLA..

Results of the present study is in agreement with that of Cachaldora *et al*. (2008) who found that the dietary fatty acid profile have a highly significant influence on the FAME of egg yolk. Cachaldora *et al*. (2008) indicated that the dietary inclusion of n-3 lipid sources such as fish oil resulted ($P < 0.001$) in a linear increase in the concentration of EPA, DPA and DHA (PUFAs n-3) within egg yolk. In support, Cherian *et al*. (2007) concluded that layer diets containing different supplementary lipid sources (yellow grease, CLA and/or fish oil) are effective in changing the FAME of egg yolk according to the dietary fatty acid profile. Celebi and Macit (2008) reported that the dietary inclusion of a short chain PUFA n-3 lipid source such as flaxseed oil resulted not only in an increased ($P < 0.0001$) concentration of PUFAs such as α-linoleic acid within egg yolk, but also of those longer type n-3 fatty acids such as EPA, DPA and DHA. Furthermore, Cherian *et al*. (1996) and Carrilo-Domínguez *et al*. (2005) concluded that hens have the ability to elongate α-linolenic acid and EPA to its longer n-3 metabolites such as DPA and DHA during the formation of yolk lipids in the liver. Comparing the dietary concentrations of α-linolenic acid, EPA, DPA and DHA (Table 3.4) of both the control n-3 and polyunsaturated n-3 treatments with their respective concentrations in egg yolk (Table 5.2), it's clearly evident that both α-linolenic acid and EPA concentrations decline on average between 80 to 87% , while the concentration of DPA (+36%) and DHA (+220 to 317%) increased considerably in the yolk, thereby supporting the findings of Carrilo-Domínguez *et al*. (2005). In another report, Alvarez *et al*. (2005) found that the dietary inclusion of HO sunflower oil as dietary n-9 lipid source, resulted in a lower ($P < 0.001$) total SFA concentration as well as palmitic and stearic acid levels and an increase ($P < 0.001$) in total MUFA and oleic acid content of egg yolk. In agreement the monounsaturated n-9 treatment of the present study (using HO sunflower oil) resulted in the lowest ($P < 0.001$)

concentration of total SFA (34.62%) as well as stearic acid (SFA) (8.61%) and the highest concentration ($P < 0.001$) of total MUFA (49.23%) and oleic acid (MUFA n-9) (44.80%).

The highest ($P < 0.0001$) concentration of total SFAs (40.33%) and lowest concentration of total UFAs (59.67%) in egg yolk of the polyunsaturated n-3 treatment were unforeseen when compared to the total concentration of the diets, especially that of the SFA treatment. However, it is interesting to note that the polyunsaturated n-3 diet had the highest ($P < 0.0001$) concentration of saturated myristic- (0.51%) and palmitic acid (30%) in the egg yolk. Furthermore, since fish oil were solely used as dietary lipid supplement in the polyunsaturated n-3 treatment, one must consider the fatty acid profile and its role in contributing to the high SFA content of egg yolk. From Table 2.1 it is also evident that the saturated palmitic acid concentration of pure fish oil could vary between 13.4 % (O'Fallon *et al*., 2007) and 20.2% (Basmacıoglu-Malayoglu, 2009). Results of the present study could however be explained by the findings of Baucells *et al*. (2000) who reported that the progressive substitution of fish oil with other lipid sources such as linseed oil (high in short chain PUFA n-3), rapeseed oil (high in short chain PUFA n-3), sunflower oil (high in PUFA n-6), and tallow (high in SFAs), hardly affected the respective levels of SFAs in egg yolk, irrespective of its significant alteration of egg yolk n-3 and n-6 PUFA concentrations. Baucells *et al*. (2000) concluded that irrespective of the dietary lipid source being used, the total SFA concentration of egg yolk ranged between 30 and 38%. Furthermore, Schreiner *et al*. (2004) also reported that the total SFA concentration of egg yolk were similar ($P > 0.05$) for both tallow (high in SFAs) and seal blubber oil (high in PUFAs of n-3 type). Schreiner *et al*. (2004) concluded that if higher levels of UFAs are fed to laying hens, they do have the capacity to keep the SFA levels in egg yolk relatively constant, which is a crucial aspect for the biological and reproductive properties of eggs.

The n-6 / n-3 ratios of both the polyunsaturated n-3 treatment (1.16 / 1) and control n-3 (1.51 / 1) are within the ideal range as prescribed by the FAO (2010), where a n-6 / n-3 ratio within human diets should be lower than 10 / 1 and preferably as low as 2 / 1. One of the beneficial aspects of these low n-6 / n-3 ratios is that consumers would be able to increase their PUFA n-3 essential fatty acid intake with sufficient dietary ingestion of these “enriched” eggs. Similarly to the results of the present study, Schreiner *et al*. (2004) reported a reduction ($P < 0.0001$) in the n-6 / n-3 ratio from 6.78 /1 to 2.50 / 1 by using seal blubber oil (PUFA n-3 type fatty acids) at a dietary inclusion level of 5% compared to tallow (SFAs). Schreiner *et*

al. (2004) also indicated that it is possible to increase the total n-3 fatty acid concentration of eggs from 33 mg n-3/ egg (tallow treatment) to 142 mg / egg (seal blubber oil) at a 5% dietary lipid inclusion level, while Hargis and Van Elswyk (1991) concluded that dietary lipid supplementation with 3 to 7% fish oil, would increase the n-3 fatty acids in eggs to more than 200 mg / egg. Results from the present study are in support to these findings where the polyunsaturated n-3 treatment had the highest ($P < 0.0001$) n-3 fatty acid content per gram egg (7.78 mg n-3 / g whole egg), while the polyunsaturated n-6 treatment resulted in the highest n-6 fatty acid content per gram of whole egg (17.69 mg n-6 / g whole egg). Results of the n-6 / n-3 ratio as well as total n-3 concentration in eggs are noteworthy to consumers and dieticians since it could be used jointly as a guideline in regulating the intake of essential fatty acids (both n-3 and n-6 type PUFAs) by means of egg consumption. Given the results of the present study (Table 5.3), one egg with an average weight of 55 g containing 7.80 mg n-3 / g whole egg, would result in a total of 429 mg n-3 which is approximately 25% (males) and 40% (females) of the daily n-3 fatty acid requirements of adults (Food & Nutrition Board, 2002). Given the same enriched egg, with a decreased n-6 concentration of 0.73 mg n-6 / g whole egg, a person would only consume 40 mg n-6 per day which is less than 0.5% of the daily n-6 requirements of adults (Food & Nutrition Board, 2002). By consuming n-3 enriched eggs, consumers would still be able to eat other food sources with higher n-6 concentrations, without compromising their egg consumption.

However, an increase in the n-3 fatty acid content of eggs also has its limitations in terms of sensory acceptability. Various authors (Holdas & May, 1966; Van Elswyk *et al*., 1992; Van Elswyk *et al*., 1995) indicated that eggs containing 200 mg or more n-3 fatty acids coming from the dietary supplementation of menhaden oil have poor sensory quality. In agreement to those reports, Hayat *et al*. (2010) found that the dietary inclusion of fish oil resulted in a significant reduction in the organoleptic quality of eggs and ascribed their results to higher levels of EPA and DHA (n-3 PUFAs) found in fish oil resulting in a consequent increase in long chain PUFAs of egg yolk. In another report, Herber-McNeill and Van Elswyk, (1996) found that although menhaden oil provides EPA, EPA and DHA (n-3 PUFAs), it is only the DHA levels that are significantly deposited in egg yolk and ascribed poor sensory characteristics of menhaden oil to this specific fatty acid. Although sensory characteristics were not evaluated during the present study, the possibility do exist that that the high levels of n-3 and therefore DHA enrichment might have resulted in eggs with a lower sensory acceptability to consumers, given documented literature.

5.3.3 Lipid oxidation of egg yolk

Results regarding the effect of dietary saturation on the oxidative stability of eggs as expressed by TBARS and PV of fresh (D_0) and stored eggs (D_{28}) are presented in Table 5.4. Dietary lipid saturation level had generally a significant effect on both TBARS ($P < 0.001$) and PV ($P < 0.05$), irrespective of egg storage period. The polyunsaturated n-3 treatment had the highest TBARS for fresh and stored eggs (0.27 mg malonaldehyde / kg yolk for both time periods), while the TBARS of the saturated and monounsaturated n-9 treatments were almost equally low in both time periods (0.13 mg malonaldehyde / kg yolk). The PV value of fresh eggs (D_0) from the polyunsaturated n-3 treatment were the highest (3.96 meq. peroxide / kg fat) whereas that of the monounsaturated n-9 treatment were the lowest for both time periods (2.77 and 2.04 meq. peroxide / kg fat respectively). The higher TBARS and PV values for the polyunsaturated n-3 treatment where fish oil were used as dietary lipid supplement were partly foreseen. However, the significant differences of both the TBARS and PV between the control n-3, polyunsaturated n-6 and polyunsaturated n-3 treatments for fresh eggs (D_0) were not anticipated given the results of the dietary PV in Table 3.5 (Chapter 3). Taking into account that a PV value above 10 meq. peroxide/kg fat and TBARS above one is considered to be high and unacceptable, and the results of the current study is well below that for all the treatments can be the reason why there are not any difference between the fresh and stored eggs. This observation may be the result of the formation of other secondary oxidation products, not measured in this study.

Since the dietary lipid oxidative stability, as measured by means of the PV, of the polyunsatured n-3 treatment (Table 3.5) were very similar to that of the control n-3 and polyunsaturated n-6 treatments for fresh diets, it is difficult to explain the significant increase in lipid oxidation of egg yolk as indicated in Table 5.4. It seems that the higher ($P < 0.0001$) level (2.42%) of α-linolenic acid (Table 5.2) found in egg yolk of the control n-3 treatment resist lipid oxidation better than the higher levels of EPA (0.95%), DPA (0.90%) and DHA (8.33%) in egg yolks from the polyunsaturated n-3 treatment. The more double bonds present in a fatty acid the more susceptible it is to lipid oxidation. EPA and DPA have five double bonds and DHA six, compared to the three double bonds of α-linolenic acid.

Table 5.4 The mean (± s.d.) effects of dietary saturation on the thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) of fresh (D_0) and stored (D_{28}) eggs at 30 weeks of age.

	Storage time	Control (n-3)	Polyunsaturated (n-3)	Polyunsaturated (n-6)	Monounsaturated (n-9)	Saturated (SFA)	Significance (P)
TBARS[1]	D_0	$0.19^{b} \pm 0.05$	$0.27^{a} \pm 0.09$	$0.16^{b} \pm 0.05$	$0.13^{b} \pm 0.04$	$0.13^{b} \pm 0.32$	< 0.0001
	D_{28}	$0.25^{a} \pm 0.09$	$0.27^{a} \pm 0.08$	$0.16^{b} \pm 0.05$	$0.14^{b} \pm 0.05$	$0.13^{b} \pm 0.05$	< 0.0001
PV[2]	D_0	$3.07^{b} \pm 0.64$	$3.96^{a} \pm 0.67$	$3.53^{ab} \pm 1.01$	$2.77^{b} \pm 0.45$	$3.23^{ab} \pm 0.73$	< 0.002
	D_{28}	$2.90^{ab} \pm 0.58$	$2.37^{ab} \pm 0.90$	$3.02^{a} \pm 0.98$	$2.04^{b} \pm 0.77$	$2.41^{ab} \pm 0.87$	< 0.05

[a,b] Row means with different superscripts differ significantly at $P < 0.05$.
[1] Thiobarbituric acid reactive substances (mg malonaldehyde / kg yolk).
[2] Peroxide value (milliequivalent peroxide / kg fat).

These results suggested that if a combination of plant- and animal oil is used for the manipulation of dietary FAME, egg yolk fatty acids would withstand lipid oxidation better than with pure animal oil (fish oil), irrespective of similar dietary oxidation levels.

The decrease in egg yolk PV between fresh (D_0) and stored (D_{28}) eggs further suggest that egg yolk peroxidation is not directly linked with the dietary PV, especially when results of the polyunsaturated n-6 diet is evaluated (Table 3.6). However, it further seems that the elevated levels of dietary lipid oxidation (Table 3.6) are not positively reflected in the level of yolk lipid oxidation. Similarly, egg production (Table 4.1) and -quality characteristics (Tables 5.1, 5.2 & 5.3) were not negatively influenced by the high levels of dietary lipid peroxidation as seen in Table 3.6. These findings could partly be explained by the results of McGill *et al.* (2011) who reported that high dietary peroxide values of 75 to 150 milliequivalent peroxide / kg fat had no negative effect on feed intake and production performances of birds, provided that an antioxidant (125 mg / kg) is included in the diet.

Shahryar *et al*. (2010) reported higher ($P < 0.01$) TBARS in egg yolk of hens fed n-3 fatty acids (fish oil) compared to n-6 fatty acids (sunflower oil) (3.29 *vs.* 2.88 mg malonaldehyde / gram yolk). This was supported by the results of the present study after a storage time of 28 days. Hayat *et al*. (2010) found that an increment in yolk n-3 fatty acids resulted in an increased risk of lipid oxidation of yolk and a subsequent reduction in egg quality and overall sensory acceptance. Hayat *et al*. (2010) further reported that the inclusion of flaxseed (high in short chain n-3 PUFAs) in layer diets resulted in a higher oxidative stability compared to fish oil (high in longer chain n-3 PUFAs) inclusion. He related this improvement in oxidative stability to the high levels of α-linolenic acid within flaxseed oil, compared to the longer chain PUFAs of fish oil. The results of Cherian *et al*. (2007) also indicated that diets supplied with fish oil (longer chain PUFAs of n-3 type) resulted in eggs with higher ($P < 0.05$) TBARS (1.5 *vs*. 1.0 mg malonaldehyde / gram yolk) on D_0 compared to diets supplemented with yellow grease (high in saturated fatty acids) and/or CLA (high in PUFAs of n-6 type in *trans*- configuration). These authors concluded that irrespective of the fatty acid profile of the dietary lipid source used, a storage period of beyond 40-days promoted lipid oxidation and the accumulation of TBARS significantly.

5.4 Conclusions

The only meaningful internal egg quality characteristic influenced by dietary lipid saturation was yolk colour. It seems that the dietary inclusion of saturated lipids resulted in the highest egg colour while that of the n-3 diets the lowest. No clear influence of dietary lipid saturation on other internal egg quality characteristics could be detected. Results from the present study clearly indicated that the fatty acid methyl esters (FAME) of egg yolk could be favourably altered by using various dietary lipid saturation profiles (PUFAs and MUFAs). Conversely, the usage of saturated tallow in the diet of layers was not characterised by a distinct influence, with the exception of margaric acid, on the SFAs concentrations of egg yolk and it seems that endogenous synthesis of these SFAs within birds might have occurred. Although the usage of PUFA n-3 diets could be successfully used to decrease the n-6 / n-3 ratios of eggs and thereby increasing the intake of essential n-3 fatty acids *via* food containing these enriched egg products, it coincide with a significant decrease in lipid oxidative stability as well as egg yolk colour. Although not investigated in the present study, a possible negative influence of high levels of PUFA n-3 in egg yolk, on sensory acceptability to consumers should be kept in mind. This aspect as well as the role of α-linolenic acid to counteract this, needs further investigation.

CHAPTER 6
GENERAL CONCLUSIONS

Polyunsaturated fatty acids (PUFAs) have been identified as essential nutrients in reducing the risks associated with cardiovascular diseases (CVD) in humans while these fatty acids are further essential for normal growth and development of children. However, the daily human diet is either deficient in PUFAs and/or the ratio of omega-6 / omega-3 (n-6 / n-3) is not within the ideal ($\leq$ 4 / 1), mainly due to the industrialised manufacturing systems used for food production and preparation. Polyunsaturated fatty acids can be incorporated into poultry meat and eggs through dietary manipulation, offering consumers a product that combines amino acids with essential fatty acids (EFA) within a complete food source. However, production performances of animals as well as quality characteristics of food products produced could be at risk using n-3 type PUFAs enriched diets due to their susceptibility to lipid oxidation. Therefore, a comprehensive study was conducted to evaluate the effects of dietary lipid saturation on nutrient digestibility, production performances of laying hens, internal and external egg quality characteristics as well as lipid oxidative stability of egg yolk during prolonged exposure (20 weeks) to specific dietary fatty acids.

Results from the present study indicated that the dietary fatty acid profile was successfully altered in agreement to those of the specific supplementary lipid sources used. It is also evident that the diets with higher concentrations of PUFAs were more susceptible to lipid oxidation, as measured by means of peroxide value (PV) and free fatty acids (FFA), compared to that of the monounsaturated n-9 and saturated fatty acid (SFA) treatments, irrespective of storage duration of feed. Results further indicate that dietary lipid saturation had no influence on feed intake as well as apparent digestibility of dry matter, organic matter or gross energy. Although the polyunsaturated n-6 treatment resulted in the lowest apparent crude protein digestibility and the SFA treatment in the lowest fat digestibility, no clear effect of dietary lipid saturation on nutrient digestibility could be recognised. Both the apparent metabolisable energy (AME) and AME corrected for nitrogen (AME_n) were influenced by dietary lipid saturation. Although the polyunsaturated n-3 treatment resulted in the highest ($P < 0.01$) AME (13.29 MJ AME/kg DM) and AME_n (12.74 MJ AME/kg DM), no clear trend regarding dietary lipid saturation on AME and AME_n could be established since both these parameters tested the lowest within the control n-3 and polyunsaturated n-6 treatments. The

results of the digestibility study seems to suggest that although diets with a highly saturated fatty acid profile have a negative influence on fat digestion, no similar negative responses were observed in terms of the AME and AME_n. In the case of adult laying hens, it seems that the constant inclusion levels of supplemental lipid sources as well as the formulation of *isoenergetic* and *isonitrogenous* diets with similar dietary mineral and fibre composition are of more importance in influencing nutrient digestibility, compared to the dietary lipid saturation as single factor.

Results regarding the effect of dietary lipid saturation on egg production performances and external egg quality suggested that the dietary fatty acid profile have no influence on most of these parameters. However, the percentage sellable eggs were the lowest ($P < 0.015$) for the SFA treatment during the present study. Although the SFA treatment resulted in the lowest percentage sellable eggs (89.17%) during peak production (20 - 40 weeks of age), no differences were recorded for eggshell weight, mean eggshell thickness, egg surface area, shell weight per unit surface area and eggshell Ca-content when compared to the other treatments. Since the SFA treatment had no negative influence on any of these eggshell quality parameters, it could be speculated that the high dietary concentrations of SFA might have influenced the ultra structure of eggshells negatively, thereby resulting in an increased egg losses *via* shell breakages and cracks. However, since the ultra structure of eggshells were not evaluated by means of microscopy or shell breaking tests, this philosophy remains only speculative in the present study, although recorded in some other documented literature and needs further investigation. Feed efficiency and percentage eggshell was the only two other parameters influenced by dietary treatment. Once again, it seems that the effect of dietary lipid saturation on these two parameters could not be clearly defined. It can be concluded from the results of the production study that, with the exception of the SFA treatment which resulted in less sellable eggs, the effects of dietary lipid saturation are similar on egg production as well as external eggshell quality parameters during peak production ($\leq$ 40 weeks of age).

It further seems that lipid saturation had no clear effect ($P > 0.05$) on most of the internal egg quality parameters. However, the SFA treatment resulted in the highest ($P < 0.0001$) yolk colour score, while the yolk colour score were the lowest for the n-3 treatments. Results of the present study confirmed that of documented literature that the FAMEs of egg yolk was successfully altered to represent that of the particular dietary treatment without any

detrimental effects on the total fat content, fat free dry matter or moisture content of egg yolk. Both the control n-3 and polyunsaturated n-3 treatments were highly effective ($P < 0.0001$) in increasing the eicosopentaenoic- and docosahexanoic acid content of egg yolk, whereas a general increase in the total n-3 concentration of egg yolk also resulted in a decreased ratio of n-6 / n-3 yolk fatty acids. Additionally, results of the present study indicate that the extent, in which the FAME of egg yolk is altered, differs between the SFA and UFAs (control n-3, polyunsaturated n-3, polyunsaturated n-6 & monounsaturated n-9) treatments, resulting in a total SFA concentration of egg yolk that ranges between 34 and 40% irrespective of dietary concentration thereof. From a metabolic point of view, it seems that the endogenous synthesis of saturated fatty acids within birds might be responsible for these results.

Dietary lipid saturation level had generally a highly significant effect on lipid oxidation of egg yolk as measured by the thiobarbituric acid reactive substances (TBARS) and peroxide value (PV), irrespective of egg storage time (fresh or 28 days of refrigerated storage). The polyunsaturated n-3 treatment resulted in the highest ($P < 0.001$) TBARS for both fresh and stored eggs (0.27 mg malonaldehyde / kg yolk), while the TBARS of both the saturated and monounsaturated n-9 treatments were almost equally low for both time periods (0.13 mg malonaldehyde / kg yolk). The significant differences in yolk lipid oxidation for fresh eggs between the control n-3 and polyunsaturated n-3 treatments as observed during the present study, could suggest that an increase in yolk concentration of n-3 fatty acids with longer carbon chain lengths (eicosopentaenoic-, docosapentaenoic- & docosahexanoic acid) resulted in an increased rate of lipid oxidation compared to n-3 fatty acids with shorter chain length (α-linolenic acid) within the birds. Although the results of the present study showed that the usage of PUFA n-3 diets could be successful to enrich the essential n-3 fatty acids of eggs, its negative effects regarding lipid oxidative stability as well as colour of egg yolk should be kept in mind. Accordingly high levels of PUFA n-3 in egg yolk could negatively influence the sensory acceptance by consumers, although not evaluated during the present study. It seems that higher levels of α-linolenic acid in egg yolk could resist lipid oxidation better than other PUFAs. The usage of PUFA lipid sources high in α-linolenic acid to improve the oxidative stability of eggs and therefore overall sensory acceptance needs urgent further investigation. Furthermore the role and manipulation of eicosapentaenoic- and docosahexaenoic in the reduction of the organoleptic quality of eggs needs further research.

Finally it can be concluded that dietary lipid saturation did not affect overall production and egg quality characteristics during peak production, even with differences recorded in terms of nutrient digestibility. Caution needs to be taken when high levels of SFAs are included in layer diets due to its associated decrease in shell quality by means of the lowest percentage sellable eggs. Furthermore, it can be concluded that the FAME of egg yolk can be manipulated through dietary manipulation without any negative influences on most internal egg quality characteristics. However, special care should be taken in monitoring the lipid oxidative stability of n-3 enriched eggs to ensure that a good quality food product with a viable shelf life could be supplied to consumers. As recommendation for future research, the aspects regarding the influence of dietary lipid oxidation on consumable food product stability when modifying the FAME of animal tissue as well as general animal welfare and immunity should be investigated.

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ABSTRACT

A study was conducted to investigate the effects of dietary lipid saturation on nutrient digestibility, egg production and egg quality characteristics of laying hens during peak production (≤ 42 weeks of age). Five *isoenergetic* (12.6 MJ AME/kg DM) and *isonitrogenous* (170 g CP/kg DM) diets were formulated with a 30 g/kg lipid inclusion level, using a blend (50 / 50) of fish- and linseed oil (control n-3), pure fish oil (polyunsaturated n-3), sunflower oil (polyunsaturated n-6), high oleic acid (HO) sunflower oil (monounsaturated n-9) and tallow (saturated fatty acid treatment). The blend of fish- and linseed oil blend were used to increase the α-linolenic acid content of the control n-3 diet, while fish oil was used in the polyunsaturated n-3 diet to increase the concentration of eicosopentaenoic- (EPA) and docosahexanoic acid (DHA) fatty acids primarily. Sunflower oil and HO sunflower oil was used to increase the linoleic- and oleic acid in the polyunsaturated n-6 and monounsaturated n-9 diets respectively, whereas tallow was used to increase palmitic- and stearic acid levels in the saturated fatty acid (SFA) treatment. Two hundred, individually caged Hy-Line Silver Brown hens (20 weeks of age) were randomly allocated to the five dietary treatments (n = 40 replicates/treatment) and received the respective experimental diets. During 24, 28, 32, 36 and 40 weeks of age, all eggs produced were recorded, individually weighed and used for analysis of internal and external egg qualities. While feed intake of hens was measured weekly, body weights were determined monthly. Data for the respective collection weeks were pooled for calculation of parameter means during statistical analysis. During the mentioned weeks eggs were evaluated for shell quality and internal egg quality. During week 30 of age, 12 eggs per treatment were also randomly selected for analyses of fatty acid methyl esters (FAME), thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) while another 12 eggs were stored at 4^{o}C for analyses after 28 days. At the end of the study (42 weeks of age) six birds per treatment were used to determine the effects of dietary lipid saturation on nutrient digestibility.

Dietary lipid saturation had no effect ($P > 0.05$) on feed intake as well as most of the nutrient digestibility coefficients, except in the case of crude protein ($P < 0.05$) and fat ($P < 0.0001$), whereby the monounsaturated n-9 diet resulted in the highest ($P < 0.05$) CP digestibility which differs statistically only with that of the polyunsaturated n-6 diet, but not with any of the other dietary treatments. Therefore, no clear influence of dietary lipid saturation on

apparent digestibility of CP could be detected. Furthermore, all poly- and monounsaturated diets had a higher fat digestibility (94.2 to 95.6%) than the SFA diet (90.4%). Although both the polyunsaturated n-6 and control n-3 treatments had the lowest ($P < 0.01$) apparent metabolisable energy (AME) and AME corrected for nitrogen (AME_n) values, no clear trend regarding dietary lipid saturation on nutrient digestibility could be established. Similarly to nutrient digestibility results, dietary lipid saturation resulted in a limited significant response on production parameters tested without any recognisable trends. The SFA treatment resulted in the lowest ($P < 0.015$) percentage sellable eggs, while feed efficiency ($P < 0.001$) and percentage eggshell ($P < 0.05$) was the lowest for the monounsaturated n-9 and polyunsaturated n-6 treatments respectively. Evaluating internal egg qualities, the control n-3 and polyunsaturated n-3 treatments had the lowest ($P < 0.0001$) egg yolk colour compared to that of the SFA which resulted in the highest colour score. Additionally, the FAME of egg yolk was successfully altered to represent that of the particular dietary treatment without any detrimental effects on the total fat content ($P = 0.24$), fat free dry matter ($P = 0.17$) or moisture ($P = 0.66$) content of egg yolk. The polyunsaturated n-3 treatment was highly effective ($P < 0.0001$) in increasing the EPA and DHA concentration of egg yolk, whereas a general increase in the dietary n-3 content resulted in a decreased ($P < 0.0001$) ratio of n-6 / n-3 for both the control n-3 and polyunsaturated n-3 diets. Both the SFA and monounsaturated n-9 treatments resulted in the lowest ($P < 0.0001$) TBARS for both time periods, whereas the polyunsaturated n-3 treatment resulted in the highest ($P < 0.001$) TBARS for both fresh and stored eggs (0.27 mg malonaldehyde / kg yolk during both time periods).

From the results of the current study it can be concluded that although fat digestion was lower for the SFA treatment, AME values did not differ between treatments. With the exception of the SFA treatment that resulted in less sellable eggs, no influence of lipid saturation on egg production and external egg shell qualities could be detected. The results showed that PUFA n-3 diets could be successfully used to enrich the essential n-3 fatty acids of eggs. However, lipid oxidation stability as well as yolk colour was negatively influenced by an increase in PUFA n-3 type fatty acids.

OPSOMMING

‘n Studie is uitgevoer om die effek van dieet lipiedversadigheid op nutriëntverteerbaarheid, eierproduksie en kwaliteitseienskappe van eiers gedurende piekproduksie (≤ 42 weke ouderdom) van lêhenne te ondersoek. Vyf energie- (12.6 MJ SME/kg DM) en proteïenekwivalente (170 g/kg RP DM) diëte is geformuleer. In die onderskeie diëte is 30 g/kg lipiedbronne ingesluit deur van ‘n vis- en lynsaadoliemengsel (50 / 50) as kontrole n-3 behandeling, visolie (polie-onversadig n-3), sonneblomolie (polie-onversadig n-6), hoë oleïensuur (HO) sonneblomolie (mono-onversadig n-9) en beesvet (versadige vetsuur dieet) gebruik te maak. ‘n Mengsel van vis- en lynsaadolie is gebruik om die α-linoleïensuur inhoud van die kontroledieet te verhoog, terwyl die visolie in die polie-onversadige n-3 dieet gebruik is om eikosapentanoë (EPA) en dokosaheksaenoë (DHA) vetsure te verhoog. In die polie-onversadige n-6 en mono-onversadige n-9 diëte is sonneblomolie en HO sonneblomolie gebruik om onderskeidelik die linoleensuur en oleïensuur inhoud in die onderskeie diëte te verhoog. Beesvet is gebruik om die vlakke van palmitien- en steariensuur in die versadige vetsuur dieet te verhoog. Twee honderd Hy-line Silwer Bruin henne (20 weke oud) wat in individuele hokke aangehou is, is ewekansig tussen die vyf behandelings (n=40 herhalings/behandeling) verdeel. Gedurende ouderdomsweke 24, 28, 32, 36 en 40 is eierproduksie daagliks aangeteken, eiers individueel geweeg en na die laboratorium vir verdere analise van eksterne- en interne kwaliteitseienskappe geneem. Voerinname is weekliks bepaal, terwyl die henne se liggaamsmassa gedurende die genoemde weke bepaal is. Data van dié onderskeie weke is vir statistiese doeleindes saam gepoel en is gedefinieerd as die piekproduksie periode. Die eierdop- en interne kwaliteit van die eiers wat geproduseer is, is in bogenoemde weke geëvalueer. Gedurende ouderdomsweek 30 is 12 eiers van elke behandeling ewekansig geselekteer om vir oksidasie stabilitiet d.m.v. peroksied waarde (PW) en tiobarbituursuur reaktiewe bestandele (TBARS) te toets. ‘n Verdere 12 eiers is ewekansig gekies en in ‘n yskas (4^{o}C) vir 28 dae gestoor waarna dit vir beide parameters ontleed is. ‘n Veteringstudie is aan die einde van die studie uitgevoer (42 weke ouderdom) om die effek van vetsuurversadigheid op nutriënt verteerbaarheid te evalueer.

Daar was geen effek van vetsuurversadigheid ($P > 0.05$) op voerinname en die skynbare verteerbaarheid van die meeste nutriënte nie, behalwe in die geval van RP en vet waar die mono-onversadigde n-9 dieet die hoogste ($P < 0.05$) RP verteerbaarheid gehad het wat slegs

met dié RP verteerbaarheid van die polie-onversadigde n-6 dieet verskil het. Al die polie- en mono-onversadigde diëte (vanaf 94.2 tot 95.6%) het ook ‘n hoër ($P < 0.0001$) vetverteerbaarheid gehad as die versadigde vetsuur dieet (90.4%). Alhoewel die polie-onversadige n-6 en kontrole behandeling beide die laagste ($P < 0.01$) skynbare metaboliseerbare energie (SME) en SME gekorrigeerd vir stikstof (SME_n) waardes gehad het, was daar geen duidelike verwantskap tussen vetsuurversadigheid en nutriëntverteerbaarheid nie. Daar was ook geen effek van vetsuurversadiging op produksie van die henne nie. Die versadigde vetsuur dieet het statisties betekenisvol ($P < 0.015$) minder bemarkbare eiers geproduseer. Die doeltreffendheid van voerbenutting ($P < 0.001$) en die persentasie eierdop ($P < 0.05$) was onderskeidelik die laagste vir die mono-onversadige n-9 en polie-onversadige n-6 behandelings. Evaluering van interne eierkwaliteite het getoon dat die kontrole n-3 en polie-onversadige n-3 behandelings die laagste eiergeel kleurtelling gehad het in vergelyking met die hoogste ($P < 0.0001$) kleurtelling van die versadigde vetsuur behandeling. Verder was dit duidelik dat die vetsuurprofiel van die eier deur dieet manipulering suksesvol verander is om die profiel van die dieet te verteenwoordig sonder dat daar enige negatiewe uitwerking op die totale vetinhoud ($P = 0.24$), vetvrye droë massa ($P = 0.17$) of voginhoud ($P = 0.66$) van die eiergeel voorgekom het. Die doeltreffendheid ($P < 0.0001$) van die polie-onversadige n-3 behandeling was duidelik sigbaar met die verhoging in EPA en DHA in die eiergeel en tesame met die kontrole n-3 behandeling het die merkbare verhoging in n-3 inhoud daartoe gelei dat ‘n drastiese verbetering ($P < 0.0001$) in die verhouding van n-6 / n-3 voorgekom het. Die TBARS vir beide mono-onversadig n-9 en versadigde vetsuur behandelings was die laagste ($P < 0.0001$) vir beide tydsperiodes, waarteenoor die polie-onversadige n-3 behandeling die swakste ($P < 0.0001$) TBARS (0.27 mg malonaldehied / kg eiergeel) vir beide tydsperiodes getoon het.

Daar is tot die slotsom gekom dat alhoewel die vetverteerbaarheid van die versadigde vetsuur behandeling die laagste was, het die SME waardes nie tussen behandelings verskil nie. Met die uitsondering van die versadigde vetsuurbehandeling wat die minste bemarkbare eiers gehad het, is geen invloed van lipiedversadigheid op eierproduksie en eksterne eierkwaliteit waargeneem nie. Die resultate het getoon dat polie-onversadige n-3 diëte met sukses gebruik kan word om essensiële n-3 vetsure in die eier te verhoog. Daarenteen is lipiedoksidasie en ook die kleur van die eiergeel negatief beïnvloed deur polie-onversadigde n-3 vetsure.