FACTORS INFLUENCING FAT DIGESTION IN POULTRY

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Abstract

The first experiment in this thesis was conducted to understand the digestion of fat along the gastrointestinal tract and ileal endogenous fat losses. The second, third and fourth experiments investigated the factors influencing fat digestion in broilers, including age of birds, cereal type, fat source and dietary calcium concentrations. In the fifth experiment, influence of unsaturated to saturated fatty acid ratio (U:S ratio) on fat digestion was examined.

The first experiment reported in Chapter 4 showed that jejunum is the major intestinal site where majority of fat and fatty acids is digested and absorbed. Long chain fatty acids showed lower digestibility than short chain fatty acids, and unsaturated fatty acids were better digested than saturated fatty acids. The fatty acid profile of ileal endogenous fat was remarkably similar to that of the bile, suggesting that the reabsorption of fat and fatty acids in bile was incomplete.

Data reported in Chapter 5 showed that the apparent metabolisable energy (AME) and total tract digestibility of fats was influenced by the age of broilers. The AME of fat was markedly lower during the first week, increased rapidly during second week and then remained constant thereafter. Total tract fat digestibility was poor during the first week and then increased until the third week of age. No further improvement was observed after the third week. The AME and fat digestibility of soybean oil, poultry fat and palm oil were determined to be higher than those of tallow. Blending of tallow and soybean oil resulted in AME and fat digestibility estimates higher than the arithmetic averages of tallow and soybean oil.

The study reported in Chapter 6 showed that the supplementation of tallow in wheat- and maize-based diets resulted in lower weight gain than that of soybean oil, but fat source had no effect on the weight gain of broilers fed sorghum-based diets. Broilers fed soybean oil supplemented diets had lower feed per gain, higher total tract retention and ileal digestibility of fat compared to those fed tallow supplemented diets. In addition, supplementation of xylanase in wheat-based diets resulted in improved weight gain and feed efficiency of broiler starters irrespective of the fat source. Xylanase supplementation increased the AME of tallow supplemented diets, but had no effect on soybean oil supplemented diets.

Data reported in Chapter 7 indicated that high dietary calcium concentrations had negative impact on broiler performance, irrespective of tallow inclusion levels. High calcium concentrations resulted in higher excreta soap and, lowered the total tract retention of fat, calcium and phosphorus. Lower calcium concentrations resulted in higher ileal digestibility of fat, nitrogen and phosphorus.

Data from the final experiment (Chapter 8) showed that the U:S ratio influenced the performance of broilers during the starter period (1 to 21 day), but had no effect on the performance over the whole trial period (1 to 35 day). Increasing the U:S ratio decreased the AME of diet and increased the total tract retention of fat. A positive linear correlation between U:S ratio and the AME of fat blends was observed, with increasing U:S ratios improving the AME of fat blends.

In conclusion, the research reported in this thesis identified several factors that influence the digestion of fat in poultry. Age of broilers influenced the digestion and absorption of fat, particularly during the first week of age. The findings consistently demonstrated that tallow was more poorly digested than soybean oil. The utilisation of these two fat sources was influenced by the cereal base used in the diets, with the effect of fat source on weight gain differing between cereal types. High dietary calcium concentrations were detrimental to the digestibility of nutrients, especially of fat. A finding of practical interest was that digestion and absorption of animal fats, containing high concentrations of saturated fatty acids, can be improved by blending with soybean oil to increase the U:S ratio.

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Table of Contents

List of Figures

Chapter 2

Chapter 4

Chapter 8

List of Tables

Chapter 2

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of Abbreviations

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
°C	Degree Celsius
Ca	Calcium
DM	Dry matter
FFA	Free fatty acid
g	Gram
GE	Gross energy
h	Hours
K	Potassium
Kg	Kilogram
meq	Milliequivalent
mg	Milligram
MJ	Mega joule
ml	Millilitre
mm	Millimetre
N	Nitrogen
Na	Sodium
ng	Nanogram
NRC	National research council
NSP	Non-starch polysaccharide
P	Phosphorus
S	Saturated
Ti	Titanium
TME	True metabolisable energy
U	Unsaturated
µl	Microlitre

CHAPTER 1

General introduction

Chickens require energy and a number of essential nutrients, including amino acids, minerals and vitamins, for maintenance, growth and egg production. Fat is usually included in diet formulations to meet the high energy requirements of broiler chickens, as the energy value of fat is at least twice as high as those of carbohydrates and protein (NRC, 1994). Other benefits of adding fats include improved palatability, improved absorption of fat-soluble vitamins and, better dust control in feed mills and poultry houses. Furthermore, fat slows down the rate of food passage through the digestive tract, allowing more time for better digestion and absorption of nutrients (NRC, 1994).

Supply of energy represents the major cost in feed formulations. Due to the recent increases in the price of cereals, there is increasing interest in exploring ways to improve the energy value of fats. Therefore, there is a need for greater understanding of the digestion and absorption of fats in poultry.

Most of the available data on the digestion and apparent metabolisable energy (AME) of fats for poultry are 20 to 40 years old and based on studies conducted with slow-growing strains of broilers (Young, 1961; Lessire *et al*., 1982; Wiseman *et al*., 1986; Huyghebaert *et al*., 1988; NRC, 1994). It is logical to expect that the genetic development in broilers over the past two decades may have improved the ability of birds to digest and utilise nutrients. Consequently, studies are warranted to investigate the factors affecting fat digestion and the strategies to improve fat digestion in modern fast-growing broiler chickens.

It is accepted that the small intestine is the major site of digestion and absorption of fat. However, the exact site of fat digestion and absorption remains a controversial issue. Renner (1965) reported that the digestion and absorption takes place beyond the jejunum. Hurwitz *et al*. (1973), however, found that both the jejunum and ileum are involved in the absorption of fatty acids. Published data on the digestion and absorption of fat and fatty acids along the digestive tract of chickens are scant. There is a continuous secretion of endogenous lipids, mainly in the form of bile, into the lumen of the intestinal tract. These endogenous lipids mix with dietary lipids and are partially digested and absorbed. The unabsorbed fraction passing beyond the ileum is considered a loss to the animal and the measurement of these inevitable losses is necessary for the calculation of

true digestibility of lipids. To the author's knowledge, endogenous losses of fat and fatty acids in chickens have not been previously determined.

The digestion and utilisation of fat in chickens are influenced by two major factors, namely, bird- and diet-related factors. Bird-related factors include age, strain, species and gender, whereas diet-related factors include fat characteristics, cereal type, and dietary calcium content.

It is well documented that the digestion and absorption of fat in newly hatched chicks is poor due to the immaturity of physiological functions (Carew *et al*., 1972). In particular, the secretion of lipase and bile seems to be first limiting during the first week of life (Krogdahl, 1985). The ability to digest and absorb dietary fat rapidly develops after the first week of life (Carew *et al*., 1972). Noy and Sklan (1995) reported that lipase secretion increased 20 to 100 fold between days 4 and 21 post hatch. The effect of age on the AME of fats has been examined in several studies (Lessire *et al*., 1982; Wiseman and Salvador 1991), but these evaluations were often limited to only one or two lipid sources and most did not include week 1. A variety of lipid supplements, such as restaurant greases, animal fats (e.g. tallow, poultry fat), and vegetable oils (e.g. soybean, corn, canola and palm oils), are available today for use by feed formulators. Fats and oils from different sources differ in their physical and chemical properties, and it is well accepted that the metabolisable energy value of fat is influenced by these characteristics (Renner and Hill, 1961a; Huyghebaert *et al*., 1988; Scheele *et al*., 1997). It is possible that the effect of age on the AME and digestibility may vary depending on the lipid source. Therefore, studies are needed to better understand the effect of age of broiler chickens on the utilisation of different fat sources.

Viscous cereals such as wheat, barley and rye contain high concentrations of non-starch polysaccharides (NSP) which exhibit anti-nutritive activity in poultry diets. Non-starch polysaccharides, such as arabinoxylans and β-glucans, increase the digesta viscosity and depress the digestibility of nutrients by impeding the diffusion of digestive enzymes and substrates, and contact between nutrients and absorption sites on the intestinal mucosa (Annison, 1993; Choct, 1997). Available data suggest that the digestion of fat is affected more than that of other nutrients by NSP and that the digestion of saturated fatty acids is affected more than that of unsaturated fatty acids (Danicke *et al*., 1997b; Danicke, 2001). Poor digestion of saturated fatty acids was attributed to intestinal viscosity which may lead to reduced gut motility and, decreased rate of

diffusion and transportation of emulsion droplets, lipase, mixed micelles, bile salts and fatty acids in the gut lumen (Smulikowska, 1998). Furthermore, saturated fatty acids are non-polar and do not form mixed micelles spontaneously, and thus are poorly emulsified (Krogdahl, 1985). While the interaction between viscous cereals and dietary fat sources is well documented, corresponding information on non-viscous cereals such as maize and sorghum are limited.

Dietary calcium content is another major factor that can influence the digestion and absorption of fats. Fatty acids, which are end products of fat digestion, have the potential to bind with minerals and form soluble or insoluble soaps in the gut lumen. If insoluble soaps are formed, then both the fatty acid and the mineral will become unavailable and excreted (Leeson and Summers, 2005). Excreta soap concentration has been shown to increase with increasing dietary calcium concentrations. In addition, excreta soap formation was higher in birds fed diets supplemented with saturated fatty acids than those fed diets containing unsaturated fatty acids (Atteh and Leeson, 1983; 1984). The interaction between dietary calcium level and fat source has been examined by Atteh and Leeson (1983, 1984) and Smith *et al*. (2003), but most of the evaluations were limited to two levels of calcium. Further investigations are warranted to understand the relationship between dietary calcium concentration and fat digestion should be considered to improve the digestion of fat in poultry.

To enhance the digestion and AME of fats, a number of strategies can be employed. Lipases and emulsifiers have been used in young broiler chickens by some researchers to improve the utilisation of fats (Polin *et al*., 1980; Meng *et al*., 2004; Zhang *et al*., 2011). Another commonly employed strategy by the feed industry is to use blends containing different ratios of unsaturated and saturated (U: S) fats (Wiseman *et al*., 1998; Danicke *et al*., 2000). There is evidence suggesting that such blending can improve the utilisation of saturated fats (Lall and Slinger, 1973; Sibbald, 1978). Sibbald (1978) reported that the AME of blends of soybean oil and tallow was higher than the sum of the means of its components parts. Similarly, Muztar *et al*. (1981) found that the AME of blends between tallow and rapeseed soapstocks was increased above the calculated AME based on its components. However, such synergistic effect of blended fats not only depends on the U: S ratio, but also depends on the dietary inclusion rates of fat (Wiseman and Lessire, 1987). In addition, most reports examining the influence of U: S fatty acid ratios on fat utilisation have been conducted with maize-based diets

and it is not known whether these findings are directly applicable to wheat-based poultry diets. This is an issue of practical interest because fat blends, rather than pure animal fat or vegetable oil, are normally used in broiler diets, and also wheat is the most widely used cereal in Australia and New Zealand.

The main issue that was addressed in this research project was; "Where exactly does fat digestion and absorption take place in the small intestine, what are the major factors affecting fat digestion and how can the utilisation of fat be improved? To answer these questions, a series of experiments were conducted.

This thesis consists of nine chapters. The first two chapters discuss the framework of the experimental research, with Chapter 1 providing the rationale for the focus of the research. A review of current literature covering various aspects of the classification of lipids and factors affecting fat digestion is presented in Chapter 2. Chapter 2 also provides an overview of potential strategies to improve fat utilisation in poultry. General materials and methods used in the experimental work reported in this thesis are described in Chapter 3. Chapters 4 through 8 present the experimental work of this thesis. Each chapter includes an abstract, introduction, materials and methods, results and discussion. These experiments were conducted to investigate,

1. The digestion of fat and fatty acids along the intestinal tract and the measurement of endogenous fat losses in broiler chickens (Chapter 4).

2. The influence of age on the AME and total tract fat digestibility of different fat sources for broiler chickens (Chapter 5).

3. The influence of cereal type and fat source on performance, energy utilisation and fat utilisation in broiler starters (Chapter 6).

4. The influence of tallow and calcium concentrations on the AME and nutrient digestibility in broilers fed maize-based diets (Chapter 7).

5. The influence of ratio of unsaturated to saturated fatty acids on performance, total tract retention of fat, AME, carcass characteristics and gut microflora counts in broilers fed wheat-based diets (Chapter 8).

Chapter 9 is a general discussion of the experimental results. This chapter addresses the major findings and, highlights the practical implications and possible areas for future research.

CHAPTER 2

Literature review

2.1. Use of fats in poultry diets

2.1.1. Introduction

The term 'fat' is generally used as a synonym for lipid. These two terms describe a diverse variety of compounds that are insoluble in water, but dissolve in organic solvents such as chloroform, acetone, alcohol and diethylether. Lipids play an important role in the biochemistry and physiology of animals and plants. From the point of view of nutrition, lipids of importance are triglycerides, phospholipids, sterols and fat-soluble vitamins (Brindley, 1984).

Fats are normally used in poultry diets as sources of energy because the energy value of fat is at least twice as high as those of carbohydrates and protein (NRC, 1994). The addition of fat to diets also has other advantages including reduced dustiness, improved palatability and lubrication of equipments used in feed milling (Firman *et al*., 2008). Furthermore, fat slows down the rate of food passage through the digestive tract, allowing more time for better digestion and absorption of nutrients (NRC, 1994).

Some fatty acids, such as linoleic and linolenic acids, are not synthesised by poultry and need to be supplied in the diet. In order to ensure adequate levels of these essential fatty acids, reduce dustiness and increase palatability, a minimum inclusion level of 10 g/kg fat in poultry diets has been suggested by Leeson and Summers (2005). Kellems and Church (2010) stated that 20 to 50 g/kg fat is often added in poultry diets if the price of fat is competitive compared to that of cereal grains. However, the addition of fat above 40 g/kg has negative effects on pellet quality (Wiseman, 1999). Lesson and Summers (2005) suggested that 30-40 g/kg is the maximum amount of fat that can be used in pelleted poultry diets.

A variety of fats and oils are currently available for use in feed formulations and these include restaurant greases (e.g. processed frying oils, known as yellow grease), rendering by-products (e.g. tallow, poultry fat), vegetable oils (e.g. soybean oil, corn oil and palm oil), acid oils (by-products of vegetable oil refining, mainly containing free fatty acids), hydrogenated fats (fats or oils which are converted to saturated fatty acids by the addition of hydrogen atom to double bonds of unsaturated fatty acids), and acid

soapstocks (free fatty acids removed from the refining process by alkali and settled as alkali soaps) (McDonald *et al*., 2002; Leeson and Summers, 2005; Kellems and Church, 2010).

The addition of fats and oils not only serve to supply calories, but also can influence carcass characteristics (Crespo and Esteve-Garcia, 2001; Azman *et al*., 2004; Nayebpor *et al*., 2007; Febel *et al*., 2008). In particular, the supplementation of fats and oils in diets has an impact on the fatty acid composition of the broiler carcass. Crespo and Esteve-Garcia (2001) found that broilers fed diets containing tallow had higher saturated fatty acids in the abdominal fat pad, thigh muscle and breast muscle than those fed diets supplemented with olive oil, sunflower oil and linseed oil. Azman *et al*., (2004) reported that birds fed diet supplemented with soybean oil had higher polyunsaturated fatty acids (PUFA) in their abdominal fat pad than those fed poultry grease or beef tallow. The observed changes in fatty acids in broilers tissue can be ascribed to the direct incorporation of dietary fatty acids into adipose tissues.

Fatty acids are also recognised as modulators of immune responses. Fritsche *et al*. (1991) reported that antibody titers to sheep red blood cells in pullets fed fish oil was higher than those fed lard, corn oil, canola oil and linseed oil. Nayebpor *et al*. (2007) also found that antibody titers against infectious bursal disease virus in broilers were enhanced with increasing dietary levels of soybean oil. Improvements in the immunity were attributed to the presence of PUFA. There is also current interest in the activity of some fatty acids on gut microflora. Medium chain fatty acids, such as 1-monoglyceride of capric acid (monocaprin), have been found to be particularly effective in controlling *Campylobacter jejuni* (Thormar *et al*., 2006). De los Santos *et al*. (2008) reported that supplementation of 7 g/kg of carprylic acid in feed reduced Campylobacter counts in broiler caeca compared to 0 and 1.4 g/kg supplementations.

With increasing feed energy costs, there is a need to better understand the digestion of fat in broilers and also the factors affecting fat digestion. The aim of this chapter is to provide an overview of classification of lipids and describe the various steps in the digestion and absorption of fat, including endogenous fat losses. Published data on the AME of different fat sources for broiler chickens and factors affecting the digestibility and AME of fats are discussed. Potential strategies to improve fat utilisation are also highlighted in this review.

2.1.2. Structure and classification of lipids

Lipids are naturally occurring substrates that are insoluble in water but dissolve in organic solvents. Alkaline hydrolysis of lipids (known as saponification) gives rise to alcohol and sodium or potassium salts of constituent fatty acids. Based on this, lipids can be divided to two main groups, namely, saponifiable and unsaponifiable groups. Lipids in the saponifiable group are simple lipids and compound lipids, whereas lipids in unsaponifiable group include some compound lipids which are alcohols and not esters (Plummer, 1987). Chemically, however, lipids are classified into three categories, namely simple, compound and derived lipids (Pond *et al*., 2005).

2.1.2.1. Simple lipids

Simple lipids are esters of fatty acids with various alcohols. Simple lipids can be categorised into two groups. The first group is fat and oils which are esters of fatty acids with glycerol. The other group is waxes which are esters of fatty acids with high molecular weight monohydric alcohols.

2.1.2.2. Compound lipids

Compound lipids are esters of fatty acids containing non-lipid substances such as minerals, protein and carbohydrates in addition to alcohol and fatty acids. Examples of compound lipids are phospholipids, glycolipids and lipoprotein. Phospholipids are fats containing phosphoric acid and frequently have nitrogen containing bases and other substituents. Glycolipids are fats containing carbohydrates, while lipoproteins are lipids bound to proteins.

2.1.2.3. Derived lipids

Derived lipids include substances derived from simple or compound lipids by hydrolysis such as fatty acids, glycerol, alcohols, fat soluble vitamins, sterols and terpernoids.

Sterols are lipids with perhydrocyclopentanophenanthrene ring as the basis of their structure. Sterols are compound lipids that are soluble in the usual lipid solvents, but most of them are not saponified. They are usually considered along with lipids

because of their similar solubility characteristics. The most abundant sterol in animal tissue is cholesterol. Cholesterol is an important substrate that has roles in membrane structure. Cholesterol is also a precursor for the synthesis of steroid hormones and bile acids.

Terpenes are compounds that usually have isoprene-type structures. Isoprene is a five carbon compound in the structure. Many terpenes found in plants are components of pigments such as carotenoids and plant hormones. In animals, some of coenzymes are terpenoids (Pond *et al.,* 2005).

2.1.2.4. Composition of fats and oils

Fats and oils have the same general structure but differ in physical and chemical properties. The major components in both are triacylglycerols, which are esters of glycerol. Triacylglycerol is a molecule of glycerol plus three fatty acids and commonly described as triglyceride. The term 'fat' refers to triacylglycerols (Figure 2.1) that are solid at room temperature, whereas the term 'oil' refers to triacylglycerols that are liquid at room temperature (Tisch, 2006).

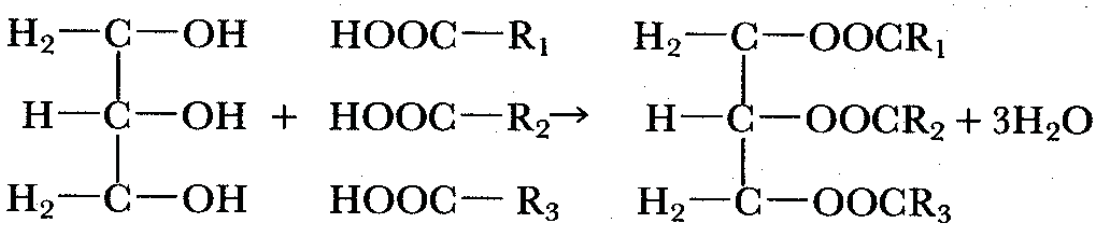

Figure 2.1. Structure of triglycerides

(Source: O'Mahony, 1988)

Different triglycerides differ in the nature and position of fatty acid residues (Enser, 1984). Fatty acid is an organic acid with a hydrocarbon chain connecting with a carboxyl group. If the same fatty acid occupies all three binding sites on the positions of glycerol molecule, the compound is termed a simple triglyceride. For example, if stearic acid is conjugated at all three positions, the compound would be called tristearin. If different fatty acids occupy the three positions, then the compound would be termed a mixed glyceride (Tisch, 2006).

2.1.3. Physical and chemical properties of fats and oils

Properties of fat and oils are determined by the composition of their component fatty acids and, the length and degree of saturation of the carbon chain. The terms 'saturated' and 'unsaturated' refer to the number of hydrogen atoms attached to the hydrocarbon tails of fatty acids as compared to the number of double bonds between carbon atoms in the tail (Figure 2.2).

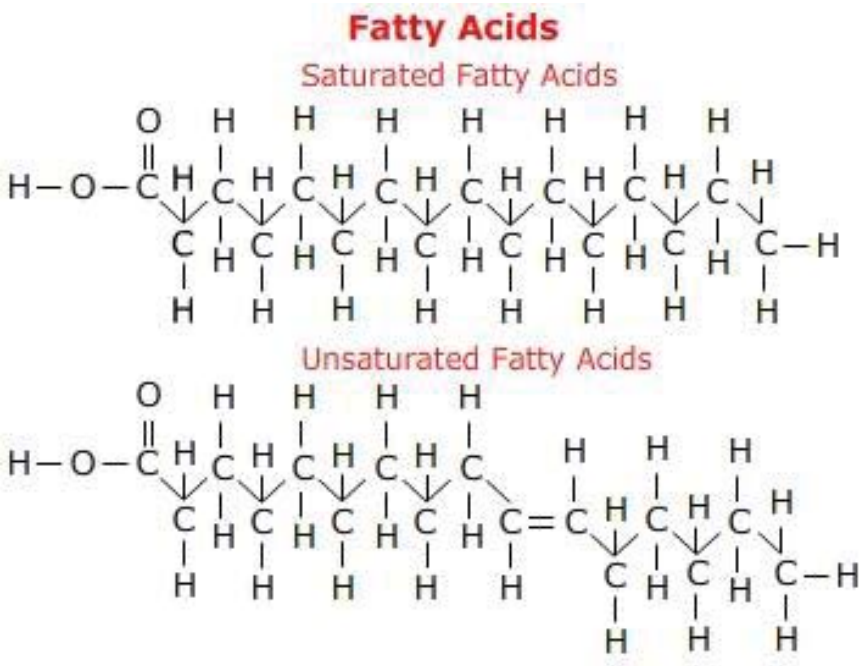

Figure 2.2. Structure of saturated and unsaturated fatty acids

(Source: Anonymous, 2006)

Tisch (2006) gives the following definitions for saturated and unsaturated fatty acids. A saturated fat is "a fat comprising of glycerol and of mostly saturated fatty acids. The fatty acids are 'saturated' with respect to hydrogen. A saturated fatty acid has no double bonds between carbon atoms and is usually solid at room temperature." On the other hand, unsaturated fat is "a fat comprising of glycerol and of mostly unsaturated fatty acids. The fatty acids are 'unsaturated' with respect to hydrogen. An unsaturated fatty acid has at least one double bond between its carbon atoms and is usually liquid at room temperature."

Fatty acids with more than one double bond are frequently referred to as polyunsaturated fatty acids (PUFA). Fatty acid chains differ in length and can be classified into four groups (Tisch, 2006): Short chain fatty acids (SCFA) are fatty acids with aliphatic tails of less than six carbons. Medium chain fatty acids (MCFA) refer to fatty acids with aliphatic tails of six to twelve carbons. Long chain fatty acids (LCFA) are fatty acids which have more than 12 carbon atoms in their tail. The last group is very long chain fatty acids (VLCFA) which refer to fatty acids with aliphatic tails of more than 22 carbon atoms. Most fatty acid chains commonly found in animal tissues are linear and contain an even number of carbons. The most abundant are fatty acids with chains between 14 and 22 carbon atoms. Fatty acids with odd numbers are more common in microorganisms. The names, number of carbons and number of double bonds of fatty acids most common in plant and animal tissues are shown in Table 2.1.

Table 2.1. Fatty acids most common in plant and animal tissues

Chemical name	Trivial name	Number of carbon	Number of double bonds	Abbreviated designation
Butanoic	Butyric	4	0	C4:0
Hexanoic	Caproic	6	0	C6:0
Octanoic	Caprylic	8	0	C8:0
Decanoic	Capric	10	0	C10:0
Dodecanoic	Lauric	12	0	C12:0
Tetradecanoic	Myristic	14	0	C14:0
Pentadecanoic	-	15	0	C15:0
Hexadecanoic	Palmitic	16	0	C16:0
Hexadecenoic	Palmitoleic	16	1	C16:1
Heptadecanoic	Margaric	17	0	C17:0
Octadecanoic	Stearic	18	0	C18:0
Octadecenoic	Oleic	18	1	C18:1
Octadecadienoic	Linoleic	18	2	C18:2
Octadecadienoic	Linolenic	18	3	C18:3
Eicosanoic	Arachidic	20	0	C20:0
Eicosatetraenoic	Arachidonic	20	4	C20:4
Docosenoic	Erucic	22	1	C22:1
Docosapentaenoic	Clupanodonic	22	5	C22:5
Tetracosanoic	Lignoceric	24	0	C24:0

Source: Pond *et al.* (2005).

The melting points of saturated differ from unsaturated fatty acids. An increase in the length of the carbon chain of a saturated fatty acid increases the melting point of the fat and the presence of a double bond decreases the melting point. The geometry

of the double bond also influences the melting point. Trans-isomer fatty acids have higher melting points than cis-isomers (Bettelheim *et al*., 2009). Trans-fats are unsaturated fats with the hydrogen atom on the opposite side of double bond, whereas cis-fats have the hydrogen atom on the same side of double bond. Most naturally occurring unsaturated fatty acids are in the cis-form. Most trans-forms are not found in nature and are formed during processing such as hydrogenation (McDonald *et al*., 2002)

Forages and cereal grains contain 20 to 40 g/kg fat on a dry matter basis but oilseeds such as soybeans and cottonseeds contain about 200 g/kg fat (Tisch, 2006). Fatty acid composition of fats and oils used in the animal feed industry differ widely. Animal fats comprise a high proportion of saturated fatty acids, whereas vegetable oils consist of high proportions of unsaturated fatty acids. The fatty acid composition of commonly used fats and oils is given in Table 2.2.

Table 2.2. Fatty acid composition (g/kg) of commonly used fats and oils

Fatty acid (Carbon atom: Double bonds)	Animal fats					Vegetable oils				
	Tallow (mutton)	Tallow (beef)	Lard	Poultry fat	Herring oil	Palm oil	Soybean oil	Maize oil	Sunflower oil	Rapeseed oil
10:0	0.2	-	0.1	-	-	-	-	-	-	-
12:0	0.3	0.1	0.1	0.1	-	0.1	-	-	-	-
14:0	5.2	3.2	1.5	0.8	6.2	1.0	0.1	0.1	0.1	0.1
14:1	0.3	0.9	-	0.2	-	-	-	-	-	-
15:0	0.8	0.5	0.1	0.1	-	-	-	-	-	-
16:0	23.6	24.3	26.0	25.3	12.7	44.4	10.6	10.9	7.0	3.8
16:1	2.5	3.7	3.3	7.2	7.5	0.2	0.1	0.2	0.1	0.3
17:0	2.0	1.5	0.4	0.1	-	0.1	0.1	0.1	0.1	-
17:1	0.5	0.8	0.2	0.1	-	-	-	-	-	-
18:0	24.5	18.6	13.5	6.5	1.1	4.1	4.0	2.0	4.5	1.8
18:1	33.3	42.6	43.9	37.7	12.9	39.3	23.2	25.4	18.7	18.5
18:2 n-6	4.0	2.6	9.5	20.6	1.1	10.0	53.7	59.6	67.5	14.5
18:3 n-3	1.3	0.7	0.4	0.8	0.7	0.4	7.6	1.2	0.8	11.0
20:0	-	0.2	0.2	0.2	-	0.3	0.3	0.4	0.4	0.7
20:1	-	0.3	0.7	0.3	15.1	-	-	-	0.1	6.6
20:4 n-6	-	-	-	-	0.3	-	-	-	-	-
20:5 n-3	-	-	-	-	6.8	-	-	-	-	-
22:0	-	-	-	-	-	0.1	0.3	0.1	0.7	0.5
22:1	-	-	-	-	22.0	-	-	-	-	41.1
22:6 n-3	-	-	-	-	5.8	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-	-	1.0
U:S ratios[1]	0.74	1.06	1.38	2.02	3.61	0.99	5.49	6.35	6.81	11.64

Sources: Tisch (2006); Sauvant *et al*. (2004).

[1] Unsaturated to saturated fatty acid ratio.

Some fatty acids are termed as essential for poultry because the birds are unable to synthesise or convert one fatty acid to another fatty acid within the same series. The essential fatty acids include linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic

acid (C20:4) (Tisch, 2006). A deficiency of essential fatty acids may result in impairment of growth and immune system function. Symptoms of linoleic acid deficiency in chicks are retarded growth, increased water consumption and reduced resistance to diseases (Balnave, 1970). In male birds, deficiency symptoms also include lower testes weight and delayed development of secondary sexual characteristics. In layers, decreased egg size is the major outcome of essential fatty acids deficiency (Watkins, 1991).

2.1.4. Digestion and absorption of fats

2.1.4.1. Digestion of fats

Two excellent reviews on the digestion of fat in poultry are available (Freeman, 1984; Krogdahl, 1985). Most dietary fats occur in triglyceride form. During digestion, two of the fatty acid molecules from the triglyceride are removed, leaving a monoglyceride (a glycerol molecule with one fatty acid attached). Thus, the digestion of triglycerides produces a monoglyceride and two fatty acids, which are the absorbable units of fat.

The digestive tract of poultry is different from that of mammals. It consists of the beak, the oesophagus which widens into crop, the lower oesophagus, proventriculus, gizzard, duodenum, jejunum and ileum. The gizzard connects with the proventriculus by a narrow and short isthmus, and with the duodenum via a narrow pylorus. Pancreatic and bile ducts open into the distal end of the duodenal loop (Duke, 1986). The gizzard is a unique feature in the digestive tract. The feed entering the gizzard is reduced in particle size mechanically by grinding and mixing vigorously. Gizzard movements are pendular and are followed by contractions of the proventriculus (Smulikowska, 1998). Digesta is shuttled between the proventriculus and gizzard to optimise enzymatic and mechanical digestive action. The liquid digesta is then pushed through the pylorus into the duodenum (Klasing, 1999). In the turkey, intestinal refluxes or digesta movment, occur 2 or 3 times per hour, and involve the entire duodenum and upper ileum (Duke, 1992). In chickens, the reflux process is continuous, enabling penetration of the gizzard by duodenal contents during the contractile period of the gizzard. This motility pattern enables the reverse passage of intestinal digesta containing pancreatic and intestinal juice, and bile into the gizzard and proventriculus (Sklan *et al.*, 1978). The presence of bile salts in the gizzard initiates fat emulsification, which is necessary for the subsequent stages of digestion and absorption in the duodenum and jejunum. The shuttling of digesta between the gizzard and duodenum also increases the time the feed

is exposed to digestive enzymes and favours fat absorption in the upper parts of the small intestine (Smulikowska, 1998).

The digestion of fat is initiated by the entry of digesta into the duodenum. The presence of fat in the duodenum stimulates the secretion of cholecystokinin which in turn regulates secretions of pancreatic enzymes and bile (Krogdahl, 1985). Bile salts are released from gall bladder to emulsify fat in the chyme. The pancreas secretes pancreatic lipase, which acts as a catalyst to hydrolyse fat with the aid of colipase (Erlanson *et al*., 1973). Pancreatic lipase activity can be inhibited by the high concentrations of bile salts. Bosc-Bierne *et al.* (1984) found that the activity of pancreatic lipase in the chicken is inhibited by bile salts, including sodium taurochenodeoxycholate, which is found in large proportions in chicken bile. Lipase activity, however, is restored by colipase.

Colipase, which consists of hydrophobic and hydrophilic amino acids, is a co-factor present in pancreatic secretions. Colipase is essential for the action of lipase on triglyceride emulsions. The function of colipase is to aid in maintaining the lipase in an active configuration at the lipid-water interface. Colipase binds to the surface of lipid droplets and acts as an anchor for lipase allowing pancreatic lipase to digest triglycerides (Borgstrom, 1980).

Triglycerides are hydrolysed by the action of pancreatic lipase (Figure 2.3). The products of this hydrolysis are free fatty acids from the sn-1 and -3 positions, and the sn-2-monoacylglycerol. These products (unsaturated long chain fatty acids, medium chain fatty acids, monoglycerides and phospholipids) spontaneously form mixed micelles with conjugated bile salts (Figure 2.4). Micelles are then transported to the mucosal surface and pass through the brush border membrane (Krogdahl, 1985).

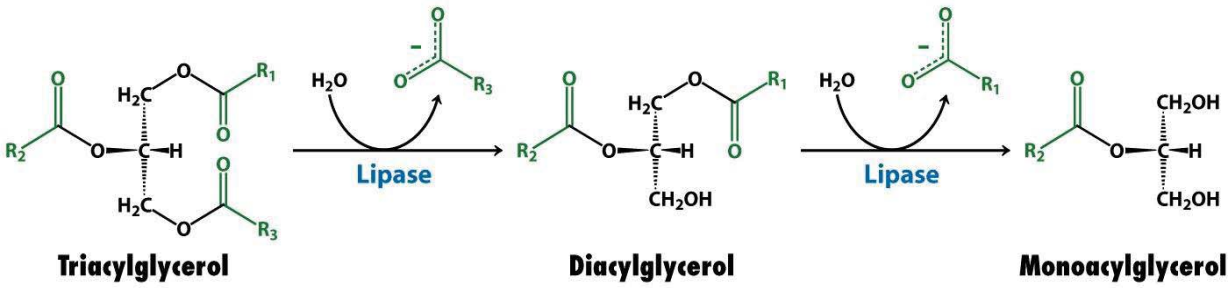

Figure 2.3. Action of pancreatic lipase on triacylglycerol.

Source: Berg *et al.* (2011).

It has been reported that the activity of lipase can be inhibited by free fatty acids. van Kuiken and Behnke (1994) stated that unsaturated fatty acids increased lipase activity, and saturated free fatty acids with eight or ten carbons had little effect, and long chain saturated fatty acids, particularly stearic acid, inhibited lipase activity. These researchers suggested that the fatty acid binding site in lipase may require the fatty acid to bend at a 141° angle, but stearic acid has an angle of 180° which make it difficult to bind with lipase. As a result, unsaturated fatty acids, which have the angle of approximately 141°, have a greater ability to increase lipase compared to long chain saturated fatty acids.

Overall, the digestion of fats is a complex process requiring adequate amounts of bile salts, pancreatic lipase and colipase. Lack of any one of these essentials will impair the digestion and absorption processes.

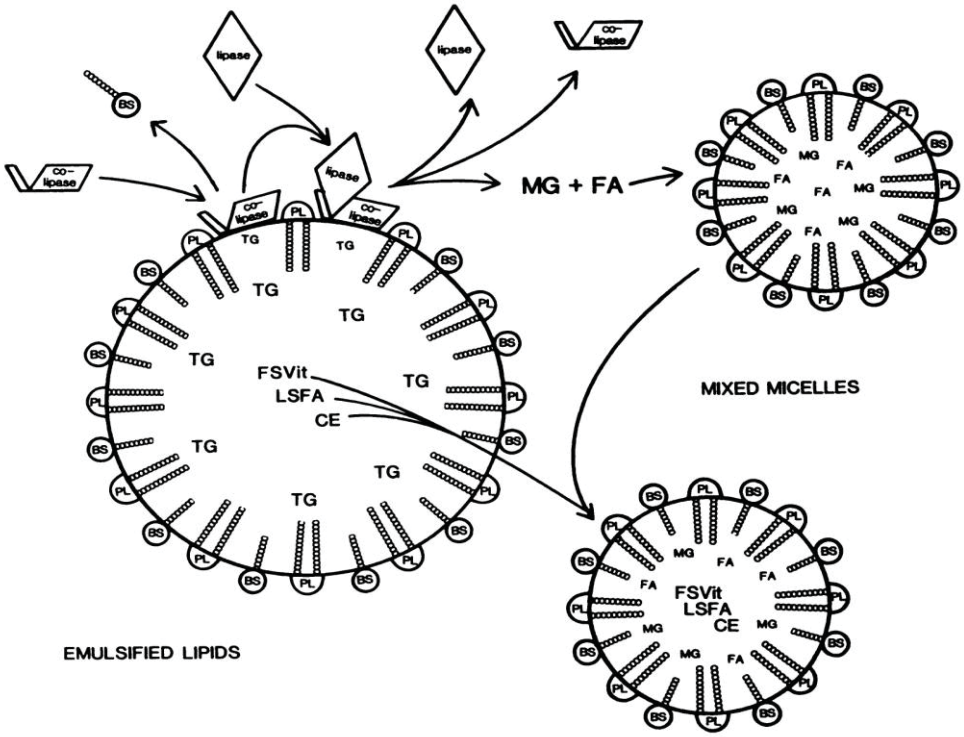

Figure 2.4. Possible sequence of events during intestinal lipolysis in poultry

(BS, bile salt; CE, cholesteryl ester; FA, free fatty acid; FSVit, fat-soluble vitamins; LSFA, long-chain saturated fatty acids; MG, monoglyceride; PL, phospholipid; TG, triglyceride).

Source: Krogdahl (1985).

2.1.4.2. Absorption of fats

It is well documented that the small intestine is the major site of digestion and absorption of fat (Freeman, 1976; Hurwitz *et al*., 1973; Krogdahl, 1985). However, the exact site of fat digestion and absorption remains a controversial issue. Renner (1965) stated that the absorption of fat was negligible in the caeca and the large intestine. Hurwitz *et al*. (1973) reported that the absorption of fat takes place mainly in the jejunum and continues in the ileum. Freeman (1976) suggested that the duodenum is the preparative and absorptive site for fat in birds. The process of fat digestion and absorption is illustrated in Figure 2.5. After the digestion, short-chain fatty acids and monoglycerides need no emulsification and are absorbed passively from the intestinal lumen to mesentery blood vessels via intestinal cells (Pond *et al*., 2005). On the other hand, long chain saturated fatty acids, diglycerides, fat soluble vitamins and cholesteryl esters require solubilisation in the hydrophobic cores of mixed micelles, which are then transported to the intestinal cells (Davenport, 1980). Ockner *et al*. (1972) suggested that the movement of fatty acids through the cytosol of the absorptive cell seems to be influenced by a soluble intracellular protein called fatty acid-binding protein (FABP). In chickens, Katongole and March (1979) found that the concentration of FABP in chickens was highest in the proximal portion of the intestine. This protein has greater affinity for unsaturated than for saturated fatty acids and has almost no affinity for medium or short chain fatty acids (Ockner and Manning, 1974). Fatty acid-binding protein also works as a protective mechanism for the absorptive cell because unbound fatty acids are potentially cytotoxic (Shiau, 1981).

Inside intestinal cells, monoglycerides and long chain fatty acids are rebuilt into new triglycerides. Triglycerides are then combined with free and esterified cholesterol, lipoprotein and phospholipids to form chylomicrons and secreted into lymphatic vessels. Since the lymphatic system of poultry is poorly developed, the chylomicrons are secreted directly to the portal circulation and are termed as portomicrons (Hermier, 1997). Portomicrons are transported to various tissues, particularly to the liver, where lipids are used in the synthesis of various compounds required by the body such as lipoprotein, phospholipids, metabolised as source of energy or stored in tissues as fat deposits (Scott *et al*., 1982).

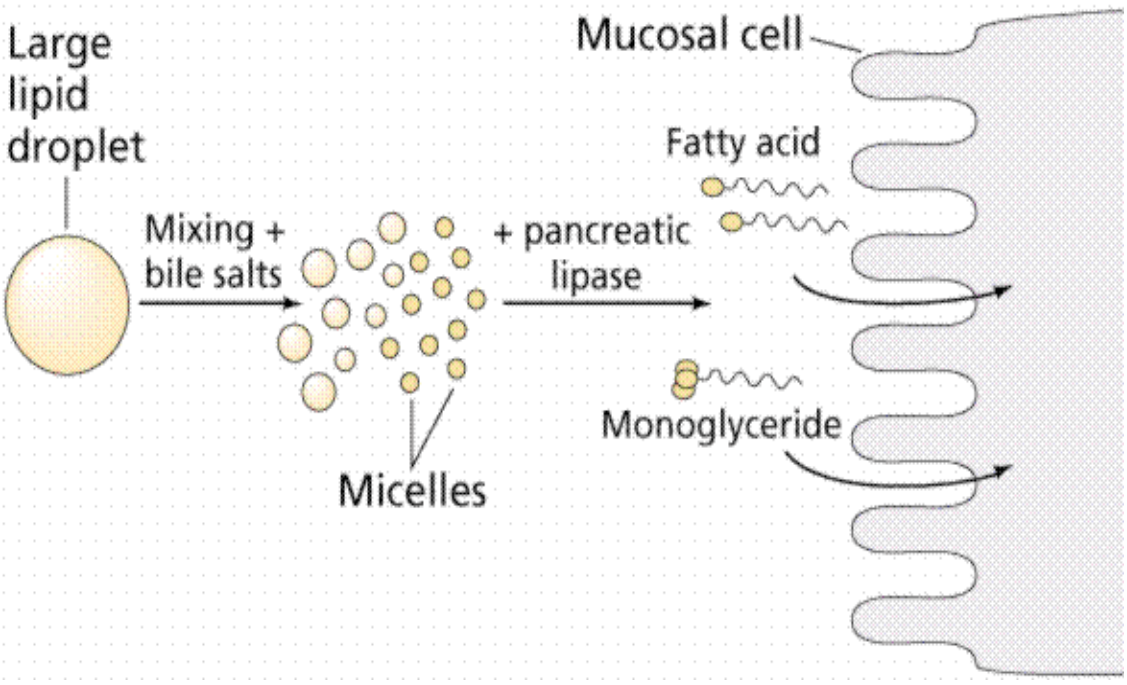

Figure 2.5. Steps in the digestion and absorption of fat

(Source: Vasanthakumari *et al*., 2011).

2.1.5. Endogenous fat secretions

There is a continuous secretion of endogenous lipids into the lumen of the intestinal tract. The primary sources of this endogenous fat are bile and desquamated intestinal epithelial cells (Clement, 1980). These endogenous lipids mix with dietary lipids and, are partially digested and absorbed. The unabsorbed fraction passing beyond the ileum is considered a loss to the animal, and measurement of these inevitable losses is necessary to calculate the true digestibility of lipids. Endogenous losses of fat have been quantified in pigs (Jorgensen *et al*., 1993; Kil *et al*., 2010). The endogenous fatty acid losses in broilers have been reported by Ajuyah *et al*. (1996). These researchers fed broilers with semi-purified diets substituted with either soybean oil or a mixture of animal-plant acid oils and measured fatty acid losses in the ileum and excreta. It was reported that the endogenous fatty acid losses in the ileal digesta and excreta of birds fed the acid oil mixture were higher than those fed soybean oil. The endogenous fatty acid profile contained mainly palmitic, stearic, oleic and linoleic acids.

Bile is the main source of endogenous fat and fatty acids and also plays an important role in the digestion and absorption of fat. Bile works as an emulsifier in the digestion process and formation of mixed micelles which are crucial for the absorption process (Krogdahl, 1985). Bile is synthesised in the hepatocytes of liver. Cholesterol, the precursor of bile, is first hydrolysed with 7-α-hydroxylase to form cholic and chenodeoxycholic acids. These bile acids are then conjugated with taurine or glycine and secreted as bile salts (Figure 2.6).

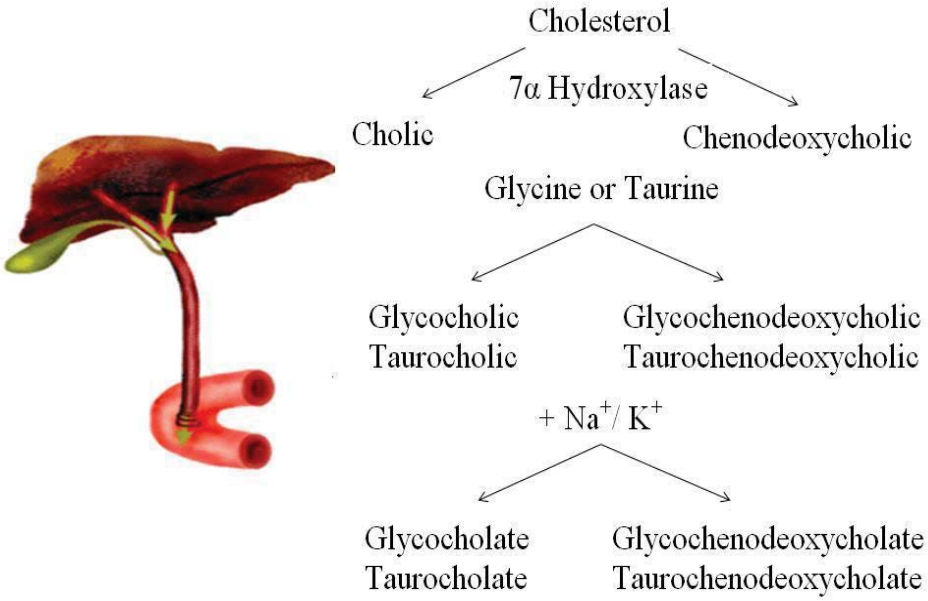

Figure 2.6. Synthesis of bile salts in the liver

Source: Wechsler (2011).

Bile consists of water, bile pigments, bile salts, phospholipids such as lecithin, neutral fats such as cholesterol, glycerides and inorganic ions and, in some mammals, the enzyme alkaline phosphatase (Haslewood, 1978). The composition of bile from humans is presented is Table 2.3. No published data are available on the composition of bile of poultry and other animals, but data on bile acids in bile of chicken and duck have been reported (Table 2.4). Bile from ducks contained high amount of taurochenodeoxycholic acid, followed by cholic acid, whereas that from chickens had high level of glycolithocholic acid, followed by taurocholic acid (Yeh and Hwang, 2001).

Table 2.3. Composition of human bile (g/dl)

Constituent	Range	Mean
Total solids	3.82-28.60	16.21
Total lipids	3.06-24.05	15.06
Bilirubin	0.07-14.13	0.89
Total bile acids	0.71-9.01	5.87
Phospholipids	0.55-6.75	3.40
Cholesterol	0.14-2.18	1.08
Free fatty acids	0.00-0.38	0.05
Monoglycerides	0.00-7.71	0.49
Diglycerides	0.00-0.09	0.02
Ca^{2+}	0.01-0.05	0.03
Na^{+}	0.36-0.62	0.47
K^{+}	0.03-0.09	0.06

Source: Haslewood (1978).

Table 2.4. Level of bile acids in the bile of the chicken and duck (mg/g)

Bile acid	Chicken	Duck
Cholic acid	9.6±0.5	45.2±2.3
Chenodeoxycholic acid	25.2±2.2	28.2±1.6
Ursodeoxycholic acid	n.d.	43.5±2.1
Deoxycholic acid	n.d.	31.6±1.9
Lithocholic acid	68.7±2.1	37.5±2.1
Taurocholic acid	152.6±3.1	16.8±1.5
Taurochenodeoxycholic acid	n.d.	97.5±3.4
Taurolithocholic acid	35.9±0.6	n.d.
Glycolithocholic acid	228.4±1.6	n.d.

n.d. = not detected.
Source: Yeh and Hwang, (2001).

2.1.6. Apparent metabolisable energy of fats

Because of its practical relevance, the AME of different fat sources have been determined in a number of studies (Table 2.5). As can be seen, the reported values are highly variable depending on the assay methodology and age of birds. In particular, the level of fat in assay diets is an important factor causing this variability, as shown by Wiseman *et al*. (1986). These researchers fed broilers a commercial fat blend substituted into a semi synthetic fat–free basal diet and a practical basal diet at concentrations of 10 g/kg to 100 g/kg in 10 g/kg increments. It was reported that increasing additions of fat decreased the AME value of fat.

Table 2.5. Apparent metabolisable energy (AME) value of various fat sources

	MJ/kg	Level of fat, g/kg [a]	Age of bird	Reference
Beef tallows				
Beef tallow	29.09-35.08	30-100	22-24 days	Guirguis (1976)
Beef tallow	29.35	70	13-17 days	Lessire *et al*.(1982)
Beef tallow	30.60	70	40-44 days	Lessire *et al*.(1982)
Beef tallow	27.75-39.13	20-60	16-19 days	Wiseman *et al*. (1986)
Beef tallow	26.02	90	13-24 days	Huyghebaert *et a*l.(1988)
Tallow	31.31	40	14-18 days	Blanch *et al*. (1995)
Tallow	42.4	40	Adult rooster	Blanch *et al*. (1996)
Animal-vegetable blends				
Beef tallow-crude soy oil	32.81	90	13-24 days	Huyghebaert *et a*l.(1988)
Tallow-refined soy oil	40.04	-	-	NRC. (1994)
Animal/vegetable blends	43.26	60	9-10 days	Pesti *et al*. (2002)
Animal/vegetable blends	41.29	60	39-40 days	Pesti *et al*. (2002)
Palm oil				
Palm oil	24.56	90	13-24 days	Huyghebaert *et a*l.(1988)
Palm oil	43.60	40	Adult rooster	Blanch *et al*. (1996)
Palm oil	27.07	60	9-10 days	Pesti *et al*. (2002)
Palm oil	21.88	60	39-40 days	Pesti *et al*. (2002)
Poultry fat				
Poultry fat	38.02	70	13-17 days	Lessire *et al*.(1982)
Poultry fat	37.35	70	40-44 days	Lessire *et al*.(1982)
Soybean oil				
Soybean oil	40.53-42.72	20-60	16-19 days	Wiseman *et al*. (1986)
Soybean oil	35.69	90	13-24 days	Huyghebaert *et a*l.(1988)
Soybean oil	44.10	40	Adult rooster	Blanch *et al*. (1996)
Soybean oil	46.48	60	9-10 days	Pesti *et al*. (2002)
Soybean oil	40.00	60	39-40 days	Pesti *et al*. (2002)

[a] Level of fats used in the test diets.

Most of the available data on the AME of fats and oils for poultry are 20 to 40 years old and from studies conducted with slow-growing strains of broilers (Young, 1961; Lessire *et al*., 1982; Wiseman *et al*., 1986; Huyghebaert *et al*., 1988; NRC, 1994 and Pesti *et al*., 2002). It is logical to expect that the advances in the genetics of broilers may have improved the ability of birds to digest and utilise nutrients and evaluations are warranted with current fast-growing strains.

2.2. Factors affecting the digestibility and AME of fats

2.2.1. Introduction

The digestibility and AME of fats are influenced by a number of factors (Krogdahl, 1985; Leeson, 1993; Wiseman, 1990; Baiao and Lara, 2005) and these can be categorised into two major factors, namely, bird- and diet-related factors. Bird-related factors include age, strain, species and gender, whereas diet-related factors include composition and quality of fats, dietary calcium level and cereal type. The effects of these factors are reviewed below.

2.2.2. Bird-related factors

2.2.2.1. Age

In newly hatched chicks, the ability to digest and absorb dietary fat is poorly developed. The secretion of bile appears to be first limiting and lipase secretion or other physiological factors may be next-limiting (Krogdahl, 1985). Noy and Sklan (1995) reported that the secretion of lipase, trypsin and amylase into the duodenum increased 20 to 100 folds between days 4 and 21 post hatch, but lipase activity was found to increase slower than other enzymes. In addition, the synthesis of FABP has been reported to be insufficient in very young birds, but increased after week 4 of life (Katongole and March, 1980). In general, the data indicate that the ability to digest and absorb fat in birds develops rapidly after the first few days of life and increase with advancing age (Renner and Hill, 1960; Carew *et al*., 1972).

Renner and Hill (1960) investigated the utilisation of maize oil, lard and tallow by crossbred chickens at different ages and found that the ability to utilise tallow improved with age. The absorbability of tallow improved from 70% at 2 weeks of age to 82% at 8 weeks of age. However, the utilisation of maize oil and lard showed a peak at 6 weeks of age and decline two weeks later. The absorbability of maize oil was 94% at 2 weeks, increased to 98% at 4 and 6 weeks and then decreased to 95% at 8 weeks of age. The absorbability of lard improved from 90% at 2 weeks to 95% at 6 weeks and then decreased to 92% at 8 weeks of age.

Carew *et al*. (1972) determined the absorption of maize oil and beef tallow during the periods of 2 to 7 and 8 to 15 days of age. The ability to absorb maize oil and tallow was found to be low during the first week of life. The average absorbability of

maize oil increased from 84 to 95%, while that of tallow increased from 40 to 79% between week 1 and 2.

The age of birds not only affects the digestion of fat but also the AME. Wiseman and Salvador (1989) determined the AME of fats in broilers fed diets containing vegetable oil and tallow at 25, 50, 75, 100 and 125 g/kg at 2, 4, 6 and 8 weeks of age. The AME of both fat sources increased between 2 to 4 weeks of age, with no further increase thereafter. The increment in AME was greater in tallow than vegetable oil.

In a subsequent study, Wiseman (1990) determined the AME of two dry emulsified fats (fat blended with bone solids, homogenised and then emulsified by spray drying). A maize-wheat-soy basal diet was supplemented with either emulsified fat A (blend of soy oil and tallow) or emulsified fat B (tallow) at 25, 50, 75 100 and 125 g/kg. Diets based on fat A were fed to broilers aged 2, 4 and 6 weeks, while those based on fat B was fed to broilers of 3, 5 and 7 weeks of age. The results showed that the AME of both fats was higher in older birds and that the AME were highest at the lowest rate of inclusion (25 g/kg). The lower AME in young birds were attributed to poor emulsification rather than to a deficiency in lipase activity.

2.2.2.2. Gender and strain

Differences in dietary nutrient requirements between different strains and gender of birds have been recognised (Sibbald and Slinger, 1963; Zelenka, 1997; Yaghobfar, 2001). It follows that similar differences may exist in the efficiency of digestion of nutrients.

Gender effects on the AME of feedstuffs for chickens were noted by Guirguis (1975; 1976), who reported significant gender effects on the AME of oats, tallow and fish meal, with AME values being higher in females. Yaghobfar (2001) studied the differences in energy utilisation of adult hens and roosters of a layer (Rhode Island Red) and a broiler (Cornish) line. It was found that gender had no significant effect on the AME content of maize. Zelenka (1997) investigated the effect of gender on nitrogen corrected AME (AME_n) of two diets with different energy: protein ratios in broiler chickens from 12 to 56 days of age. No differences were observed between the males and females.

The effect of the strain of bird on energy utilisation was investigated by Sibbald and Slinger (1963). These researchers reported that White Leghorn chicks utilised more energy per unit of feed compared with White Rock chicks. The observed differences

were attributed to genetic variations in the ability to digest and absorb nutrients. Katongole and March (1980) determined the utilisation of tallow and maize oil in different genetic strains between weeks 3 and 11 of age. It was found that the absorbability of tallow and maize oil in New Hampshire chicks was higher than broiler-type or White Leghorn chicks from 3 to 5 weeks of age. After six weeks, there were no further increases in the efficiency of fat utilisation and there were no differences among the strains.

In contrast, others have observed no differences in energy utilisation or fat digestibility between different strains of chickens. Young *et al.* (1963) fed White Plymouth Rock chicks and crossbred of Rhode Island Red and Barred Plymouth Rock chicks with diets containing 150 g/kg of either lard fatty acids or tallow fatty acids and reported no difference between the strains on fat digestibility.

2.2.3. Diet-related factors

2.2.3.1. Degree of saturation of fatty acids

Energy-yielding potential of a fat is influenced by its chemical structure (Freeman, 1984; Krogdahl, 1985). Carbon chain length, saturation degree and the position of double bonds of fatty acids all have an impact on the digestion and absorption of fats (Renner and Hill, 1961a; Baiao and Lara, 2005).

It is now recognised that the degree of saturation of fatty acids has a major influence on the AME of fats (Wiseman *et al.*, 1991). Animal fats such as tallow containing high amount of saturated fatty acids, especially palmitic and stearic acids, are poorly digested and absorbed by poultry (Scott *et al*., 1982). Saturated fatty acids need bile salts to emulsify them and form micelles prior to digestion. Garrett and Young (1975) reported that the solubilisation and absorption of saturated fatty acids are more negatively affected in the absence of bile salts than that of unsaturated fatty acids. Both palmitic and stearic acids are non-polar, and cannot form mixed micelles spontaneously, but only in the presence of micelles formed from unsaturated fatty acids and conjugated bile salts (Smulikowska and Mieczkowaka, 1996). As a result, oils from plants such as soybean oil which contains high amount of unsaturated fatty acids are more digested and absorbed than animal fats (Sklan, 1979). Ward and Marquardt (1983) determined the effects of chain length and degree of saturation on fat absorption in two experiments. In Experiment 1, White Leghorn chicks were fed either a wheat- or rye-based diet containing

50 g/kg of different pure glycerides (tristearin (C18:0), triolein (C18:1) or trilinolein (C18:2)). It was found that the absorption of fat in birds fed wheat- or rye-based diets with saturated fat (tristearin) was lower compared to those fed unsaturated fats (triolein and trilinolein). In Experiment 2, White Leghorn chicks were fed wheat- or rye-based diets containing 50 g/kg of different pure saturated glycerides (tricaprylin (C8:0), trilaurin (C12:0), tripalmitin (C16:0) and tristearin (C18:0)). The absorption of fat in birds fed diets with the short chain fatty acid (tricaprylin) was higher than the other fatty acids. These data suggested that longer the chain length, lower was the absorption of fat.

There is some evidence suggesting that blending of saturated and unsaturated fats may improve fat absorption, and that there may be a synergistic response with such blends (Sibbald *et al*., 1962; Lall and Slinger, 1973; Wiseman and Lessire, 1987). This phenomenon is particularly important for the absorption of long chain saturated fatty acids such as palmitic and stearic acids. Sibbald (1978) studied the effect of blending soy oil and tallow on the TME. Tallow was blended with soy oil at ratios of 100:0, 99:1, 98:2, 96:4, 92:8, 84:16, 68:32, 36:64 and 0:100. The TME of tallow in the ratios 100:0 and 99:1 were similar (33.13 and 33.09 MJ/kg). On the other hand, ratios of 98:2, 96:4, 92:8, 84:16, 68:32, 36:64 and 0:100 resulted in TME values of 33.55, 33.89, 34.43 , 34.97 , 35.31 and 37.36 MJ/kg, respectively.

Wiseman and Lessire (1987) studied the effect of the mixtures of tallow and rapeseed oil at five ratios (100:0, 95:5, 90:10, 80:20 and 0:100), supplemented at 40, 80 and 120 g/kg of a basal diet in 14-day broilers and adult roosters. Age of bird affected the AMEn, with values being higher in the roosters. The ratio of unsaturated to saturated fats also influenced the AMEn, with an increasing proportion of unsaturated fat (rapeseed oil) associated with higher AMEn.

2.2.3.2. The position of fatty acid

Another factor affecting the digestibility of fat is the positional distribution of fatty acids in the glycerol molecule. During digestion, triglycerides are hydrolysed by the action of pancreatic lipase at sn-1 and -3 positions. End products of this process are two free fatty acids and sn-2-monoacylglycerol. The reported differences in fat digestibility between animal fats and vegetable oils may be attributed, in part, to the fact that animal fats consists of high proportions of saturated fatty acids in 1 and 3 positions (Meng *et*

al., 2004). Sibbald and Kramer (1977) reported that 73 to 81% of palmitic and stearic acids in beef tallow are located at 1- and 3- positions.

2.2.3.3. Quality of fats

In the feed industry, the quality of fat is determined primarily based on the level of impurities, free fatty acids and the rancidity of fat. Impurities in fat are usually measured as moisture, impurities and unsaponifiables (MIU) (Leeson, 1993). The maximum acceptable value of moisture is 10 g/kg fat. Impurities are determined as the percentage insoluble fraction of the fat in petroleum ether and should be lower than 10 g/kg fat. Unsaponifiables, such as sterol, pigments and hydrocarbon, are substrates that are not saponified after treatment with caustic soda. The maximum accepted level of unsaponifiables is 10 g/kg fat (Baiao and Lara, 2005). Non-elutable material is another quality measure of fat (Wiseman, 1999) and refers to the total amount of non-nutritional materials in fats including moisture, impurities, oxidised, unsaponifiable and glycerol which are determined by gas liquid chromatography (Edmunds, 1990). Quality criteria for fats used in feed industry is presented in Table 2.6.

Table 2.6. Quality criteria for fats and oils

Parameters	g/kg
Monomeric fatty acids	Minimum 920
Moisture and impurities	Maximum 10
Free fatty acids	Maximum 500
Non-elutable material	Maximum 80
Oxidised fatty acids	Maximum 20

Source: Ross (2009).

Level of free fatty acids (FFA) is often used by the feed industry as an indication of fat quality. Fatty acids in fats are normally bound to triglycerides. When not attached to any molecules, they are referred to as ‘free fatty acids’. It has been reported that digestibility and AME of fats are depressed with increasing concentrations of FFA (Freeman, 1976; Sklan 1979).

Wiseman and Salvador (1991) investigated the effect of FFA on the AME of 2 and 8 week old broilers fed diets containing tallow (TO) and tallow acid oil (TAO) blends (TO:TAO; 0.75:0.25, 0.50:0.50, 0.25:0.75), palm oil (PO) and palm acid oil (PAO) blends (PO:PAO; 0.75:0.25, 0.50:0.50, 0.25:0.75) and, soybean oil (SO) and soybean acid oil (SAO) blends (SO:SAO; 0.75:0.25, 0.50:0.50, 0.25:0.75). It was observed that increasing FFA concentrations decreased the AME in both age groups.

Wiseman and Blanch (1994) fed broilers aged 12 and 52 days with a blend of coconut oil and palm kernel oil (CP; FFA content, 13.8 g/kg) and coconut oil and palm kernel acid oil (CPAO; FFA content, 839 g/kg). The two blends (CP: CPAO) were mixed in the following proportions: 75:25, 50:50 and 25:75. Five oils (coconut oil, palm kernel oil and the three mixtures) were included in a basal diet at 40, 80 and 120 g/kg. It was found that the AME of fats in young broilers was lower than that in older birds. The fat with the lowest FFA had the highest AME in both young and older birds (33.1 and 34.6 MJ/kg DM, respectively), while the fat containing the highest FFA had the lowest AME in both age groups (25.8 and 33.0 MJ/kg DM, respectively).

Vila and Esteve-Garcia (1996b) determined the effect of degree of saturation of FFA substituted for either tallow or sunflower oil on fat digestibility in broilers. It was found that the substitution of saturated FFA for tallow or sunflower oil markedly depressed fat digestibility, whereas substitution of unsaturated FFA for tallow and sunflower oil had no effect.

Oxidative rancidity is another major cause of loss of quality of the fat. Oxidative rancidity is a degradation process that occurs in unsaturated fatty acids due to the oxidation of the double bond of triglycerides. It affects the odour, colour and flavour, and decreases the nutritive value of the fat (Baiao and Lara, 2005). Several methods are used to evaluate oxidative rancidity in fat and oils including peroxide value and active oxygen method (AOM). Peroxide value is the widely used indicator of fat oxidation and expressed as meq of peroxide per kg fat. Animal fats develop a slight rancid odour when peroxide levels reach 20 meq/kg, whereas the rancid odour in vegetable oils start developing at around 80 meq/kg (Leeson and Summers, 2005). Vegetable oils which contain high amount of unsaturated fatty acids tend to be more prone to oxidation, particularly when stored at high temperatures. Oxidation of fats has negative effects on poultry performance and the quality of meat (Jensen *et al*., 1997). Cabel *et al.* (1988) fed broilers with diets containing oxidised poultry fat at levels of 0, 50, 100 and 175 meq/ kg

fat. It was found that body weights at 21 and 42 days of age were lowest in birds fed diets containing 175 meq/kg. Similarly, Tavarez *et al*. (2011) reported that broilers fed diets supplemented with oxidised soy oil at 180 meq/ kg fat showed lower body weight and feed intake compared to those fed diets with 0 meq/kg fat. It was concluded that the oxidised oil contained substrates such as aldehydes, ketones and esters, which may have lead to the development of rancid flavours and odours, and reduced the palatability of the feed.

Iodine value is another method used to measure fat stability. Iodine value measures the level of unsaturation of fats and oils, and is expressed as grams of iodine absorbed per 100 grams of sample. Unsaturated fatty acids have higher iodine values compared to saturated fatty acids (O'Brien, 2009*)*. The range of iodine value for commonly used fats and oils is presented in Table 2.7.

Table 2.7. The range of iodine values for common fats and oils

	Iodine value (g iodine/100 g fat or oils)
Soybean oil	123-139
Corn oil	118-128
Sunflower oil	125-136
Palm oil	46-56
Coconut oil	7.5-10.5
Lard	45-70
Tallow	40-49
Fish oil (Menhaden oil)	150-165

Source: O'Brien (2009).

2.2.3.4. Interaction between cereal type and fat source on fat digestion

Available data indicate the existence of significant interaction between cereal type and fat source in terms of fat digestion. Antoniou *et al.* (1980) reported that the performance and fat digestibility were markedly depressed in broilers fed rye-based diets containing tallow, but these effects were less significant in diets containing soybean oil. Danicke *et al*. (1997b) similarly found that broilers fed a rye-based diet supplemented with soybean

oil were heavier compared to those fed the diet supplemented with tallow. Ward and Marquardt (1983) reported that the combination of tallow and rye depressed the digestibility of fat more than the combination of tallow and wheat. Viscous cereals such as wheat, barley and rye contain high concentrations of non-starch polysaccharides (NSP) which exhibit anti-nutritive activity in poultry diets. Non-starch polysaccharides such as arabinoxylans and β-glucans increase the digesta viscosity and depress the digestibility of nutrients by impeding the diffusion of digestive enzymes and substrates, and contact between nutrients and absorption sites on the intestinal mucosa (Annison, 1993; Choct, 1997).

The digestion of fat is affected more than that of other nutrients by NSP and the digestion of saturated fatty acids is affected more than that of unsaturated fatty acids (Danicke, 2001). Poor digestion of saturated fatty acids was attributed to the increased intestinal viscosity caused by the NSP. High digesta viscosity can slow the gut motility and impair the diffusion and convective transport of droplets of emulsified fat, fatty acids, mixed micelles, bile salts and lipase within intestinal contents (Smulikowska, 1998). Furthermore, soluble NSP may stimulate microbial growth in the small intestine. Annison and Choct (1991) stated that when diets with high level of NSP are ingested, NSP are solubilised in the upper gut of chickens. In the hind gut, solubilised NSP can be a fermentable carbohydrate source aiding the proliferation of anaerobe bacteria. Increasing bacterial population in the intestinal tract may have a systemic effect on gut secretions and intestinal morphology. Increase of bacterial activity may also increase the deconjugation of bile acids. Deconjugated bile cannot be reabsorbed and will be excreted. Poor digestion of fat, therefore, may also occur due to the reduced recycling and the resultant low concentration of bile salts in birds fed diets containing high levels of NSP (Smits and Annison, 1996).

Saturated fatty acids are non-polar and cannot form mixed micelles spontaneously, but can be emulsified by micelles formed from unsaturated fatty acids and conjugated bile salts (Wiseman, 1990). Therefore, the digestion and absorption of saturated fatty acids are impaired by the indirect effects of NSP on bile acid deconjugation and micelle formation.

2.2.3.5. Dietary calcium levels

End-products of the digestion of triglyceride are monoglycerides and free fatty acids. These free fatty acids then have the opportunity of reacting with other nutrients, particularly they can form soluble or insoluble soaps. If insoluble soaps are formed, there is the possibility that both the fatty acid and the mineral will be unavailable to the bird (Leeson and Summers, 2005). It has been suggested that phytate, as Ca-phytate, may be involved in the formation of insoluble metallic soaps in the gut (Ravindran *et al*., 2000).

Type of fatty acid and dietary level of calcium are reported to impact calcium metabolism and soap formation in broilers. Atteh and Leeson (1983) fed broilers a control diet without supplemental fatty acids and diets supplemented with 80 g/kg of mixture of linoleic and oleic acids (2.5:1), oleic acid, palmitic acid, and stearic acid at two levels of calcium (8 and 12 g/kg). Increasing calcium concentrations was found to reduce the retention of fat only in birds fed the diet supplemented with palmitic acid. Birds fed diets with palmitic and stearic acids had higher concentrations of faecal soaps than those fed diets supplemented with oleic acid and the mixture of linoleic and oleic acids. Therefore, calcium retention was affected by the type of fatty acid, with birds fed diets with palmitic and stearic acids having lower retention than those fed diets with oleic acid and mixture of linoleic and oleic acids.

Atteh and Leeson (1984) examined the effect of saturated or unsaturated fatty acids (80 g/kg of mixture of oleic and palmitic acids, oleic acid and palmitic acid) and calcium level (8, 12 and 16 g/kg) on fat retention and faecal soap formation in broilers. Significant interactions were observed between fatty acids and calcium concentration for fat retention and faecal soap formation. Birds fed diets containing 16 g/kg calcium showed a significant decrease in fat retention. Increasing the level of calcium above 8 g/kg resulted in higher faecal soap contents in diets supplemented with palmitic acid. Soap formation in birds fed blend of oleic and palmitic acids was increased in diets containing 16 g/kg calcium. It was also found that birds fed the diet with palmitic acid excreted more faecal soap than those fed the mixture of oleic and palmitic acids and oleic acid at all three calcium concentrations. These data showed that the potential for soap formation is greater in saturated fatty acids compared to unsaturated fatty acids such as oleic acid (Atteh and Leeson, 1983; 1984). Higher concentrations of calcium in

diets containing animal fats may further increase metallic soap formation and lower the energy derived from lipids (Leeson, 1993).

Smith *et al.* (2003) evaluated the effect of dietary calcium level (9 and 15 g/kg) and fat source (corn oil, animal fat, fish oil and dry blend of animal and vegetable fat) on the performance, mineral utilisation of broilers at different room temperatures (23.9 and 23.9 to 35 °C). No interaction between dietary calcium and fat source was observed for any of the parameters. High level of calcium did not affect the performance and the concentration of calcium in tibia of broiler; however, birds fed diets with 15 g/kg calcium had higher plasma calcium than those fed 9 g/kg calcium. Fat source influenced the weight gain and feed efficiency. Birds fed animal fat had the highest weight gain and feed efficiency. Fat source also affected mineral concentration in tibia with birds fed diets supplemented with fish oil having the lowest tibia calcium and zinc concentrations.

2.3. Strategies to improve fat digestion

2.3.1. Introduction

The ability to digest and absorb fat is poorly developed in young birds, especially during the first few weeks of life (Carew *et al*., 1972; Wiseman and Salvador, 1991). It is not clear whether this low capacity to digest fats during week 1 is due to a deficiency in lipase, bile or both. The digestion of fats is a relatively complex process that requires sufficient quantities of bile salts, which are essential for emulsification, and enzyme lipase. It follows that any strategy to improve fat digestion must consider the supplementation of both the enzyme and emulsifiers. Another commonly employed strategy to improve fat digestion is to consider the use of fats containing high proportions of unsaturated fatty acids or blends of fats.

2.3.2. Supplemental enzymes

2.3.2.1. Lipase

Limited studies have evaluated the effect of lipase addition on the growth performance and fat utilisation in poultry. Lipases tested in these evaluations came from two sources; crude porcine lipase (Polin *et al.*, 1980; Kermanshahi *et al*., 1998) and microorganisms such as *Rhizopus arrhizus*, *Aspergillus niger* and *Pseudomonas* spp. (Kermanshahi *et al*., 1998). Mammalian lipases normally hydrolyse 1- and 3-positions of the triglyceride,

but microbial lipases have a broad range of selectivity including specificity to hydrolyse 1- and 3- positions (Kermanshahi, 1998).

Polin *et al.* (1980) fed White Leghorn chicks with diets containing 40 g/kg tallow supplemented three levels of crude porcine lipase (0, 0.1, and 1 g/kg) and two levels of cholic acid (0 and 0.4 g/kg). Fat absorption was higher in birds fed diet supplemented with 1 g/kg of lipase compared to those fed 0 and 0.1 g/kg. However, the addition of cholic acid alone to the diet resulted in better fat absorption (82.6%) than the combination of cholic acid and 1 g/kg lipase (82.3%).

Al-Marzooqi and Leeson (1999) evaluated the addition of lipase and crude porcine pancreas on fat utilisation in young broilers in three experiments. In Experiment 1, two levels of animal-vegetable fat blend (40 and 80 g/kg) and three enzyme treatments (none, 7.14 g/kg crude pancreatic enzyme and 7.14 g/kg pancreatin) were supplemented to a maize-based diet to study the effect of enzyme on the performance, fat digestibility and soap formation. Pancreatic and pancreatin enzymes improved fat digestibility and AMEn compared to the control without enzyme. Excreta soap formation in birds fed diets without enzyme was higher than those fed diets with enzymes. However, enzyme supplementation reduced feed intake and weight gain of birds resulting in higher feed per gain. In Experiment 2, diets containing 40 g/kg animal-vegetable oil blend were supplemented with pancreatic enzyme at 0, 2.14, 4.29, 6.43, 8.57 and 10.071 g/kg and effects on the performance, digestibility of fat and soap formation were determined. It was found that increasing levels of enzyme increased fat digestibility and AMEn, and lowered soap formation. However, increasing levels of enzyme reduced feed intake and weight gain, and increased feed per gain. In Experiment 3, broilers were fed diets containing 40 g/kg animal-vegetable oil blend supplemented with ground dried crude porcine pancreas at 0, 3.21, 5.35, 7.50, 9.64, 11.78 and 13.92 g/kg. No significant effect was observed on the performance of broilers. It was concluded that lipase improved fat digestion in broilers and speculated that the contamination with cholecystokinin may be responsible for the poor feed intake.

In a follow-up series of studies, Al-Marzooqi and Leeson (2000) determined the effect of crude porcine pancreatic enzyme supplementation on gut morphology, gastric motility and performance of broilers in three experiments. In Experiment 1, lipase was used at graded levels of 0, 2.14, 4.29, 6.43, 8.57, and 10.71 g/kg to examine the effect on gut structure. Experiment 2 was designed to investigate the effect of lipase at 0, 2.68,

5.36, 8.04, 10.71, and 13.39g/kg on gastric motility. No effect of enzyme was found on gut morphology and gastric motility. In Experiment 3, the enzyme was supplemented at four levels (0, 3.75, 7.50, or 11.25 g/kg) in the starter diet. Starter diets were fed from day 1 to 21 and then replaced with grower diets containing no enzyme. During the starter period, there was a linear decrease in feed intake and weight gain, but no differences in feed intake and weight gain were observed among treatment groups from 21 to 42 days of age.

2.3.2.2. Glycanases

It is known that fat digestion suffers the most pronounced impairment in diets based on viscous cereals (Ward and Marquardt, 1983; Choct and Annison, 1992). Viscous cereals such as wheat, barley and rye contain NSP, which increase intestinal digesta viscosity and adversely affect nutrient digestion and bird performance (Choct and Annison, 1992). Diets based on these cereals are routinely supplemented with exogenous glycanases (glucanase and xylanase) to overcome the problem of high digesta viscosity and, to improve nutrient metabolisability and bird performance.

Danicke *et al.* (1997b) studied the interaction between rye-based diets containing soybean oil or tallow (100 g/kg), supplemented without and with xylanase. They reported that feed intake and live weight of broilers fed rye-based diets with soybean oil were higher than those fed diets supplemented with tallow. It was also shown that xylanase supplementation to tallow diets improved feed per gain to the same level as of birds fed soybean oil without enzyme (1.573 and 1.464 kg/kg, respectively). Enzyme addition decreased the viscosity of ileal digesta and improved fat digestibility (70.8%) compared to birds fed the diet without enzyme (52.6%).

Langhout *et al*. (1997) investigated the effect of endo-xylanase supplementation (0 or 0.1 g/kg) and fat source (65 g/kg of soybean oil or blend of 60 g/kg animal fat and 5 g/kg soybean oil) on fat digestion in broilers fed wheat- and rye-based diets. They reported that endo-xylanase had significant effect on fat digestibility and the effect of enzyme was more pronounced in birds fed diets supplemented with the blend than those fed diets supplemented with soybean oil. Endo-xylanase improved digestibility of the blend from 0.605 to 0.699, whereas digestibility of soybean oil diet increased from 0.783 to 0.802.

Overall, these results demonstrated that the addition of enzymes to broiler diets based on rye, wheat or barley and containing animal fat resulted in greater improvements in fat digestibility.

2.3.3. Emulsifiers

Fats are insoluble in water and do not solubilise in the gastrointestinal tract. They must be emulsified before they can be digested by lipolytic enzymes. The ease of emulsification depends on the characteristics of the fat such as chain length, position of fatty acids on triglycerides and fat saturation (Gu and Li, 2003). Emulsifiers therefore have the potential to improve the utilisation of lipids, especially of animal fats. Emulsifiers may also play a role in overcoming the inadequacies of naturally low bile production and recirculation in young birds.

Emulsifiers which are normally used in feed industry can be categorised as two groups, namely, natural and synthetic emulsifiers. Natural emulsifiers include those produced in animal body such as bile and phospholipids, and those from foods such as soy lecithin (Soares and Lopez-Bote, 2002). Synthetic emulsifiers are modified emulsifiers such as lysolecithin or lysophosphatidylcholine (Zhang *et al*., 2011).

2.3.3.1. Bile acid and salts

Bile is the excretory fluid of the liver which plays an important role in fat digestion. As noted earlier, bile is formed in hepatocytes and then transported for storage in the gallbladder (Koeppen and Stanton, 2008). Bile contains bile pigments, bile salts, cholesterol, electrolytes and some proteins (Krogdahl, 1985). Bile acids act as emulsifiers by reducing the tension of the oil-water interface. Bile activates pancreatic lipase as well as prevents denaturation of this enzyme when it leaves the surface of emulsified fat droplets. Bile is an effective natural emulsifier because bile salts are flat amphiphilic molecules with a hydrophobic surface on one side that interacts with the oil phase of the emulsion and a hydrophilic surface on the other that interacts with water (Chen *et al*., 1975).

Secretion of bile is thought to be limiting in young birds, especially during the first week of life, resulting in reduced fat digestion and absorption (Krogdahl, 1985). In addition, young birds are unable to replenish bile salts like older birds and decreased pool size of bile salts may also contribute to the poor digestion and absorption of fat in young birds (Serafin and Nesheim, 1970). For this reason, bile acid derivatives such as

bile salts and cholic acid have been evaluated in diets for young birds to improve fat digestion and absorption.

Gomez and Polin (1976) studied the absorption of tallow in chicks by adding bile acid (cholic and chenodeoxycholic acids) and bile salts (taurocholate) at three levels (0, 0.25 and 0.5 g/kg) to a maize-based diet containing 82 g/kg tallow. Addition of bile acids and bile salts increased fat absorption at 7 and 19 days of age compared to birds fed the diet without bile. The addition of cholic acid improved the absorption of tallow better than chenodeoxycholic acid and taurocholate.

Polin *et al*. (1980) also observed the effect of bile acids on the absorption of tallow by feeding chicks with diets containing 40 g/kg tallow supplemented with one of the bile acids (cholic acid, chenodeoxycholic acid, dehydrocholic acid or deoxycholic acid) or bile salt (sodium taurocholate) at 0.4 g/kg. It was reported that fat absorption was significantly improved at 1 and 3 weeks of age. The chenodeoxycholic acid supplemented group showed higher fat absorption compared to the unsupplemented control group at one week of age (89.7 and 84.1%, respectively). At 3 weeks of age, fat absorption was higher in the cholic acid group compared to the control and chenodeoxycholic acid group (87.1 versus. 84.8 and 81.0%, respectively).

Detary supplementation of ox bile to improve fat utilisation was studied by Fedde *et al*. (1960) in two experiments. In Experiment 1, chicks were fed diets containing beef tallow (200 g/kg) supplemented without or with bile (5 g/kg). It was found that the absorption of fat in birds fed diets supplemented with bile was higher than those fed diets without bile. In Experiment 2, chicks were fed diets containing 200 g/kg beef tallow supplemented with increasing levels of bile (0, 0.5, 1, 5, 10, 20, 40 and 80 g/kg). It was found that the body weight of birds fed diets with 40 and 80 g/kg bile were lower than the other groups. The weight of the gall bladders of birds fed diets supplemented with bile was higher than the control. Fat absorption in birds fed diets supplemented with 5 g/kg bile was significantly higher than those fed diets containing 0, 0.5, 1 and 20 g/kg bile, but there were no differences between birds fed 5, 10, 40 and 80 g/kg bile. Based on these results, it was speculated that addition of exogenous bile may aid directly in fat absorption or it may stimulate the liver cells to secrete more bile.

Alzawqari *et al*. (2011) studied the effect of dried ox bile on the performance and fat digestibility of broilers by feeding diets containing tallow (50 g/kg) supplemented with three levels of bile (0, 2.5 and 5 g/kg). Supplementation of bile at 5

g/kg resulted in higher weight gain and lower feed per gain than 2.5 and 0 g/kg. Fat digestibility was higher in the 5 g/kg bile treatment (84.4%) and lower in 2.5 and 0 g/kg (79.3 and 59.3%, respectively).

2.3.3.2. Lecithin

Lecithin, a by-product from the processing of soybean oil, has been evaluated to improve fat digestion in diets for young pigs (Overland *et al*., 1993; Soares and Lopez-Bote, 2002) and broilers (Polin, 1980). Lecithin (a phospholipid) is a mixture of surface-active agents, consisting of a hydrophobic portion with affinity for fats, and a hydrophobic portion with an affinity for water (Gu and Li, 2003). Polin (1980) supplemented a diet containing 40 g/kg tallow with 0.2, 2 and 20 g/kg lecithin and reported that the absorption of tallow was increased in broilers fed 20 g/kg lecithin compared to those fed 0.2 and 2 g/kg.

Azman and Ciftci (2004) studied the effect of replacing dietary fat with lecithin on broiler performance. Broiler starters were fed diets containing 40 g/kg soybean oil (control) and, 40 g/kg soybean oil and soy-lecithin mixtures (in 75:25 and 50:50 proportions) or 40 g/kg of beef tallow and soy lecithin mixtures (in 50:50 proportions) from 5 to 21 days of age. From days 22 to 35, birds were fed grower diets containing 60 g/kg soybean oil or tallow mixed with the same proportion of soy lecithin as in the starter diets. It was found that feed per gain of broilers fed the tallow- lecithin mixture was higher than those fed soybean oil and soybean oil-lecithin mixture. There were, however, no differences in the feed per gain of broilers fed either soybean oil or soybean oil mixed with soy lecithin.

2.3.3.3. Commercial synthetic emulsifiers

The use of bile supplementation or natural emulsifiers to improve fat utilisation is not currently economically viable. Synthetic emulsifiers are cheaper and a number of commercial products are available. Examples of synthetic products are blends of hydrolysed lecithin (AD.Emulsifier, Ad.biotech, New York, USA), lysophosphatidylcholine (Lysoforte^{TM}, Kemin Industries, Singapore) and glycerol polyethylene glycol ricinolate (Volamel Extra, Nulamel Inc., Olen, Belgium).

Lysophosphatidylcholine, a powerful biosurfactant, is the mono-acyl derivative of phosphatidylcholine which is produced by the action of enzyme phospholipase A_2.

Lysophospholipids are more hydrophilic than phospholipids because they have one fatty acid residue per molecule and are capable of forming spherical micelles in aqueous solutions leading to improved emulsification in the gastrointestinal tract (Vasanthakumari *et al*., 2011). Lysophospholipids also have the ability to form small sized micelles and are more effective than bile and soy lecithin (Melegy *et al*., 2010).

Zhang *et al*. (2011) evaluated the effect of commercial lysophosphatidylcholine (Lysoforte^{TM}) on the performance and AME of broilers fed diets with three fat sources (soybean oil, tallow and poultry fat) at 30 g/kg in starter diets and at 40 g/kg in grower diets. It was found that supplementation of lysophosphatidylcholine increased weight gain in birds fed all three fat sources during the starter phase, but no differences were observed during the grower phase. Supplementation of lysophosphatidylcholine tended to increase the AME during the starter phase and increased the AME during the grower phase with the highest AME being determined for birds fed diets with poultry fat and emulsifier compared to those fed diets with soybean oil or tallow plus emulsifier (13.02, 12.93 and 12.77 MJ/kg, respectively).

The emulfying potential of glycerol polyethylene glycol ricinolate, an ester of ethylene oxide and ricinus oil, was tested by Roy *et al*. (2010). These researchers fed broilers with maize based diets containing palm oil at 35 g/kg in the starter period and 28 g/kg in the grower period supplemented with three levels of glycerol polyethylene glycol ricinolate (0, 10 and 20 g/kg). The addition 10 g/kg emulsifier resulted in higher live weight and lower feed per gain compared to 0 and 20 g/kg additions.

2.3.4. Type of added fat

The animal fats normally used in the feed industry are tallow and poultry fat, while the common vegetable oils used are soybean oil, palm oil and maize oil. A number of studies have examined the effects of adding different types of fats and oils on the fat digestion, AME and growth performance in broiler chickens (Zumbado *et al.,* 1999; Dei *et al*., 2006; Wongsuthavas *et al*., 2007b).

Pesti *et al*. (2002) conducted two experiments to study the effect of eight fat types (feed grade poultry grease, pet grade poultry grease, restaurant grease, white grease, animal/vegetable oil blend, palm oil, yellow grease and soybean oil) on the AME and growth performance of broilers. In Experiment 1, birds were fed a basal diet containing 60 g/kg of different fat sources. Diets containing poultry grease (feed grade

and pet grade) and palm oil had lower AME values, while those with soybean oil, yellow grease and animal/vegetable oil blend had higher AME values followed by white grease and restaurant grease. In Experiment 2, birds were fed a basal diet supplemented with 30 or 60 g/kg of each fat source. Different fat sources had no effect on the performance. However, feed per gain of broilers fed diets containing 60 g/kg fat was significantly lower than birds fed diets containing 30 g/kg fat.

Firman *et al*. (2008) investigated the performance of broilers fed diets supplemented with 30 g/kg of soybean oil, yellow grease, poultry fat, tallow, vegetable and animal fat blend, lard and palm oil over a 7-week trial period and found no differences between different fat sources in terms of broiler performance.

Blends of animal fats and vegetable oils represent an alternative option for the poultry feed industry. Animal fats contain a high proportion of long chain saturated fatty acids, while vegetable oils have a high proportion of unsaturated fatty acids. As discussed above, saturated fatty acids are poorly digested compared with unsaturated fatty acids. The use of blends of animal fats and vegetable oils, with different ratios of unsaturated and saturated (U:S) fatty acids, has been studied by several researchers (Wiseman and Lessire, 1987; Scaife *et al*. 1994; Wiseman *et al*., 1998; Danicke *et al*., 2000). Ketels and De Groote (1989) reported that increasing U:S ratios increased the utilisation of saturated fatty acids, but there was no effect on unsaturated fatty acids. Leeson and Summers (2005) suggested that a ratio of 3:1 (U:S) is a good compromise for optimum fat digestibility in birds of all ages. It is well documented that blending animal fats with vegetable oils can produce a synergistic effect which can improve the utilisation of saturated fats (Lall and Slinger, 1973; Sibbald, 1978). Sibbald (1978) reported that the AME of mixtures of soybean oil and tallow was higher than the sum of the means of its components. Similarly, Muztar *et al*. (1981) found that the AME of blends of tallow and rapeseed soapstocks was higher (by over 4%) than the calculated AME of its components. Danicke *et al.* (2000) fed broilers with rye-based diets containing 100 g/kg fat based on blends of beef tallow and soybean oil (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0) which gave U:S ratios of 5.47, 3.23, 2.11, 1.45, 1.00 and 0.69, respectively. The results indicated that increasing proportions of tallow decreased weight gain and AMEn, and increased feed per gain.

The effect of blending animal fats with vegetable oils was also studied by Preston *et al.* (2001), who fed broilers wheat-based diets containing 60 g/kg tallow,

soybean oil or a 2:1 blend of tallow to soybean oil and found no significant effects of fat type on bird performance, but fat type had an effect on the digestibility of fat. Fat digestibility coefficients for soybean oil, blend and tallow were 0.85, 0.76 and 0.69, respectively.

2.4. Conclusions

The genetic development of modern broilers chickens has made a large contribution to improve the growth performance and may also have improved the ability of birds to digest and utilise nutrients. Fats and oils are rich sources of energy and are normally used to increase the dietary energy density of broiler diets. It is important to better understand the characteristics of different fat sources and also the process of fat digestion and absorption in order to optimise the efficiency of fat utilisation. This chapter presents an overview of fat classification and the digestion and absorption processes of fats, including endogenous fat losses. Information on bile which plays an important role in fat digestion and absorption is provided. The wide variability in published AME values of different fat sources is highlighted. Furthermore, the efficiency of fat utilisation by poultry is dependent on number of factors, including age of birds, interaction between cereal types, dietary calcium levels and fat sources. Studies on the factors affecting fat digestion in modern fast-growing broiler chickens are required to achieve the full benefit of fat supplementation. Possible strategies which can be employed to improve digestion and absorption of fat are also discussed.

CHAPTER 3

General Materials and Methods

All experimental procedures described in this thesis were approved by the Massey University Animal Ethics Committee (MUAEC approval numbers: 08/10 and 08/11) and complied with the New Zealand Revised Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching (Anonymous, 2008).

3.1. Birds and housing

For all experiments, day-old male broilers (Ross 308) were obtained from a commercial hatchery. Birds were individually weighed and assigned to cages in electrically heated battery brooders on weight basis so that the average weight of birds per cage was similar. On day 12, birds were transferred to grower cages and were maintained on the same diets until day 21. Battery brooders and grower cages were housed in environmentally controlled rooms. The temperature was maintained at 31 ºC on d 1, and was gradually reduced to 24 ºC by 21 day of age. Birds were maintained under 20 hours of fluorescent illumination per day. Diets were offered *ad libitum* and water was freely available at all times.

In growth trials, body weights and feed intake were recorded at weekly intervals throughout the trial. Mortality was recorded daily. Feed per gain values were corrected for the body weight of any bird that died during the course of the experiment.

3.2. Apparent metabolisable energy determination

Feed intake and total excreta output of each cage were recorded for four consecutive days for the determination of apparent metabolisable energy (AME). Daily excreta collections from each cage were pooled, mixed well in a blender, sub-sampled and freeze-dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Dried excreta samples were then ground to pass through 0.5 mm sieve and stored in airtight plastic containers at -4 ºC pending analysis. The diet and excreta samples were analysed for dry matter and gross energy.

3.3. Ileal digesta collection

In experiments which involved ileal digestibility measurements (Chapters 4, 6 and 7), four birds from each cage were euthanised at the end of the experiment by intravenous

injection of sodium pentobarbitone solution (1 ml per 2 kg live weight) (Provet NZ Pty Ltd., Auckland, New Zealand). After euthanasia, the small intestine was immediately exposed and the ileum was excised. Contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction. Digesta were pooled within a cage, immediately frozen (-20 ºC), lyophilised, ground to pass through a 0.5-mm sieve and stored at -4 ºC in airtight containers until laboratory analysis.

3.4. Chemical analysis

Dry matter content was determined using standard procedures (method 930.15; AOAC, 2005). Duplicate samples were weighed and placed in a drying oven for 24 h at 105 ºC and the weight was recorded after two hours of cooling in a desiccator.

Gross energy was determined by adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardised with benzoic acid.

Nitrogen (N) content was determined by combustion (method 968.06; AOAC, 2005) using a CNS-200 carbon, nitrogen, and sulphur auto analyser (LECO® Corporation, St. Joseph, MI, USA). Pre-weighed samples were placed into a furnace at 850 ºC with excess oxygen (O_2) and totally combusted. The combustion products, mainly carbon dioxide (CO_2), water (H_2O), nitrous oxides (NO_x) and nitrogen gas (N_2) were passed through a series of columns to remove H_2O, convert NOx to N_2, and to remove the remaining oxides and excess O_2. The gaseous N_2, carried by helium, was then measured by thermal conductivity and expressed as a percentage of the sample.

Fat content was determined by the Mojonnier method (method 954.02; AOAC, 2005). Samples were first hydrolysed by hydrochloric acid and transferred to Mojonnier tubes. Crude fat was extracted by combination of diethyl ether and petroleum ether. Solvents were then decanted into the pre-weighed conical flask and evaporated by placing the flask on the steam bath and drying the flask in the oven at 100 ºC for 90 minutes. After cooling down, the weight of the flasks with fat was recorded and fat contents were calculated.

For calcium (Ca) and phosphorus (P) analysis, samples were prepared by standard procedures (method 968.08D; AOAC, 2005). Samples were first ignited at 500 °C to burn off all organic materials. The ash was digested with 6M hydrochloric acid to release calcium and phosphorus. After the preparation step, calcium contents were determined

following the procedures of Gitelman (1967) and Gindler and King (1972) by using o-cresolpthalein complexone (CPC) in alkaline solution to develop colour. The absorbance was measured by spectrophotometer (Shimadzu UV mini-1240, Shimadzu Corporation, Tokyo, Japan) at the wavelength 578 nanometre. Phosphorus contents were determined following the procedures of ISO 6491 (1998) by using ammonium molydate reagent and ANSA (Amino-napthol sulphonic acid) reagent to develop the colour. The absorbance was measured by spectrophotometer at the wavelength 680 nanometre.

Titanium dioxide was determined following the procedures of Short *et al*. (1996). The samples were ignited at 500 °C to burn off all organic materials and the ash was then digested using 66% sulphuric acid to release titanium which was then determined using colorimetric assay.

Fatty acid composition was determined by the procedure reported by Sukhija and Palmquist (1988). Fatty acids were extracted by using methanolic hydrochloric acid (mixture of methanol and acetyl chloride) in culture tubes. Samples were vortexed and heated at 70 °C for methylation. After extraction and methylation, potassium carbonate and chloroform were added. Samples were vortexed and centrifuged to separate the methyl esters. Methyl esters were then transferred to the vials for fatty acid determination by using gas chromatography (Shimadzu GC-17A, Shimadzu corporation, Tokyo, Japan), equipped with a flame ionisation detector.

3.5. Determination of microflora

The microflora in caecal contents was determined by Fluorescence *in situ* hybridisation (FISH) method. The procedure described by Dinoto *et al.* (2006) was followed with some modification (Molan *et al*., 2009). In brief, caecal contents from two birds per replicate cage were collected, pooled and representative samples were obtained. A sample of 1 g of caecal content was mixed with 9 ml of sterile-filtered phosphate buffer (PBS; pH 7.2) and the bacteria contained in the supernatant were fixed in 4% (w/v) of paraformaldheyde in PBS (pH 7.2). After the fixing step, the samples were applied to Teflon-coated microscopic slides (Biolab, North Shore City, New Zealand) and air dried. Bacterial cells were then dehydrated with ethanol solutions and fixed on glass slides. Slides were hybridised by hybridisation buffer, labelled with Cy3-labeled oligonucleotide specific probes (50 ng/µl) and placed in plastic box containing wet sponges (soaked in hybridisation buffer) at 46 °C for 2 hours. Slides were rinsed with warm hybridization buffer at 48 °C and washed in pre-warmed washing buffer for 20 minutes at 48 °C.

After the washing step, slides were rinsed with ice-cold distilled water and thoroughly dried before being observed using a fluorescence scanning microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) under 400X magnification. The images were captured using an Optronics MagnaFIRE SS99802 digital camera with MagnaFIRE frame-grabbing software on a Pentium IV computer. Fluorescent cells were counted automatically using the ImageJ program of Abramoff *et al*. (2004). The image files were opened by the ImageJ program and the process command and find maxima command were selected. Preview point selection command was then selected to count the number of fluorescent cells. The probes (Geneworks, Hindmarsh, Australia) used in this method were Bif 164 for bifidobacteria with a sequence of 5-CATCCGGCATTACCACCC-3, Lab 158 for lactobacilli with a sequence of 5-GGGTATTAGCATCTGTTTCCA-3, Cajej for *Campylobacter jejuni* with a sequence of 5-AGCTAACCACACCTTATACCG-3, Chis150 for clostridia with a sequence of 5-TTATGCGGTATTAATCTCCCTTT-3 and Bac 303 for Bacteroides species with a sequence of 5-CCAATGTGGGGGACCTT-3.

3.6. Calculations

The AME of diets were calculated using the following formula with appropriate corrections made for differences in DM content.

$$\text{AME (MJ/kg)} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

Apparent ileal digestibility coefficients of N, fat, fatty acids, Ca and P were calculated, using titanium dioxide as indigestible marker, as shown below:

$$\text{Apparent digestibility of fat or fatty acid or nutrient} = \frac{(NT/Ti)_d - (NT/Ti)_i}{(NT/Ti)_d}$$

where, $(NT/Ti)_d$ = ratio of fat, or fatty acid or nutrient to titanium in the diet, and

$(NT/Ti)_i$ = ratio of fat, or fatty acid or nutrient to titanium in ileal digesta.

Total tract retention coefficients of N, fat, Ca and P were calculated using the following formula with appropriate corrections made for differences in DM content.

$$\text{Total tract retention coefficients of nutrient} = \frac{\text{(Nutrient intake) - (Nutrient output)}}{\text{Nutrient intake}}$$

3.7. Statistical analysis

Data from experiments reported in Chapters 4 and 5 were analysed by repeated measures analysis and those from experiments reported in Chapters 6 and 7 were analysed by two-way analysis of variance (ANOVA). Performance data from Chapter 7 were analysed separately by one-way ANOVA. Data from the experiment reported in Chapter 8 were analysed by orthogonal polynomials contrast. The cage means were used to derive performance data. All data were analysed by using the General Linear Models procedure of SAS (2004). Differences were considered significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

CHAPTER 4

Digestion of fat and fatty acids along the intestinal tract of broiler chickens

4.1. Abstract

Two experiments were conducted. The first experiment investigated the digestion of fat and fatty acids from soybean oil and tallow along the intestinal tract of broiler chickens. The second experiment was conducted to determine endogenous fat and fatty acid losses and the fatty acid profile of chicken bile. In experiment 1, two-week old broilers were fed maize-soy diets supplemented with 50 g/kg of soybean oil or tallow for seven days and digesta were collected from the duodenum, upper jejunum, upper ileum and lower ileum. Apparent digestibility coefficients were calculated using the titanium marker ratio in diets and digesta. Digestibility of fat was determined to be highly negative in the duodenum, indicating marked net secretion of fat into this segment. Fat was rapidly digested and absorbed in the jejunum, with digestibility coefficients of 0.601 to 0.644 being determined at the end of jejunum. The digestion and/or absorption of fat continued in the upper ileum. The digestibility coefficients of fat in soybean oil and tallow diets at the lower ileum were 0.823 and 0.739, respectively. Linoleic acid was absorbed throughout the intestinal tract, while the digestion and absorption of palmitic, stearic and oleic acids started only in the jejunum. Measurements at the lower ileal level showed that the unsaturated fatty acids (linoleic and oleic acids) were well digested (0.900 to 0.941), irrespective of the source of fat. In contrast, the digestibility of saturated fatty acids (palmitic and stearic acids) was influenced by the fat source. Digestibility coefficients of palmitic and stearic acids at lower ileum were markedly higher in the diet containing soybean oil (0771 to 0.848) compared to that containing tallow (0.577 to 0.684).

In experiment 2, two-week old broilers were fed a fat-free purified diet for two days and digesta were collected from the lower ileum. Bile was also collected. The results showed the ileal endogenous fat loss to be 1714 mg/kg dry matter intake. Endogenous fat was composed mainly of palmitic, stearic, oleic, linoleic and arachidonic acids, which was remarkably similar to the fatty acid profile of the bile. These results suggest that the re-absorption of fat and fatty acids from the bile was incomplete in young broiler chickens.

4.2. Introduction

The digestion of fats and fatty acids in poultry has been generally measured over the total tract (Hurwitz *et al*., 1973; 1979). Such evaluations, however, do not provide information on the site of intestinal absorption. Identification of the sites of fatty acid absorption is critical to understand the dynamics of fat digestion. Studies investigating the intestinal site of fat digestion and absorption are not only limited, but also contradictory. Hurwitz *et al.* (1973) reported that both the jejunum and ileum were involved in the absorption of fatty acids in laying hens, and that fatty acids were absorbed rapidly in the jejunum, although the absorption of some fatty acids, particularly linoleic, stearic and palmitic acids, continued in the ileum. Sklan *et al.* (1975) reported that the absorption of fatty acids took place mainly in the duodenum and upper jejunum. On the other hand, Hurwitz *et al*. (1979) also reported that 60% of the fat was absorbed in the duodenum and that the absorption was completed in the upper ileum of turkeys. Renner (1965) stated that the absorption of fat was negligible in the caeca and large intestine.

In any study investigating fat digestion along the intestinal tract, fatty acid composition should also be considered, as the degree of fatty acid unsaturation and chain length are known to influence the digestion and absorption of fat and fatty acids (Renner, 1965; Hurwitz *et al.*, 1973; Sklan *et al.*, 1973). Renner (1965) reported that the absorption of fat in birds fed diets containing tallow was lower in the jejunum and ileum compared to those fed diets containing lard and soybean oil. Sklan *et al.* (1973) observed that the degree of absorption was positively correlated with degree of unsaturation. It was shown that linoleic acid was better absorbed in the duodenum and upper jejunum compared to palmitic and stearic acids. Hurwitz *et al*. (1979) also reported that the absorption of fatty acids in turkeys was increased with the degree of unsaturation. Chain length of fatty acids may be another factor influencing the site of fat digestion and absorption. Hurwitz *et al*. (1979) observed that the absorption of stearic acid (18:0) in the duodenum was lower compared to that of palmitic acid (16:0).

Significant losses of nutrients, including lipids, occur during digestion and absorption along the intestinal tract and these inevitable losses from an important part of the digestion process. Measurement of, and correction for, these losses is necessary for the estimation of true ileal digestibility of lipids. Estimations for ileal endogenous losses of protein and amino acids have been published (Ravindran and Bryden, 1999; Lemme

et al., 2004; Cowieson *et al*., 2009), but studies on endogenous fat losses are scant (Ajuyah *et al*. 1996). Bile is possibly the predominant source of endogenous lipids, but to our knowledge, no published data are available on the composition of bile from chickens.

The present evaluation consisted of two experiments. In the first experiment, the digestion of fat and fatty acids along the intestinal tract of broiler chickens fed diets supplemented with either soybean oil or tallow were investigated. In the second experiment, ileal endogenous losses of fat and fatty acids were determined following the feeding of a fat-free diet. The fatty acid composition of the bile was also determined.

4.3. Materials and methods

Experiment 1 Digestion of fat and fatty acids along the intestinal tract

Day-old male broilers (Ross 308) were obtained from a commercial hatchery and, raised in floor pens and fed a commercial broiler starter diet. On day 14 post-hatching, the birds were individually weighed and 32 birds of uniform weight were selected and assigned to eight cages. Housing conditions have been described in Chapter 3 Section 3.1.

Two maize-soy diets containing 50 g/kg soybean oil or tallow were formulated (Table 4.1). Titanium oxide (3 g/kg) was included in both diets as an inert marker. The diets, in mash form, were offered *ad libitum* for seven days and water was freely available throughout the trial.

On day 21, all birds were euthanised by intravenous injection of sodium pentobarbitone and, digesta from duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum were collected and processed as described in Chapter 3 section 3.3. Samples of diets and digesta were ground to pass through a 0.5 mm sieve and stored in airtight containers at - 4 $^{\circ}$C for analyses of dry matter (DM), titanium, fat and fatty acids.

Dry matter, titanium, fat and fatty acids were determined as described in Chapter 3, section 3.4.

Table 4.1. Ingredient and calculated composition of experimental diets (g/kg as fed)

Ingredient	Maize-soy oil	Maize-tallow
Maize	522.2	525.8
Soybean meal, 480 g/kg	382.5	378.7
Soybean oil[1]	50	0
Tallow[2]	0	50
Salt	2.5	2.5
DL. Methionine	2.0	2.0
L-Lysine.HCl	1.4	1.5
Dicalcium phosphate	20.0	20.0
Limestone	11.7	11.8
Sodium bicarbonate	1.6	1.6
Vitamin-trace mineral premix[3]	3.1	3.1
Titanium oxide	3.0	3.0
Provision		
ME, (MJ/kg)	13.22	12.88
Crude protein	23.00	23.00
Calcium	1.00	1.00
Available phosphorus	0.50	0.50
Methionine	0.55	0.55
Methionine + cysteine	0.92	0.92
Lysine	1.38	1.38
Threonine	0.86	0.86
Tryptophan	0.31	0.31

[1]ME content of soybean oil was assumed to be 38.0 MJ/kg.
[2]ME content of tallow was assumed to be 27.0 MJ/kg.
[3]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg, Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1.0 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

Apparent digestibility coefficients of fat and individual fatty acids in each intestinal segment were calculated according to the calculation described in Chapter 3, Section 3.6.

Data were analysed by repeated measures analysis using SAS (2004). Differences were considered significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

Experiment 2 Endogenous losses of fat and fatty acids

Day-old male broilers (Ross 308), obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diet. On day 14 post-hatching, the birds were individually weighed and, 24 birds of uniform weight were selected and assigned to four cages of six birds each. Housing conditions have been described in Chapter 3 Section 3.1.

A fat-free purified diet, containing titanium oxide (3 g/kg) as an inert marker, was formulated (Table 4.2). After 10 days on the commercial starter diet, in mash form, the purified diet was introduced on day 24 and offered *ad libitum* for two days. Water was freely available throughout the experiment. On day 26, all birds were euthanised by intravenous injection of sodium pentobarbitone and, digesta were collected from the terminal ileum and processed as described in Chapter 3 section 3.3. Bile was also collected from the gall bladder, pooled within a cage and lyophilised.

Processed digesta and bile were ground to pass through a 0.5 mm sieve and stored in airtight containers at - 4 $^{\circ}$ C for analyses of DM, titanium, fat and fatty acids in digesta and for analyses of DM, fat and fatty acids in the bile.

Dry matter, titanium, fat and fatty acids were determined as described in Chapter 3, section 3.4.

The flow of fat and individual fatty acids at the terminal ileum were calculated, as milligrams lost per ingestion of kilogram of feed dry matter (DM), using the following formula (Rutherfurd *et al*., 2004).

$$\text{Fat or fatty acid flow (mg/kg DM intake)} = \text{Fat or fatty acid concentration in ileal digesta (mg/kg)} \times \frac{\text{Diet titanium (mg/kg)}}{\text{Ileal titanium (mg/kg)}}$$

The standardised ileal digestibility coefficient (SIDC) of fat and fatty acids were calculated following the formula described by Ravindran (2004).

$$\text{SIDC} = \text{AID} + \frac{\text{Ileal endogenous fat or fatty acids flow (g/kg DM intake)}}{\text{Fat or fatty acid content of the diet (g/kg DM)}}$$

Where, AID = Apparent ileal digestibility coefficient of fat or fatty acids

Table 4.2. Composition of the fat-free purified diet (g/kg)

Ingredient	
Casein	180.0
Dextrose	722.4
Solkafloc	35.0
Dicalcium phosphate	24.0
Sodium bicarbonate	20.0
Dipotassium phosphate	5.0
Magnesium oxide	2.0
Titanium oxide	3.0
Trace mineral premix[1]	5.0
Vitamin premix[2]	1.0
Salt	2.6
Analysed value, fat	0.7

[1]Supplied per kilogram of diet: Co, 0.6 mg; Cu, 6.0 mg; Fe, 50 mg; I, 2.0 mg; Mn, 250 mg; Mo, 1.0mg; Se, 400 µg; Zn, 120 mg.
[2]Supplied per kilogram of diet: antioxidant, 166 mg; biotin, 0.33 mg; calcium pantothenate, 21.2mg; cholecalciferol, 99.6µg; cyanocobalamin, 0.028 mg; folic acid, 8.6 mg; menadione, 6.6 mg; niacin, 58 mg; pyridoxine, 16.6 mg; trans-retinol, 5.52 mg; riboflavin, 19.9 mg; thiamine, 4.9mg; dl-α-tocopheryl acetate, 99.6 mg; choline chloride, 1.05 g.

4.4. Results

Experiment 1

The fatty acid composition of the experimental diets is summarised in Table 4.3. The major fatty acids in both diets were palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids. Saturated fatty acid (16:0 and 18:0) content was high in the tallow diet and unsaturated fatty acid (18:1 and 18:2) content was higher in the soybean oil diet. The unsaturated to saturated fatty acid ratios (U:S) in soybean oil and tallow diets were 4.41 and 1.33, respectively.

Table 4.3. Analysed fat content and fatty acid composition of diets used in Experiment 1 (g/kg dry matter basis)

	Maize-soybean oil	Maize-tallow
Fat	90.3	93.8
Saturated fatty acids		
C14:0 Myristic	0	1.71
C16:0 Palmitic	10.31	17.20
C17:0 Margaric	0.24	1.12
C18:0 Stearic	3.05	13.32
C20:0 Arachidic	0.31	0.23
C21:0 Heneicosanoic	0	0.30
C22:0 Behenic	0	0.14
C24:0 Lignoceric	0	0.13
Unsaturated fatty acids		
C14:1 Myristoleic	0	0.27
C16:1 Palmitoleic	0.09	1.29
C18:1 Elaidic	0	0.20
C18:1 Vaccenic	0	1.18
C18:1 Oleic	17.17	22.09
C18:1 Vaccenic	0.81	0.60
C18:2 Linoleic	43.12	17.34
C18:3 linolenic	0.21	1.52
C20:1 Eicosenoic	0	0.16
C20:2 Eicosadienoic	0.04	0
C22:5 Docosapentaenoic	0	0.95
Saturated fatty acids	13.91	34.15
Unsaturated fatty acids	61.44	45.60
Unsaturated to saturated fatty acid ratio	4.41	1.33

The apparent digestibility of fat from soybean oil and tallow diets in different intestinal segments is presented in Table 4.4. A significant interaction ($P < 0.01$) between fat source and intestinal segments was observed for fat digestibility. This interaction was due primarily to a greater change in fat digestion between the upper jejunum and upper ileum in birds fed the soybean oil diet.

Table 4.4. Apparent digestibility coefficients of fat and fatty acids in different intestinal segments of broilers fed diets supplemented with soybean oil or tallow[1]

		Apparent digestibility				
		Fat	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)
Soybean oil	Duodenum	-1.897^{g}	-2.608^{f}	-11.179^{e}	-0.421	0.463^{d}
	Upper jejunum	0.291^{f}	0.231^{e}	-0.514^{e}	0.525	0.822^{ab}
	Lower jejunum	0.644^{de}	0.706^{b}	0.443^{c}	0.802	0.887^{ab}
	Upper ileum	0.800^{ab}	0.825^{a}	0.717^{a}	0.886	0.937^{a}
	Lower ileum	0.823^{a}	0.848^{a}	0.771^{a}	0.900	0.941^{a}
Tallow	Duodenum	-1.630^{g}	-1.290^{f}	-2.148^{e}	-0.265	0.173^{e}
	Upper jejunum	0.316^{f}	0.353^{d}	0.239^{d}	0.582	0.641^{cd}
	Lower jejunum	0.601^{e}	0.590^{c}	0.486^{bc}	0.820	0.755^{bc}
	Upper ileum	0.712^{cd}	0.658^{bc}	0.544^{bc}	0.891	0.849^{ab}
	Lower ileum	0.739^{bc}	0.684^{b}	0.577^{b}	0.901	0.903^{ab}
SEM [2]		0.0164	0.0165	0.0243	0.0127	0.0362
Main effects						
Fat type						
Soybean oil		0.511	0.522	0.386	0.622	0.810
Tallow		0.473	0.457	0.369	0.639	0.664
SEM [2]		0.0073	0.0073	0.0108	0.0056	0.0162
Intestinal segment						
Duodenum		-1.763	-1.949	-6.664	-0.343^{d}	0.318
Upper jejunum		0.304	0.292	0.119	0.553^{c}	0.731
Lower jejunum		0.622	0.648	0.465	0.811^{b}	0.821
Upper ileum		0.756	0.742	0.631	0.888^{a}	0.893
Lower ileum		0.781	0.766	0.674	0.901^{a}	0.922
SEM [2]		0.0116	0.0116	0.0172	0.0090	0.0256
Probabilities, P ≤						
Fat type		**	***	NS	0.06	***
Intestinal segment		***	***	***	***	***
Fat type x intestinal segment		**	***	***	NS	*

NS, not significant; * P < 0.05; ** P < 0.01;*** P < 0.001.

[1] Each value represents the mean of four replicate samples.

[2] Pooled standard error of mean.

[a-g] Means in a column not sharing a common superscript are significantly different (P < 0.05).

Digestibility of fat was determined to be highly negative in the duodenum. However, fat was rapidly digested and absorbed in the jejunum, with digestibility coefficients of 0.601 to 0.644 being determined at the end of jejunum. The digestion and/or absorption of fat continued in the ileum. The digestibility coefficients of fat in the soybean oil and tallow diets at the lower ileum were 0.823 and 0.739, respectively.

The apparent digestibility of the four major fatty acids, namely palmitic, stearic, oleic and linoleic acids, in soybean oil and tallow diets in different intestinal segments is

shown in Table 4.4. The main fatty acids secreted into the duodenum were palmitic and stearic acids, as indicated by their highly negative digestibility coefficients. There was also some net secretion of oleic acid, but no net secretion of linoleic acid. Linoleic acid was absorbed throughout the intestinal tract. The net absorption of palmitic and oleic acids started in the upper jejunum, while that of stearic acid started only in the lower jejunum. Measurements at the lower ileal level showed that unsaturated fatty acids (linoleic and oleic acids) were well digested (0.90 to 0.94), irrespective of the source of fat. In contrast, the digestibility of saturated fatty acids (palmitic and stearic acids) was influenced by the fat source. Digestibility coefficients of stearic and palmitic acids at the lower ileum were higher ($P < 0.05$) in the diet containing soybean oil (0.771 and 0.848, respectively) compared to that containing tallow (0.577 and 0.684, respectively). Chain length of saturated fatty acids influenced the digestion, with the ileal digestibility of stearic acid being lower than that of palmitic acid in both fat sources.

Cumulative fat digestibility determined in different intestinal segments is shown in Figure 4.1 (data from duodenum are not shown because of the negative digestibility coefficients). Fat digestibility did not differ between fat sources at the jejunal level, but the digestibility in the upper and lower ileum was higher ($P < 0.05$) in birds fed the diet supplemented with soybean oil than those fed the diet supplemented with tallow.

The proportion of fat digested in different intestinal segments is shown in Figure 4.2 (data for duodenum are not shown because of the negative digestibility coefficient). Irrespective of the fat source, almost 75% of the fat was digested and absorbed in the upper and lower jejunum. Digestion and absorption continued into the ileum, with 15-25% being absorbed in the upper ileum. There was little digestion or absorption in the lower ileum.

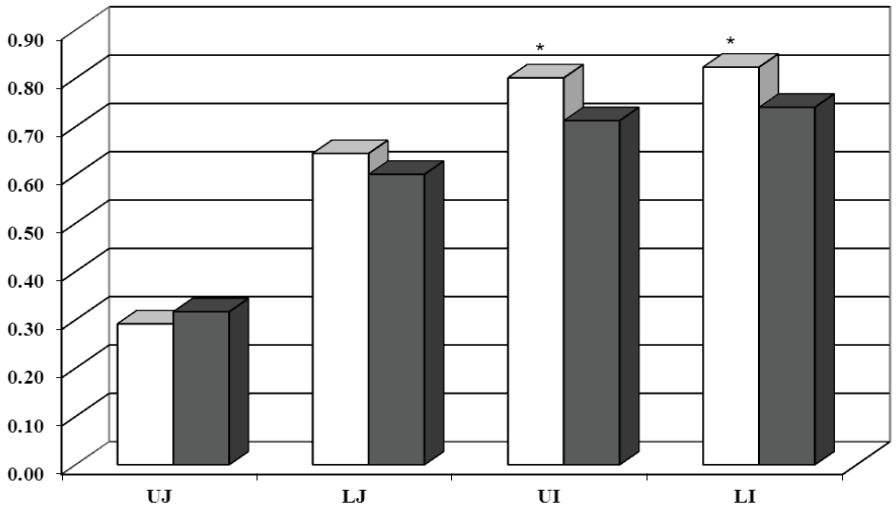

Figure 4.1. Apparent digestibility of fat determined in different intestinal segments of broilers fed diets supplemented with soybean oil (□) and tallow (■). Asterisks denote significant difference at $P < 0.05$.

(UJ, upper jejunum; LJ, lower jejunum; UI, upper ileum; LI, lower ileum)

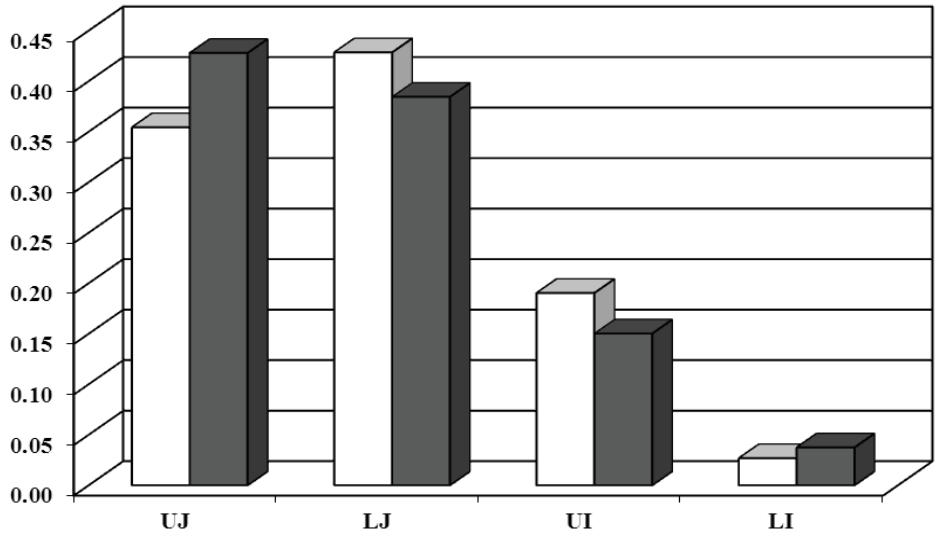

Figure 4.2. Digestion of fat, as proportion of total digestion determined at the lower ileum, along the small intestine of broilers fed diets supplemented with soybean oil (□) or tallow (■).

(UJ, upper jejunum; LJ, lower jejunum; UI, upper ileum; LI, lower ileum)

The apparent digestibility coefficient of the four major fatty acids determined at different intestinal segments and the digestion as proportion of total digestion at the lower ileum are shown in Figures 4.3 and 4.4., respectively. Figure 4.3 clearly shows that the digestion and absorption of linoleic acid started at the duodenum. Net absorption of palmitic and oleic acids started at the upper jejunum, and that of stearic acid only at the lower jejunum. The digestibility of saturated fatty acids (palmitic and stearic acids) and linoleic acid differed between fat sources. In general, digestibility coefficients of these fatty acids in most segments were higher in the diet containing soybean oil compared to that containing tallow. The digestion of oleic acid along the intestine was remarkably similar in both fat sources. Degree of saturation had an impact on fatty acid digestion. Unsaturated fatty acids (oleic and linoleic acids) were better digested than saturated fatty acids (palmitic and stearic acids). Chain length of fatty acids also influenced the digestion, with the digestibility of stearic acid (18:0) being lower than that of palmitic acid (16:0).

The data presented in Figure 4.4 show that, of the four major fatty acids, only linoleic acid was absorbed in the duodenum. Net absorption of linoleic acid in the duodenum was 45% in the soybean oil diet and 17% in the tallow diet. As can be seen, most of the digestion and absorption of fatty acids took place in the jejunum. It is noteworthy that unsaturated fatty acids (oleic and linoleic acids) were digested and absorbed mostly in the duodenum and upper jejunum,while saturated fatty acids (palmitic and stearic acids) were digested and absorbed in upper and lower jejunum.

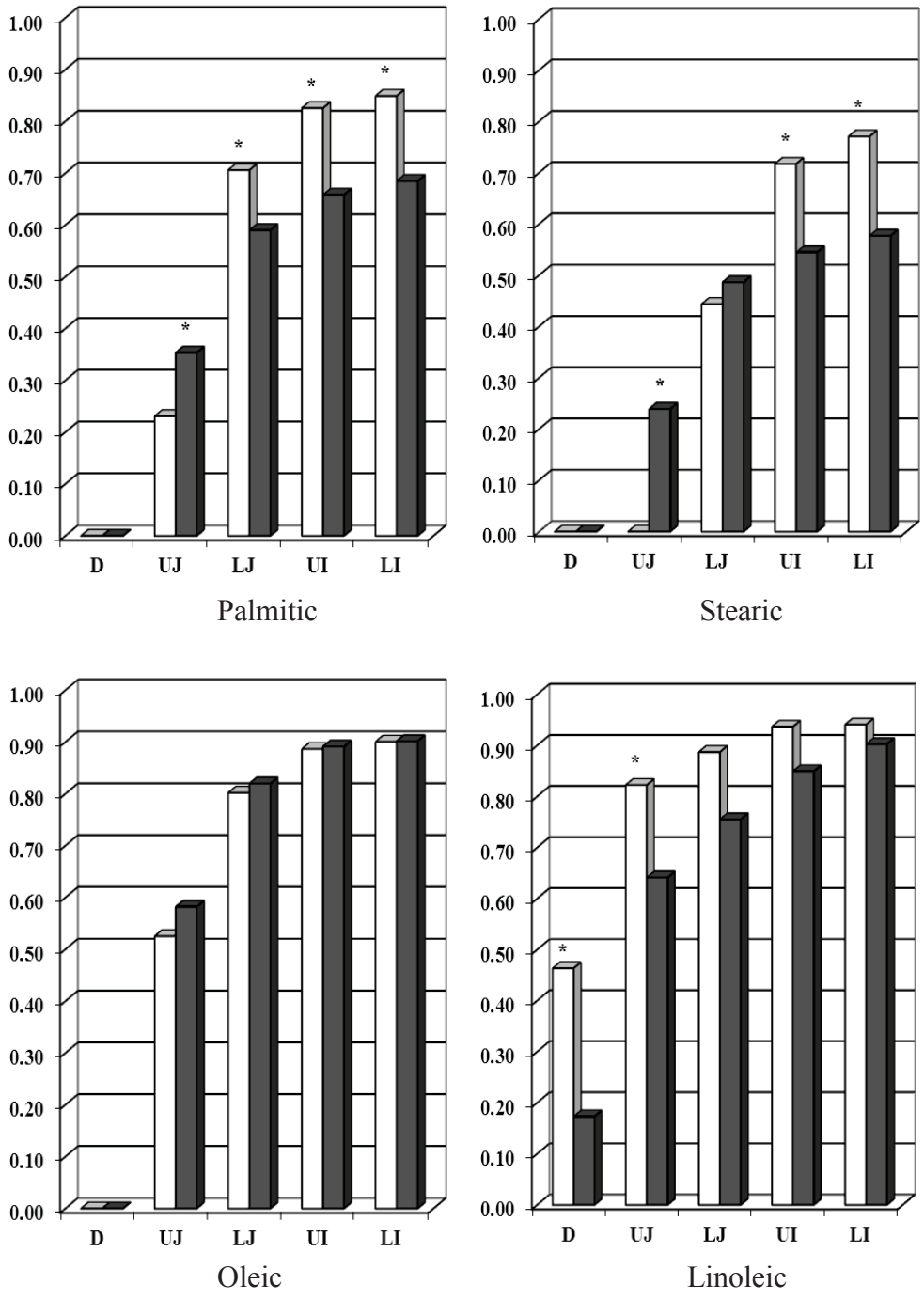

Figure 4.3. Digestibility of fatty acids determined at different intestinal segments of broilers fed diets supplemented with soybean oil (□) or tallow (■) (* $P < 0.05$).

(D, duodenum; UJ, upper jejunum; LJ, lower jejunum; UI, upper ileum; LI, lower ileum)

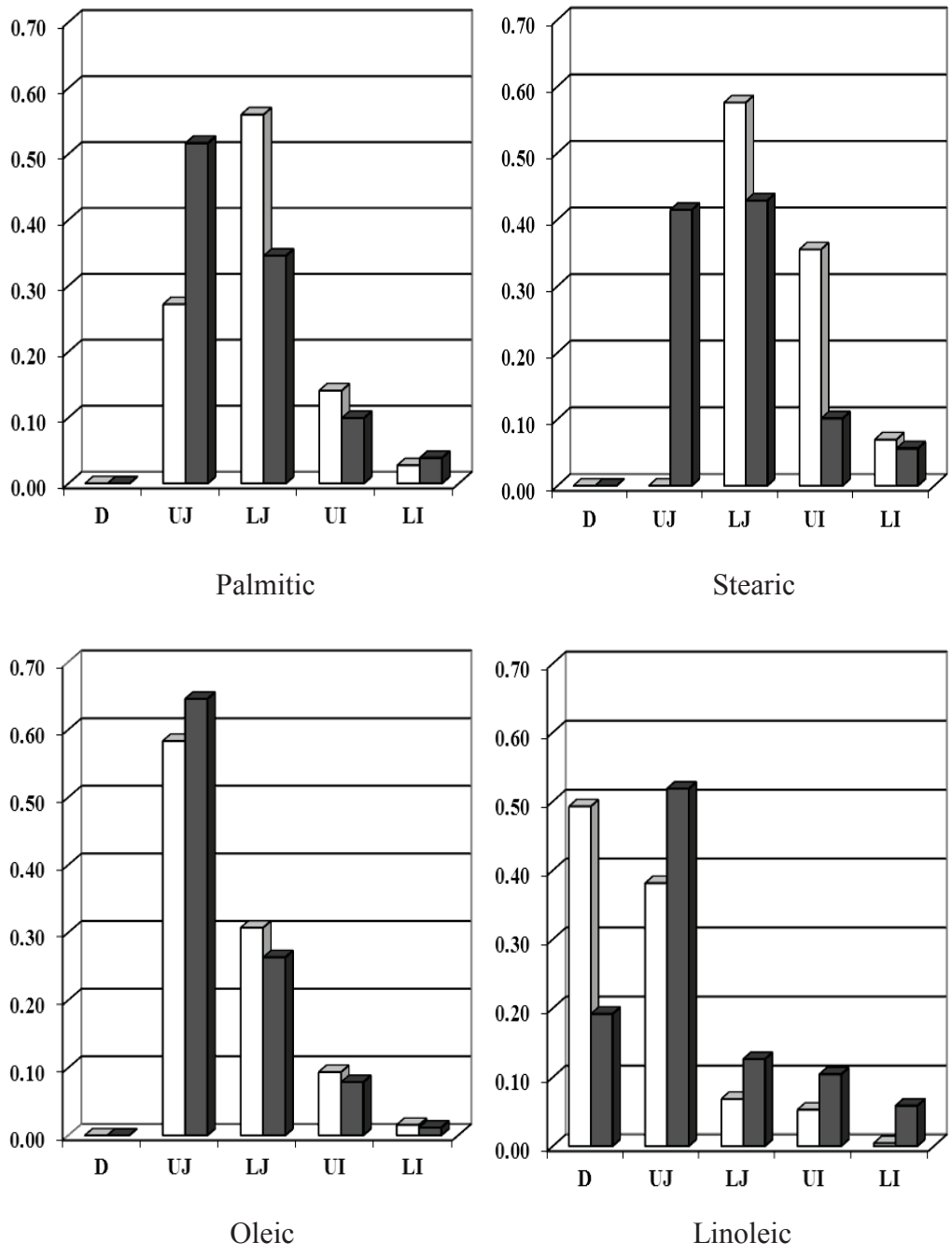

Figure 4.4. Fatty acid digestion, as proportion of total digestion at lower ileum, along the intestinal tract of broilers fed diets supplemented with soybean oil (□) or tallow (■).

(D, duodenum; UJ, upper jejunum; LJ, lower jejunum; UI, upper ileum; LI, lower ileum)

Experiment 2

Data on the ileal endogenous flow of fat and fatty acids are presented in Table 4.5. Ileal endogenous losses of fat and total fatty acids were determined to be 1714 and 825 mg/kg DM intake, respectively. The major saturated fatty acids in the ileal endogenous fat were palmitic and stearic acids, whereas the main unsaturated fatty acids were oleic, linoleic and arachidonic acids.

Table 4.5. Ileal endogenous flow of fat and fatty acids (mg/kg DM intake) in broiler chickens[1]

Fat	1714.3
Saturated fatty acids	
C16:0 Palmitic	127.9
C17:0 Margaric	16.3
C18:0 Stearic	225.2
Unsaturated fatty acids	
C18:1 Oleic	125.4
C18:2 Linoleic	227.1
C20:4 Arachidonic	103.2
Total fatty acids	825.1

[1]None of the other fatty acids were detected.

The fatty acid profile of endogenous fat is shown in Table 4.6. Only 48.1% of the endogenous fat was of fatty acid origin. The profile showed that slightly more unsaturated fatty acids (266 g/ kg fat) were present in the endogenous fat than saturated fatty acids (216 g/ kg fat).

Table 4.6. Fatty acid profile of endogenous fat (g/kg fat)

Saturated fatty acids	
C16:0 Palmitic	74.6
C17:0 Margaric	9.5
C18:0 Stearic	131.4
Unsaturated fatty acids	
C18:1 Oleic	73.2
C18:2 Linoleic	132.5
C20:4 Arachidonic	60.2
Total fatty acids	481.0

The major unsaturated fatty acids were linoleic, oleic and arachidonic acids (132.5, 73.2 and 60.2 g/kg fat, respectively), whereas the major saturated fatty acids were stearic, palmitic and margaric acids (131.4, 74.6 and 9.5 g/kg fat, respectively).

The fat content and fatty acid composition of the chicken bile are presented in Table 4.7. The contents of fat and total fatty acids in the bile were determined to be 2399 and 1542 mg/kg DM, respectively. The fatty acid profile was dominated by unsaturated fatty acids. Bile contained 868 mg/kg unsaturated fatty acids, as against 674 mg/kg saturated fatty acids. Linoleic acid was the major fatty acid in the bile (406 mg/kg), followed by stearic and palmitic acids (347 and 317 mg/kg, respectively).

Table 4.7. Fat content and fatty acid composition of the chicken bile[1]

	mg/kg dry matter [2]
Fat	2399
Saturated fatty acids	
C14:0 Myristic	1.9
C16:0 Palmitic	316.8
C17:0 Margaric	7.3
C18:0 Stearic	346.9
C20:0 Arachidic	<1.0
C21:0 Heneicosanoic	1.0
Unsaturated fatty acids	
C16:1 Palmitoleic	6.2
C18:1 Elaidic	1.5
C18:1 Vaccenic	4.5
C18:1 Oleic	109.6
C18:1 Vaccenic	13.3
C18:2 Linoleic	405.9
C18:3 Linolenic	15.9
C20:1 Eicosenoic	1.5
C20:2 Eicosadienoic	5.5
C20:3 Eicosatrienoic	14.2
C20:4 Arachidonic	216.7
C20:5 Eicosapentaenoic	9.1
C22:5 Docosapentaenoic	15.4
C22:6 Docosahexaenoic	48.4
Total fatty acids	1541.6

[1] Each value represents the mean of four replicates samples.
[2] Dry matter content of bile was 27.2%.

The fatty acid profile of bile presented in Table 4.8 shows that fatty acids, accounts for 64% of the fat. Palmitic and stearic acids were the major saturated fatty acids, whereas oleic, linoleic and arachidonic acids were the main unsaturated fatty acids. Bile consisted of similar concentrations of palmitic and stearic acids (132.1 and 144.6 g/kg), but both were lower than that of linoleic acid (169.2 g/kg). Arachidonic and oleic acids concentrations were 90.3 and 45.7 g/kg, respectively.

Table 4.8. Fatty acid profile of chicken bile

	g/kg fat
Saturated fatty acids	
C14:0 Myristic	0.8
C16:0 Palmitic	132.1
C17:0 Margaric	3.0
C18:0 Stearic	144.6
C20:0 Arachidic	< 0.4
C21:0 Heneicosanoic	0.4
Unsaturated fatty acids	
C16:1 Palmitoleic	2.6
C18:1 Elaidic	0.6
C18:1 Vaccenic	1.9
C18:1 Oleic	45.7
C18:1 Vaccenic	5.5
C18:2 Linoleic	169.2
C18:3 Linolenic	6.6
C20:1 Eicosenoic	0.6
C20:2 Eicosadienoic	2.3
C20:3 Eicosatrienoic	5.9
C20:4 Arachidonic	90.3
C20:5 Eicosapentaenoic	3.8
C22:5 Docosapentaenoic	6.4
C22:6 Docosahexaenoic	20.2
Total fatty acids	642.6

The SIDC of fat and fatty acids of diets supplemented with either soybean oil tallow is summarised in Table 4.9. The SIDC of fat in birds fed diet supplemented with soybean oil was higher than those fed diets with tallow. The SIDC of saturated fatty acids (palmitic and stearic acids) of the diet supplemented with soybean oil was higher than that of the diet supplemented with tallow. The SIDC of oleic acid was found to be

similar in both two diets, but the SIDC of linoleic in the diet supplemented with soybean oil was higher than that supplemented with tallow.

Table 4.9. Standardisation ileal digestibility coefficient (SIDC) of fat and fatty acids of broiler chickens fed diets supplemented with soybean oil or tallow

	Soybean oil	Tallow
Fat	0.842	0.758
Saturated fatty acids		
Palmitic	0.861	0.692
Stearic	0.845	0.594
Unsaturated fatty acids		
Oleic	0.908	0.907
Linoleic	0.946	0.916

4.5. Discussion

The highly negative apparent digestibility of fat and fatty acids determined in the duodenum is indicative of marked net secretion of fat into this segment. Bile is a major source of fat secretion into the duodenum and may account for the observed negative digestibility. The net secretion fat and fatty acids into the duodenum has also been observed by other researchers (Hurwitz *et al*., 1973; Sklan *et al*., 1973; 1975). Hurwitz *et al*. (1973) stated that the net secretion of lipid in the duodenum can be attributed to digestive juices from liver, pancreas and small glands in the duodenal wall. The retrograde movement of digesta from duodenum to gizzard could also partly account for the observed negative values. Digesta and bile are shuttled between the gizzard and duodenum to optimise the action of enzymatic and mechanical digestion. The reflux process is continuous, enabling penetration of the gizzard by duodenal contents during the contractile period of the gizzard (Sklan *et al.*, 1978; Smulikowska, 1998). Thus, the net concentration of lipid may be increased by the reverse passage of digesta containing pancreatic and intestinal juices, and bile into the duodenum of birds.

The results of this study showed that jejunum was the predominant site of fat and fatty acid absorption in broiler chickens. Fat was rapidly digested and absorbed in

the jejunum, and the digestion and/or absorption continued in the upper ileum. These findings are in agreement with those of Hurwitz *et al*. (1973) who reported that both jejunum and ileum were involved in the absorption of fatty acids in laying hens and that fatty acids were absorbed rapidly in the jejunum. However, these findings are in contrast with those of Sklan *et al.* (1975) who reported that the absorption of fatty acids took place mainly in the duodenum and upper jejunum.

Differences were noted between fatty acids in terms of site of absorption. Linoleic acid was absorbed throughout the intestinal tract, whereas the digestion and absorption of palmitic, stearic and oleic acids started only in the jejunum. Reasons for the commencement of digestion and absorption of linoleic acid in the duodenum are not clear. However, the start of digestion and absorption of palmitic, stearic and oleic acids in the jejunum can be explained, in part, to the insufficiency of bile in the duodenum, as bile ducts in chickens enter only at the distal end of duodenum (Hurwitz *et al*., 1973). Moreover, passage time in the duodenum of chickens is very short (Hurwitz *et al*., 1979), which may not give sufficient time to digest and absorb saturated fatty acids, which require emulsification.

Ileal digestibility data showed that the estimates for unsaturated fatty acid (oleic and linoleic acids) were higher than those for saturated fatty acids (palmitic and stearic acids). The present results are consistent with those of Hurwitz *et al*. (1979) who studied the absorption of fatty acids in turkeys. These researchers reported that the absorption of linoleic acid was higher than that of oleic, palmitic and stearic acids, and the absorption of palmitic acid was higher than that of stearic acid. The present findings may be explained by the degree of unsaturation and chain length. Once digested, unsaturated free fatty acids such as linoleic and oleic acids spontaneously form mixed micelles with monoglycerides and conjugated bile salts. These mixed micelles are then transported to the mucosal surface and pass through the brush border of small intestine (Krogdahl, 1985). The higher the degree of fatty acid unsaturation the better is the ability of it to form micelles and to be absorbed. The lower digestibility of palmitic and stearic acids was due to the non-polarality and need to be emulsified prior to absorption.

The current results also demonstrated that the digestibility of stearic acid was lower than that of palmitic acid. Renner and Hill (1961b) studied the utilisation of saturated fatty acids in broilers and laying hens, and reported that the utilisation of saturated fatty acids decreased as the chain length increased. The low digestibility of

stearic acid may be attributed to its longer chain length. Van Kuiken and Behnke (1994) stated that long chain saturated fatty acids, particularly stearic acid, may inhibit the activity of lipase. It was suggested that the fatty acid binding site in lipase requires the fatty acid to bend at a 141° angle, but stearic acid has an angle of 180° which makes it difficult to bind with lipase.

The digestion and absorption of fat and fatty acids differed depending on the source of fat. Measurements at the end of ileum showed that unsaturated fatty acids (linoleic and oleic acids) were well digested, irrespective of the source of fat. In contrast, the digestibility of saturated fatty acids (palmitic and stearic acids) was influenced by the fat source. Birds fed the soybean oil diet had markedly higher ileal digestibility of fat, palmitic and stearic acids than those fed the tallow diet. These finding are in accordance with those of Danicke *et al*. (2000) who determined the apparent ileal digestibility of fat and fatty acids in broilers. They reported that birds fed a soybean oil diet had higher digestibility of fat, palmitic and stearic acids than those fed the tallow diet. High digestibility of palmitic and stearic acids in the soybean oil diet may be attributed to the high dietary concentration of unsaturated fatty acids (Table 4.3), which are natural emulsifiers and, assist in mixed micelle formation and absorption. Friedman and Nylund (1980) stated that the absorption of long chain saturated fatty acids is limited by their incorporation rate into micelles. Saturated fatty acids are less rapidly incorporated into micelles because of their non-polarity, which makes them rely on an adequate presence of bile salts and unsaturated fatty acids for efficient emulsification (Polin *et al*., 1980; Danicke, 2001). After the mixed micelles of unsaturated fatty acids and conjugated bile salts are formed, the hydrophobic cores of these micelles are able to solubilise long chain saturated fatty acids which are then absorbed (Scott *et al*., 1982; Krogdahl, 1985).

Data on ileal endogenous fat and fatty acid flows showed that the major saturated fatty acids in the endogenous fat were palmitic, margaric and stearic acids whereas the major unsaturated fatty acids were oleic, linoleic and arachidonic acids. These findings are in general agreement with those of Ajuyah *et al*. (1996) who reported that the main endogenous fatty acids in broiler chickens were palmitic, stearic, oleic and linoleic acids. In the study of Ajuyah *et al*. (1996), however, semi-purified diets containing soybean oil or mixed animal-vegetable acid oil were used and this could have influenced the measurement of endogenous fatty acids losses. Interestingly, the

fatty acid profile of endogenous fat measured in the current study corresponded closely to that of the bile. These observations may suggest that the reabsorption of fat and fatty acids in bile was incomplete. Hurwitz *et al*. (1973), however, reported that over 90% of the bile was reabsorbed in the small intestine of laying hens.

Only 48% of the endogenous fat was accounted for fatty acids. The balance 52% comes from non-fatty acid sources such as cholesterol and phospholipids in the bile (McKee and McKee, 2009), desquamated intestinal epithelial cells (Clement, 1980) or fats of microbial origin (Ajuyah *et al*., 1996).

The fatty acid composition of the bile showed that palmitic acid and stearic acids were the major saturated fatty acids and oleic and linoleic acids were the major unsaturated fatty acids. To our knowledge, no published data are available on the fatty acid composition of bile from chickens. Nakayama and Blomstrand (1961) stated that 90% of fatty acids in bile were associated with phospholipids. According to Alvaro *et al*. (1986) and van Berge Henegouwen *et al*. (1987), 90 to 95% of phospholipids in the bile are secreted as phosphatidylcholine which is consists of two molecular species containing palmitic acid in the sn-1 position and either linoleic or oleic acids in the sn-2 position. The fatty acid composition of phospholipids in animals and human has been reported in the literature. For example, Kawamoto *et al*. (1980) reported that palmitic and linoleic acids were the major fatty acids found in bile phosphatidylcholine of the rat. Alvaro *et al*. (1986) reported that the molecular species composition of phospholipids in bile were similar in various animal species (chicken, dog, sheep, rat, ox, pig, guinea-pig and human). The sn-1 and sn-2 positions of phospholipids were predominated filled by palmitic and linoleic acids. The fatty acid composition of phospholipids in the chicken bile showed that palmitic and linoleic acids dominated at the sn-1 and sn-2 positions. van Berge Heneqouwen *et al*., (1987) studied fatty acid composition of phospholipids in human bile and reported that palmitic acid was the major saturated fatty acid (41.4%) followed by stearic acid (5.5%), while linoleic acid was the major unsaturated fatty acids (32.8%) followed by oleic acid (12.1%). Palmitoleic and arachidonic acids were also observed in human bile phospholipids (2.7 and 5.6% respectively). Results from the present study confirm that the fatty acid profile of chicken bile was similar to that in the human bile and other animals reported by Kawamoto *et al*. (1980) and Alvaro *et al*. (1986). In contrast to that of Alvaro *et al*. (1986), however, the present data showed that linoleic acid was found in higher concentrations than stearic and palmitic acids in the

chicken bile. The current data also showed that there was a relatively high concentration of arachidonic acid in chicken bile which may be attributed to the synthesis of arachidonic acid by the elongation and desaturation of linoleic acid (McKee and McKee, 2009).

4.6. Conclusions

Overall, the current study improves the understanding of the dynamics of fat digestion and absorption, and also of the inevitable endogenous losses of fat and fatty acids during digestion. It was shown that the jejunum is the major intestinal site where majority of fat and fatty acids is digested and absorbed. The only exception was linoleic acid, almost half of which was digested and absorbed in the duodenum. Both chain length and degree of saturation influenced the digestibility of fatty acids. Long chain fatty acids showed lower digestibility than short chain fatty acid and unsaturated fatty acids were better digested than saturated fatty acids. The present data also suggest that lipids with high U: S ratios can improve the digestion and absorption of saturated fatty acids. The flow of ileal endogenous fat and fatty acids originate partly from unabsorbed bile.

The digestion of fat can be influenced by a number of other factors such as the age of birds and fat source. Further studies are warranted to investigate the effect of age of broiler chickens on the digestibility and energy utilisation of different fat sources.

CHAPTER 5

Influence of age on the apparent metabolisable energy and total tract fat digestibility of different fat sources for broiler chickens

5.1. Abstract

The influence of age of broiler chickens on the apparent metabolisable energy (AME) and total tract digestibility of fat in five sources (tallow, soybean oil, poultry fat, palm oil and a 50:50 blend of tallow and soybean oil) was investigated. The assay diets were developed by substituting the different fats for 40 g/kg (w/w) of a maize-soy basal diet and the measurements were made during weeks 1, 2, 3 and 5 post hatch. The AME of fats was strongly influenced ($P < 0.001$) by the age of broilers. The AME was markedly lower ($P < 0.05$) during week 1, but improved during week 2. There were no further improvements ($P > 0.05$) in the AME after week 2. There was no interaction ($P > 0.05$) between fat source and age of broilers for the AME, indicating that the effect of age on the AME was similar for all fat sources. Total tract fat digestibility generally followed the same trend as the AME of fats. The AME of palm oil, soybean oil and poultry fat were determined to be similar ($P > 0.05$), but higher ($P < 0.05$) than that of tallow. The AME of the 50:50 blend of tallow and soybean oil was 9% greater than the mean of the individual fat sources alone, indicating a synergistic effect. Overall, the present data highlight the physiological limitation in newly hatched chicks to effectively digest and utilise fats.

5.2. Introduction

Genetic development of modern broiler strains has increased their growth potential and the need for high intake of energy, which necessitates the feeding of high energy diets. Fats and oils are widely used in broiler diets to increase the dietary energy density because apparent metabolisable energy (AME) content of dietary fats is almost three times as high as that of other feedstuffs (NRC, 1994). For this reason, fat has become an essential component in the formulation of high energy broiler diets. Other benefits of adding fats include improved feed texture, reduced dustiness and increased palatability (NRC, 1994).

A diverse variety of fats and oils, including animal fats, vegetable oils and restaurant greases, are available to use in poultry diets. However, the digestibility and

AME of different fat sources vary widely, depending on their chemical characteristics (Lessire *et al*., 1982; Wiseman and Salvador, 1989; Blanch *et al*., 1995). In particular, fatty acid composition and, the length and saturation degree of the carbon chain have major effects on the utilisation of fats (Wiseman, 1984). Oils from plants, consisting of high proportions of unsaturated fatty acids, are relatively easily digested by birds. On the other hand, animal fats contain high proportions of saturated fatty acids which are poorly digested (Whitehead and Fisher, 1975).

The age of the birds is another major factor affecting the digestibility and AME of fats and oils. The physiological ability to digest and absorb fats is poorly developed in young birds, especially during the first week of life, but improves with increasing age (Carew *et al*., 1972; Freeman, 1984; Krogdahl, 1985). The saturation degree also has an influence on the digestion and absorption of fat in young chickens. Renner and Hill (1960) found that the ability to absorb fats with high proportions of palmitic and stearic acids was limited in young birds.

Most of the AME data for fats currently used by the feed industry are 20 to 40 years old and determined in studies conducted with slow-growing strains of broilers (Lessire *et al*., 1982; Wiseman *et al*., 1986; NRC, 1994). It is likely that genetic development in broilers during the past two decades may have improved the ability of birds to digest and utilise nutrients. Consequently, studies are warranted to investigate whether the AME values generated with older strains are applicable to modern fast-growing broiler chickens. Moreover, although the effect of age on the AME of fats has been examined in several studies (Renner and Hill, 1960; Carew *et al*., 1972; Wiseman and Salvador, 1989), the evaluations were often limited to only one or two fat sources and most did not include week 1 of life. The objective of the present experiment was, therefore, to investigate the effect of five sources of fat on the AME and total tract digestibility of fats for modern broilers from week 1 to 5 post-hatch.

5.3. Materials and methods

5.3.1. Birds and Housing

Two hundred and sixteen day-old male broilers (Ross 308) were obtained from a commercial hatchery. Birds were individually weighed and assigned on a weight basis to 36 cages (6 birds per cage). Each of the six dietary treatments was then randomly assigned to six cages. Housing conditions have been described in Chapter 3, section 3.1.

5.3.2. Diets

A maize-soy basal diet was formulated (Table 5.1). Five assay diets were then developed by substituting tallow, soybean oil, poultry fat, palm oil and, a 50:50 blend of tallow and soybean oil for 40 g/kg (w/w) of the basal diet. Diets, in mash form, were offered *ad libitum* and water was freely available throughout the trial.

Table 5.1. Composition of the basal diet used in the AME assay (g/kg)

Ingredient	(g)
Maize	607.0
Soybean meal, 480 g/kg	351.8
Dicalcium phosphate	21.7
Limestone	7.8
Salt	2.0
Sodium bicarbonate	2.3
DL Methionine	2.6
L-lysine	1.8
Trace mineral-vitamin premix[1]	3.0

[1]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4.0 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1.0 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

5.3.3. AME determination

The AME was determined using the classical total excreta collection method during weeks 1 (d 5 to 7), 2 (d 13 to 15), 3 (d 19 to 21) and 5 (d 33 to 35) post hatch as described in Chapter 3, section 3.2.

5.3.4. Chemical analysis

The diet and excreta samples were analysed for fat and GE as described in Chapter 3, section 3.4. Fatty acid composition of fat sources were analysed as described in Chapter 3, section 3.4.

5.3.5. Calculations

The AME of the basal diet and test diets was calculated using the formula described in Chapter 3 section 3.6.

The AME of fat sources was then calculated using the difference method, which assumes that there is no interaction between the basal diet and the fats.

$$\text{AME of fat} = \frac{(\text{AME of test diet}) - (\text{AME of basal diet} \times 0.96)}{0.04}$$

Apparent total tract digestibility coefficient of fat sources was calculated as follows:

$$\text{Total tract digestibility of test fat} = \frac{(ADC_{Td} \times Fat_{Td}) - (ADC_{Bd} \times Fat_{Bd} \times 0.96)}{(0.04 \times Fat_{Tf})}$$

where, ADC_{Td} = Apparent digestibility coefficient of test diet,

Fat_{Td} = Fat content of test diet,

ADC_{Bd} = Apparent digestibility coefficient of basal diet

Fat_{Bd} = Fat content of basal diet, and

Fat_{Tf} = Fat content of test fat

5.3.6. Data analysis

The data were analysed by repeated measures analysis (SAS, 2004). Differences were considered significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

5.4. Results

The fatty acid composition and profile in different fat sources are shown in Tables 5.2 and 5.3, respectively. Palm oil and tallow contained high concentrations of saturated fatty acids, mainly palmitic and stearic acids, whereas poultry fat and soybean oil contained high concentrations of unsaturated fatty acids, mainly oleic and linoleic acids. Soybean oil had the highest unsaturated to saturated fatty acid (U:S) ratio (5.06), following by poultry fat (2.07), whereas palm oil and tallow showed similar U:S ratios (0.93 and 0.80, respectively).

Table 5.2. Fatty acid composition of palm oil, poultry fat, soybean oil and tallow (g/kg)

	Palm oil	Poultry fat	Soybean oil	Tallow
Saturated fatty acids				
C8:0 Caprylic	0.3	-	0.3	0.2
C10:0 Capric	-	-	-	0.7
C12:0 Lauric	2.5	0.3	-	1.2
C14:0 Myristic	10.2	6.1	0.8	24.5
C16:0 Palmitic	428.3	225.4	103.1	184.9
C17:0 Margaric	1.3	2.2	1.1	14.7
C18:0 Stearic	41.6	57.3	40.3	187.6
C20:0 Arachidic	2.9	0.5	2.9	1.3
C21:0 Heneicosanoic	-	0.8	-	1.8
C22:0 Behenic	0.5	-	3.6	-
C23:0 Tricosanoic	-	1.9	-	-
C24:0 Lignoceric	0.5	-	1.2	-
Unsaturated fatty acids				
C14:1 Myristoleic	-	1.6	-	2.7
C15:1 Pentadecenoic	-	-	0.1	-
C16:1 Palmitoleic	1.5	61.3	0.7	21.4
C17:1 Heptadecenoic	-	1.0	0.4	4.0
C18:1 Elaidic	0.6	2.8	0.3	3.8
C18:1 Vaccenic	0.3	1.2	0.2	28.7
C18:1 Oleic	355.5	397.4	202.5	253.5
C18:1 Vaccenic	4.9	15.5	10.0	5.8
C18:2 Linoleic	86.5	114.9	491.5	9.1
C18:3 Linolenic	1.6	8.3	63.8	3.3
C20:1 Eicosenoic	1.0	3.4	6.1	0.7
C20:2 Eicosadienoic	-	1.0	0.4	-
C20:3 Eicosatrienoic	-	0.8	-	-
Total fatty acids (g/kg)	940.00	903.7	929.3	749.9

Table 5.3. Fatty acid profile of palm oil, poultry fat, soybean oil and tallow (g/kg fat)

	Palm oil	Poultry fat	Soybean oil	Tallow
Saturated fatty acids				
C8:0 Caprylic	0.4	-	0.3	0.3
C10:0 Capric	-	-	-	0.9
C12:0 Lauric	2.6	0.4	-	1.5
C14:0 Myristic	10.8	6.7	0.9	32.6
C16:0 Palmitic	455.6	249.4	110.9	246.6
C17:0 Margaric	1.4	2.5	1.2	19.6
C18:0 Stearic	44.2	63.4	43.4	250.2
C20:0 Arachidic	3.1	0.5	3.2	1.8
C21:0 Heneicosanoic	-	0.8	-	2.4
C22:0 Behenic	0.5	-	3.9	-
C23:0 Tricosanoic	-	2.1	-	-
C24:0 Lignoceric	0.5	-	1.3	-
Unsaturated fatty acids				
C14:1 Myristoleic	-	1.8	-	3.6
C15:1 Pentadecenoic	-	-	0.1	-
C16:1 Palmitoleic	1.6	67.9	0.8	28.5
C17:1 Heptadecenoic	-	1.1	0.4	5.3
C18:1 Elaidic	0.7	3.1	0.3	5.0
C18:1 Oleic	378.2	439.8	217.9	338.1
C18:1 Vaccenic	5.6	18.6	11.0	46.1
C18:2 Linoleic	92.1	127.2	528.8	12.2
C18:3 Linolenic	1.7	9.1	68.7	4.4
C20:1 Eicosenoic	1.0	3.7	6.5	0.9
C20:2 Eicosadienoic	-	1.1	0.4	-
C20:3 Eicosatrienoic	-	0.8	-	-
Saturated fatty acids	519.1	325.8	165.1	555.9
Unsaturated fatty acids	480.9	674.2	834.9	444.1
Unsaturated to saturated ratio	0.93	2.07	5.06	0.80

The influence of the age of broilers on the AME and total tract fat digestibility of the five fat sources is summarised in Table 5.4. The AME of fat was significantly affected ($P < 0.001$) by age of birds. For all fat sources, AME values were very low during week 1, almost doubled ($P < 0.05$) in week 2, with small non-significant ($P > 0.05$) increases in week 3, and then no further increase beyond week 3. There was no interaction ($P > 0.05$) between the fat source and age of birds for the AME.

Table 5.4. Influence of age of broilers on the AME (MJ/kg dry matter) and total tract fat digestibility coefficient of the five sources of fat[1]

Fat source	Age (Week)	AME	Total tract fat digestibility coefficient
Tallow	1	12.25	0.368
	2	22.21	0.653
	3	26.85	0.736
	5	26.64	0.726
Soybean oil	1	16.76	0.591
	2	35.44	0.898
	3	37.86	0.965
	5	37.27	0.948
Tallow: Soybean oil blend	1	17.70	0.500
	2	31.30	0.831
	3	33.06	0.830
	5	35.32	0.856
Poultry fat	1	18.05	0.600
	2	33.77	0.845
	3	35.08	0.928
	5	34.16	0.911
Palm oil	1	15.90	0.603
	2	32.79	0.806
	3	34.65	0.836
	5	35.22	0.843
SEM [2]		2.40	0.029
Treatment effects			
Fat source			
Tallow		21.99[b]	0.621[d]
Soybean oil		31.83[a]	0.850[a]
Tallow: Soybean oil blend		29.35[a]	0.754[c]
Poultry fat		30.27[a]	0.821[ab]
Palm oil		29.64[a]	0.772[bc]
SEM [2]		1.17	0.014
Age (week)			
1		16.13 [b]	0.532[c]
2		31.10 [a]	0.807[b]
3		33.50 [a]	0.859[a]
5		33.72 [a]	0.857[a]
SEM [2]		1.04	0.013
Probabilities, P ≤			
Fat source		* * *	* * *
Age		* * *	* * *
Fat source x Age		NS	NS

NS, not significant; *** P < 0.001.

[1] Each value represents the mean of six replicates.

[2] Pooled standard error of mean.

[a,b,c,d] Within each main effect, means in a column not sharing a common superscript are significantly different (P < 0.05).

In general, the influence of age and fat source on total tract fat digestibility coefficients followed the same trend as that for the AME. The only exception was that the fat digestibility coefficient of soybean oil was found to be higher ($P < 0.05$) than those of palm oil. The average fat digestibility was low ($P < 0.05$) during week 1, increased ($P < 0.05$) until week 3 and plateaued beyond week 3. No fat source x age interaction ($P > 0.05$) was observed for total tract fat digestibility.

The AME of fat was influenced ($P < 0.001$) by the source of fat. The AME of tallow was lower ($P < 0.05$) than those of soybean oil, palm oil and poultry fat. The AME of soybean oil, poultry fat and palm oil were determined to be similar ($P > 0.05$). There was no difference ($P > 0.05$) between the AME of soybean oil and the blend of tallow and soybean oil.

5.5. Discussion

The AME and total tract digestibility of fat are influenced by the age of broilers. In the present study, the AME and digestibility of fats were found to be very low during the first week of life. These results are in agreement with those of Carew *et al*. (1972) who measured the digestibility of maize oil and beef tallow during days 2 to 7 and days 8 to 15 of age. It was reported that the digestibility of maize oil and beef tallow were lower during day 2 to 7 (85 and 40%, respectively) but increased (to 95 and 79%, respectively), during day 8 to 15. Low digestibility and AME of fat during the first week can be explained by the poor physiological ability of newly hatched chicks to digest and absorb dietary fats. During the first week of life, the secretion of lipase, trypsin and amylase in chicks is reported to be very low. Noy and Sklan (1995) reported that the secretion of trypsin and amylase increased 20 to 100-fold between days 4 and 21 post hatch. However, the increase in lipase secretion was lower and slower than those of other enzymes. Leeson and Summers (2005) stated that the low digestibility of saturated fats in young birds can be attributed to low level of bile salts, inefficient on the recycling process of bile salts or inadequate of fatty acid binding protein.

The current results also showed that the AME and total tract digestibility of fats improved with increasing age. The AME of fats during week 2 of life almost doubled compared to week 1 and then remained constant thereafter. Digestibility of fat during week 2 increased by 52% compared to week 1 and a small, but significant, increase of 6.4% was observed during week 3. No further improvement in fat digestibility was noted after week 3. These findings are in agreement with those of Lessire *et al.* (1982)

who showed that the AME of tallow increased with age. They determined the AME of beef tallow at 2 and 6 weeks of age and reported that the AME increased by 4.3% between weeks 2 and 6. Wiseman and Salvador (1989) also demonstrated that the AME of various fat sources improved between 2 and 4 weeks of age. It was observed that the AME of tallow increased by 15.9% between weeks 2 and 4, whereas the AME of vegetable oil increased by only 4.4% and no further improvements were observed after week 4. Scheele *et al.* (1997) evaluated the AME and digestibility of animal fat in broilers at 2, 4, 6 and 8 weeks of age and reported that the AME and digestibility of fat were low during week 2 and increased significantly with increasing age. The AME was determined to be 27.96, 29.02, 32.40 and 33.19 MJ/kg during weeks 2, 4, 6 and 8 weeks, respectively.

The AME and total tract digestibility of fat were influenced by the source of fat. Tallow had a lower AME and digestibility of fat compared to soybean oil, poultry fat and palm oil. These results are consistent with those of Lessire *et al.* (1982); Wiseman and Salvador (1989) and Scheele *et al.* (1997) who also found that tallow had lower AME and fat digestibility than poultry fat, soybean oil and palm oil. The low AME and digestibility of tallow can be attributed to its fatty acid composition. It is well documented that the utilisation and AME of fats in poultry diets are correlated with the degree of saturation (Renner and Hill, 1961a; Sklan, 1979; Wiseman *et al.*, 1991; Baiao and Lara, 2005). Fats rich in saturated fatty acids are more poorly digested and absorbed than fats containing high concentrations of unsaturated fatty acids. Tallow consists of high concentrations of long chain saturated fatty acids, mainly palmitic and stearic acids, which are poorly digested and absorbed by poultry (Scott *et al*., 1982). Saturated fatty acids require bile salts for emulsification and to form micelles. Garrett and Young (1975) reported that the solubilisation and absorption of saturated fatty acids are more negatively affected in the absence of bile salts than those of unsaturated fatty acids. Both palmitic and stearic acids are non-polar and only form mixed micelles in the presence of conjugated bile salts and unsaturated fatty acids (Smulikowska and Mieczkowaka, 1996).

The fat digestibility of soybean oil was higher than that of palm oil and tallow, but similar to that of poultry fat. The high digestibility of soybean oil and poultry fat can be attributed to high concentrations of unsaturated fatty acids which are better digested and absorbed by poultry.

The AME of palm oil was found to be similar to that of soybean oil, poultry fat and the blend of tallow and soybean oil, but higher than tallow. Although palm oil had a U:S ratio similar to that of tallow, the AME and fat digestibility were both higher than tallow. These results are in contrast with those of Huyghebaert *et a*l. (1988) who reported that palm oil had lower AME than tallow. Moreover, Blanch *et al*. (1996) and Scheele *et al.* (1997) reported that the AME of palm oil was similar to that of tallow. Higher AME and digestibility of palm oil than that of tallow in the present study were unexpected, but an explanation may be provided by taking into account of the fatty acid composition of the two fats. Both palm oil and tallow had high concentrations of palmitic and stearic acids. As a percentage of total saturated fatty acids, tallow contained 44% palmitic acid and 45% stearic acid, whereas palm oil contained 88% palmitic acid and 9% stearic acid. As highlighted by Van Kuiken and Behnke (1994), long chain saturated fatty acids, particularly stearic acid, inhibit the activity of lipase. It is possible that the high concentrations of stearic acid in tallow may have inhibited the activity of lipase resulting in the lower AME and digestibility.

The AME of tallow, soybean oil and poultry fat at week 5 of age fall within the range reported in the literature (Table 5.5). However, the AME value of palm oil determined in the present study was higher than that reported by previous researchers (Wiseman and Salvador, 1991; Scheele *et al*., 1997; Zumbado *et al*., 1990; Pesti *et al*., 2002). The observed discrepancy may be attributed to different palm oil products being used in these studies. Palm oil products normally used in broiler diets include crude palm oil, refined palm oil (refined , bleached, deodorized, known as RBD), palm olein and palm acid oils (Walker, 2011). Unfortunately, the type of palm oil used in the previous AME studies is not adequately described. In the present study, refined palm oil was evaluated, which was similar to that used by Wiseman and Salvador (1991). However, the AME determined in the present study was 9% higher than that reported by these researchers. The AME of crude palm oil has been determined by Zumbado *et al.* (1999). Scheele *et al.* (1997) and Pesti *et al*. (2002) also measured the AME of palm oil, but the type of palm oil product used in their assays was not specified. The AME of palm oil determined in the current study was 37, 23 and 61% higher than those reported by Scheele *et al.* (1997), Zumbado *et al.* (1999) and Pesti *et al*. (2002), respectively.

Table 5.5. Comparison of the AME determined in the present study with published data

Fat source	Present data (at 5 weeks of age), MJ/kg	Published data (at 4-8 weeks of age), MJ/kg
Tallow	26.64	24.50 – 32.90[1]
Soybean oil	37.27	35.40 - 40.00[2]
Poultry fat	34.16	30.10 - 37.35[3]
Palm oil	35.22	21.88 – 32.30[4]

[1]Young (1961), Lessire *et al*. (1982), Wiseman and Salvador (1991), Scheele *et al.* (1997), Zumbado *et al*. (1999).
[2]Wiseman and Salvador (1991), Scheele *et al.* (1997), Pesti *et al*. (2002).
[3]Lessire *et al*. (1982), Scheele *et al.* (1997).
[4]Wiseman and Salvador (1991), Scheele *et al.* (1997), Zumbado *et al.* (1999), Pesti *et al*. (2002).

In the current study, the AME and fat digestibility coefficient in the blend of tallow and soybean oil was determined to be higher than the arithmetic averages of the tallow and soybean oil. The AME and fat digestibility were 9% and 2.5% higher in the blend than those predicted from its components. These results are in agreement with those of Lall and Slinger (1973) and Sibbald (1978) who reported that AME of the mixture between animal fats and vegetable oils was greater than the sum of the means of its components. Similarly, Muztar *et al*. (1981) reported that the AME of the blend of tallow and rapeseed soapstocks (1:1) was more than 4% higher than the AME calculated from its components. It is well documented that the utilisation of saturated fatty acids is improved in the presence of high concentrations of unsaturated fatty acids (Young and Garrett, 1963; Garrett and Young 1975; Leeson and Summers, 1976). Scheele *et al*. (1997) stated that a blend of hard fat with liquid oil tends to have better digestibility than that calculated from the digestibility of it components. This synergistic effect can be explained by high concentrations of unsaturated fatty acids acting as emulsifiers to enhance the emulsification of saturated fatty acids (Scheele *et al*., 1997). Overall the present results indicated that blending of tallow and soybean oil resulted in a synergistic effect on the AME and fat digestibility.

5.6. Conclusions

The present study demonstrated that the age of broilers influenced the AME and total tract digestibility of fat. Lower AME and fat digestibility were determined in week 1 of life confirming that the physiological ability to digest and absorb fats is immature at hatch, but this ability developed rapidly during week 2 and continued until week 3.

Soybean oil, poultry fat and palm oil showed higher AME and fat digestibility than that of tallow. The blending of tallow and soybean oil resulted in AME and fat digestibility estimates that were higher than the arithmetic averages of the individual ingredients. This synergistic effect suggests that the low AME and fat digestion in saturated fats could be partly overcome by such blending.

Overall, the present data highlight the limited ability of newly hatched chicks to digest and absorb fats, and the need to assign lower AME values to fats in the formulation of pre-starter diets. It may also be advisable to choose the right fat source for young birds, especially during week 1. The present results also suggest that soybean oil, poultry fat and palm oil can be the choice of ingredients for the feed industry. Blending of tallow with vegetable oils may provide an effective solution to improve the energy utilisation and digestibility of tallow.

Age is one of the bird-related factors influencing fat digestion in poultry, but there are also numbers of diet-related factors such as cereal type. Future studies are warranted to evaluate the influence of cereal type and fat source on performance, energy utilisation, fat digestibility and changes in gut microflora in broilers.

CHAPTER 6

Influence of cereal type and fat source on the performance, energy utilisation and fat utilisation in broiler starters

6.1. Abstract

An experiment was conducted to determine the influence of cereal type and fat source on the performance and, utilisation of energy and fat in broiler starters. The experimental design was 3 × 2 factorial arrangement of treatments, which included three cereals (wheat, maize or sorghum) and two fat sources (soybean oil or tallow). Broiler starter diets, based on each cereal and supplemented with 60 g/kg soybean oil or tallow, were formulated. Weight gain was not affected ($P > 0.05$) by fat source in sorghum-based diets, but increased ($P < 0.05$) with soybean oil supplementation compared to tallow supplementation in wheat- and maize-based diets. Feed per gain was lower ($P < 0.05$) in birds fed soybean oil diets compared to those fed tallow supplemented diets. The apparent metabolisable energy (AME) was affected ($P < 0.001$) by cereal type, with birds fed sorghum-based diets having the highest AME. The AME of tallow diets was found to be higher ($P < 0.001$) than that of soybean oil diets. However, total tract retention and ileal digestibility of fat were higher ($P < 0.05$) in birds fed soybean oil diets compared to those fed tallow supplemented diets.

In addition, by including an exogenous xylanase in the two wheat-based diets supplemented with soybean oil or tallow, the effects of enzyme supplementation on performance, nutrient utilisation and caecal microflora numbers were evaluated. Enzyme supplementation increased ($P < 0.01$) weight gain and decreased ($P < 0.01$) feed per gain. Birds fed soybean oil diets had higher ($P < 0.001$) weight gain, fat retention and fat digestibility, and lower feed per gain compared to those fed tallow supplemented diets. Addition of enzyme improved ($P < 0.01$) the AME in diets supplemented with either of fat sources, but the effect was more pronounced in birds fed tallow diets. The number of lactobacilli was unaffected by enzyme supplementation in birds fed soybean oil diets, but decreased ($P < 0.05$) in those fed tallow diets supplemented with enzyme. The number of clostridia was found to be higher ($P < 0.01$) in birds fed enzyme-supplemented diets. Overall, present results suggest that the effect of fat source on weight gain of broiler starters differed depending on the cereal base used in diet formulations.

6.2. Introduction

Current broiler strains require high daily energy intake to achieve their rapid growth potential and this often necessitates the dietary inclusion of lipids (fats and oils), which contain at least twice the available energy value as those of carbohydrates and protein (NRC, 1994). Animal fats and vegetable oils are widely used in poultry diets to increase the energy concentration. It is known that the utilisation of lipids by poultry is influenced by the characteristics of lipids (Renner and Hill, 1961a; Ward and Marquardt, 1983; Baiao and Lara, 2005). Soybean oil consists of high proportions of unsaturated fatty acids and is better utilised by poultry, whereas tallow contains long chain saturated fatty acids, which are nonpolar and difficult to digest and absorb (Krogdahl, 1985).

The cereal base used in feed formulation is another factor which may affect the digestion and absorption of lipids. It has been shown that high concentrations of non-starch polysaccharides (NSP) in wheat, barley and rye influence the digestion and absorption of lipids in poultry, and that the effect is more evident when tallow is used as the lipid source (Danicke *et al*., 1997b; Danicke, 2001). Antoniou *et al.* (1980) reported that the performance and fat digestibility were markedly depressed in rye-fed birds when the fat source was tallow, but the effects were much smaller when soybean oil was used. Feeding broilers on rye-based diets containing soybean oil resulted in higher body weights compared to those fed the same basal diets containing tallow (Danicke *et al*., 1997b). Poor digestion of saturated fats was attributed to higher intestinal viscosity, reduced gut motility and, decreased rate of diffusion and transportation of emulsion droplets, lipase, mixed micelles, bile salts and fatty acids in the gut lumen (Smulikowska, 1998).

Globally, maize is the most commonly used cereal to supply the energy in poultry diets, followed by wheat and sorghum. Wheat contains relatively high concentrations of NSP compared to maize and sorghum (Choct, 1997). While the interaction between viscous cereals and dietary fat sources is well documented (Danicke *et al*., 1997b; 1999a; Danicke, 2001), corresponding information on non-viscous cereals such as maize and sorghum are limited. It was hypothesised that the effect of dietary lipid source will differ in birds fed diets based on different cereals.

Wheat-based diets are routinely supplemented with exogenous glycanases (β-glucanase and xylanase) to overcome the problem of high digesta viscosity, and to improve nutrient digestibility and bird performance. Several studies have shown that the

effect of supplemental enzyme is influenced by the fat sources (Langhout *et al*., 1997; Danicke *et al*., 1999a). The enzyme effect on bird performance was found to be greater in diets supplemented with animal fats rather than vegetable oils (Smulikowska and Mieczkowska, 1996; Langhout *et al*., 1997; Danicke *et al*., 1999a). The influence of enzyme supplementation on gut microflora profile has been studied (Vahjen *et al*., 1998; Danicke *et al*., 1999b; Choct, 2006), but no studies have examined the interaction between enzyme supplementation and fat source on gut flora.

The aims of the present study were to investigate the influence of cereals (wheat, maize and sorghum) and dietary lipids (tallow and soybean oil) on the performance, apparent metabolisable energy (AME) and, the total tract retention and ileal digestibility of fat in young broiler chickens. A secondary aim was to examine the influence of enzyme supplementation and fat source on the caecal microflora counts in birds fed wheat-based diets.

6.3. Materials and methods

6.3.1. Birds and Housing

Three hundred and eighty four day-old male broilers (Ross 308) were obtained from a commercial hatchery, and individually weighed and allocated on the basis of body weight to 48 cages (8 birds per cage) in electrically heated battery brooders. Diets, in mash form, were offered *ad libitum* from day 1 to 21 post-hatch. Water was freely available at all times. Housing conditions were described in Chapter 3, section 3.1.

6.3.2. Diets

Six diets, based on each of the three cereals (wheat, maize and sorghum) with either tallow or soybean oil, were formulated to meet the Ross 308 strain recommendations (Ross, 2007) for major nutrients for broiler starters (Table 6.1). In addition, two more diets were developed by including a xylanase product (Avizyme®, Danisco Animal Nutrition, Marlborough, UK) to wheat-based diets supplemented with either soybean oil or tallow. All diets were formulated to be isocaloric and isonitrogenous. The calculated differences in AME between the diets containing tallow and soybean oil were overcome by the inclusion of cellulose, sand and maize starch.

6.3.3. Performance data

Performance data were recorded as described in Chapter 3, section 3.1.

Table 6.1. The composition and calculated analysis of the experimental diets (g/kg as fed)

Ingredient	Wheat-Soy oil	Wheat-Tallow	Maize-Soy oil	Maize-Tallow	Sorghum-Soy oil	Sorghum-Tallow	Wheat-Soy oil	Wheat-Tallow
Wheat	603.2	603.2	0	0	0	0	603.2	603.2
Maize	0	0	521.3	521.3	0	0	0	0
Sorghum	0	0	0	0	577.1	577.1	0	0
Soybean meal, 480 g/kg	76.7	76.7	170.4	170.4	113.5	113.5	76.7	76.7
Soy protein isolates	131.2	131.2	111.6	111.6	127.9	127.9	131.2	131.2
Soy oil[1]	60.0	0	60.0	0	60.0	0	60.0	0
Tallow[2]	0	60.0	0	60.0	0	60.0	0	60.0
Salt	2.6	2.6	2.7	2.7	2.6	2.6	2.6	2.6
DL-methionine	3.1	3.1	2.6	2.6	3.0	3.0	3.1	3.1
Lysine. HCl	3.4	3.4	1.8	1.8	3.1	3.1	3.4	3.4
L-Threonine	1.0	1.0	0.3	0.3	0.5	0.5	1.0	1.0
Dicalcium phosphate	20.8	20.8	21.0	21.0	20.6	20.6	20.8	20.8
Limestone	12.3	12.3	12.2	12.2	12.8	12.8	12.3	12.3
Trace mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[4]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Titanium dioxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Maize starch	4.7	47.3	0	42.6	0	42.6	4.7	47.3
Cellulose	50.0	20.0	50.0	30.0	50.0	30.2	50.0	20.0
Sand	24.9	12.3	40.0	17.4	22.8	0	24.9	12.3
Xylanase[5]	0	0	0	0	0	0	1.0	1.0
Total	1000	1000	1000	1000	1000	1000	1000	1000
Provision								
Metabolisable energy (MJ/kg)	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9
Crude protein	230	230	230	230	230	230	230	230
Calcium	10	10	10	10	10	10	10	10
Available phosphorus	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Methionine + Cysteine	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
Lysine	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8
Threonine	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Tryptophan	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7

[1]ME content in soy oil was assumed to be 38.0 MJ/kg.
[2]ME content in tallow was assumed to be 27.0 MJ/kg.
[3]Supplied per kilogram of diet: Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1.0 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.
[4]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4.0 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg.
[5]Avizyme®, Danisco Animal Nutrition, Marlborough, UK.

6.3.4. AME determination

Total collection of excreta was carried out between days 17-20 post-hatch to determine of AME, as described in Chapter 3, section 3.2.

6.3.5. Fat retention measurements

Excreta collected between days 17-20 post-hatch were processed and analysed for fat content as described in Chapter 3, section 3.4 and total tract fat retention was calculated.

6.3.6. Ileal digestibility determination

On day 21, four birds per replicate cage were euthanised by intravenous injection of sodium pentobarbitone, and digesta were collected and processed as described in Chapter 3, section 3.3.

6.3.7. Determination of microflora

On day 21, two more birds per replicate cage from the four wheat-based treatments were euthanised by intravenous injection of sodium pentobarbitone. Caecal cotents were collected and kept at -20 ºC before determination of microflora (*Lactobacillus*, *Bifidobacterium*, *Clostridium* and *Bacteroides* species) by fluorescent *in situ* hybridisation (FISH) method as described in Chapter 3, section 3.5.

6.3.8. Chemical analysis

Dry matter, gross energy, fat and titanium were analysed as described in Chapter 3, section 3.4. Insoluble, soluble and total NSP content of the diets were determined using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable alpha-amylase, protease and amyloglucosidase.

6.3.9. Calculations

The AME, and total tract retention and apparent ileal digestibility coefficient of fat were calculated using the formula described in Chapter 3, section 3.6.

6.3.10. Data analysis

Two separate statistical analyses were conducted. Data from the three cereal sources and two fat sources were analysed as a 3 x 2 factorial arrangement of treatments to determine the effect of cereal type and fat source. Data from wheat-based diets with two fat sources and two xylanase levels were analysed as a 2 x 2 factorial arrangement of treatments to determine the effect of fat source and enzyme xylanase, as described in Chapter 3, section 3.7.

6.4. Results

The fatty acid composition and fatty acid profile of the tallow and soybean oil used in the experiment are presented in Tables 6.2 and 6.3, respectively. Linoleic and oleic acids were the major unsaturated fatty acids in the soybean oil, whereas oleic acid was the major unsaturated fatty acid in the tallow.Tallow contained higher amounts of palmitic and stearic acids compared to that of soybean oil. Fatty acid profiles showed that the unsaturated to saturated fatty acid ratio of soybean oil and tallow were 4.80 and 0.63, respectively. Analysed NSP contents of the diets are summarised in Table 6.4. As expected, the wheat-based diet had higher soluble NSP level than maize- and sorghum-based diets. Diets supplemented with soybean oil had higher insoluble and total NSP contents than those supplemented with tallow.

Cereal type x fat source interactions

6.4.1. Performance

Mortality during the experiment was negligible. Only seven out of 288 birds died and the deaths were not related to any specific treatment.

An interaction between cereal type and fat source ($P < 0.05$) was observed for the weight gain (Table 6.5). Weight gain was unaffected by fat source in sorghum-based diets, but was higher with soybean oil supplementation compared to tallow supplementation in wheat- and maize-based diets.

The main effect of cereal type was significant ($P < 0.001$) for feed intake. Birds fed wheat-based diets had higher feed intake than those fed maize- and sorghum-based diets. Neither the main effect of fat source nor the interaction between cereal type and fat source was significant ($P > 0.05$) for feed intake.

The main effect of fat source was significant ($P < 0.001$) for feed per gain, with birds fed diets supplemented with tallow having higher feed per gain than those fed diets supplemented with soybean oil. Neither the main effect of cereal type nor the interaction between cereal type and fat source was significant ($P > 0.05$) for feed per gain.

Table 6.2. Fatty acid composition of soybean oil and tallow (g/kg)

Fatty acid	Soybean oil	Tallow
Saturated fatty acids		
C10:0 Capric	-	0.4
C12:0 Lauric	-	0.6
C14:0 Myristic	1.3	29.8
C16:0 Palmitic	105.3	225.7
C17:0 Margaric	1.7	18.3
C18:0 Stearic	44.4	237.5
C20:0 Arachidic	3.4	1.9
C21:0 Heneicosanoic	0.3	3.3
C22:0 Behenic	4.0	-
C24:0 Lignoceric	1.5	-
Unsaturated fatty acids		
C14:1 Myristoleic	-	4.5
C16:1 Palmitoleic	0.8	18.9
C18:1 Elaidic	-	3.3
C18:1 Vaccenic	-	20.7
C18:1 Oleic	216.9	257.5
C18:1 Vaccenic	11.1	5.7
C18:2 Linoleic	476.9	5.8
C18:3 Linolenic	3.3	-
C18:3 linolenic	63.8	6.6
C20:1 Eicosenoic	2.6	0.8
C20:2 Eicosadienoic	1.4	-
Total fatty acids (g/kg)	938.7	841.3

Table 6.3. Fatty acid profile of soybean oil and tallow (g/kg fat)

Fatty acid	Soybean oil	Tallow
Saturated fatty acids		
C10:0 Capric	-	0.5
C12:0 Lauric	-	0.7
C14:0 Myristic	1.4	35.4
C16:0 Palmitic	112.2	268.3
C17:0 Margaric	1.8	21.8
C18:0 Stearic	47.3	282.3
C20:0 Arachidic	3.6	2.3
C21:0 Heneicosanoic	0.3	3.9
C22:0 Behenic	4.3	-
C24:0 Lignoceric	1.6	-
Unsaturated fatty acids		
C14:1 Myristoleic	-	5.3
C16:1 Palmitoleic	0.8	22.5
C18:1 Elaidic	-	3.9
C18:1 Oleic	231.1	306.1
C18:1 Vaccenic	11.8	31.3
C18:2 Linoleic	508.0	6.9
C18:3 Linolenic	71.5	7.8
C20:1 Eicosenoic	2.8	1.0
C20:2 Eicosadienoic	1.5	-
Saturated fatty acids (g/kg)	172.5	615.2
Unsaturated fatty acids(g/kg)	827.5	384.8
Unsaturated to saturated ratio	4.80	0.63

Table 6.4. Analysis of insoluble, soluble and total NSP content (g/ kg DM [1]) of the diets

Cereal	Fat source	Insoluble NSP	Soluble NSP	Total NSP[2]
Wheat	Soybean oil	138	16	154
	Tallow	111	18	129
Maize	Soybean oil	139	11	150
	Tallow	109	15	124
Sorghum	Soybean oil	122	13	135
	Tallow	99	11	110

[1] Dry matter contents of wheat-soybean oil diet, 912 g/kg; wheat-tallow diet, 913 g/kg; maize-soybean oil diet 903 g/kg; maize-tallow diet 905 g/kg; sorghum-soybean oil diet 907 g/kg; sorghum-tallow diet 908 g/kg.
[2] Total NSP = Insoluble NSP + Soluble NSP.

Table 6.5. Influence of cereal type and fat source on the weight gain (g/bird), feed intake (g/bird) and feed per gain (g feed/g gain) of broiler starters, 1-21 days post-hatch[1]

	Fat source	Weight gain	Feed intake	Feed per gain
Wheat	Soybean oil	880[a]	1118	1.262
	Tallow	819[bc]	1093	1.336
Maize	Soybean oil	860[ab]	1055	1.239
	Tallow	782[c]	1054	1.338
Sorghum	Soybean oil	809[c]	1009	1.259
	Tallow	813[bc]	1052	1.300
SEM[2]		16.4	15.3	0.014
Main effects				
Cereal type				
Wheat		850	1106[a]	1.299
Maize		821	1054[b]	1.289
Sorghum		811	1030[b]	1.280
Fat source				
Soybean oil		850	1060	1.254[b]
Tallow		804	1066	1.325[a]
Probabilities, P ≤				
Cereal type		0.06	***	NS
Fat source		***	NS	***
Cereal type x Fat source		*	NS	NS

NS, not significant; * P < 0.05; *** P < 0.001.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.
[a,b,c] Means in a column not sharing a common superscript are significantly different (P < 0.05).

6.4.2. AME, and total tract retention and ileal digestibility of fat

The influence of dietary treatments on the AME, and, total tract retention and apparent ileal digestibility of fat is summarised in Table 6.6. The main effect of cereal type was significant (P < 0.001) for AME, with higher AME value being determined for sorghum-based diet compared to wheat- and maize-based diets. The main effect of fat source was also significant (P < 0.001) for AME, with tallow supplemented diets showing higher AME than soybean oil supplemented diets.

The main effect of cereal type was significant ($P < 0.05$) for the total tract retention of fat. Birds fed wheat-based diets had a lower fat retention coefficient compared to those fed maize- and sorghum-based diets. The main effect of fat source was significant ($P < 0.001$) for total tract retention and ileal digestibility coefficient of fat. Birds fed diets supplemented with soybean oil had higher values than those supplemented with tallow. No interaction ($P > 0.05$) was observed between cereal type and fat source for AME and, total tract retention and ileal digestibility of fat.

Table 6.6. Influence of the cereal type and fat source on the AME (MJ/kg DM) and, total tract fat retention and ileal fat digestibility of broiler starters[1]

	Fat source	AME	Total tract fat retention	Ileal fat digestibility
Wheat	Soybean oil	14.37	0.819	0.877
	Tallow	14.62	0.672	0.717
Maize	Soybean oil	14.45	0.850	0.892
	Tallow	14.61	0.697	0.666
Sorghum	Soybean oil	14.92	0.852	0.906
	Tallow	15.11	0.685	0.694
SEM[2]		0.07	0.007	0.016
Main effects				
Cereal type				
Wheat		14.49[b]	0.746[b]	0.797
Maize		14.53[b]	0.774[a]	0.779
Sorghum		15.01[a]	0.768[a]	0.800
Fat source				
Soybean oil		14.58[b]	0.840[a]	0.892[a]
Tallow		14.78[a]	0.685[b]	0.692[b]
Probabilities, P ≤				
Cereal type		***	*	NS
Fat source		***	***	***
Cereal type x Fat source		NS	NS	NS

NS, not significant; * $P < 0.05$; *** $P < 0.001$.

[1] Each value represents the mean of six replicates.

[2] Pooled standard error of mean.

[a,b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

6.4.3. Performance

The main effects of fat source ($P < 0.001$) and supplemental xylanase ($P < 0.01$) were significant for weight gain. Weight gain was higher in soybean oil supplemented diets compared to those supplemented with tallow (Table 6.7). Birds fed diets supplemented with xylanase showed higher weight gain than those without xylanase. There was no interaction ($P > 0.05$) between fat source and xylanase supplementation for weight gain.

Neither the main effects nor the interaction between fat source x xylanase was significant ($P > 0.05$) for feed intake.

Table 6.7. Influence of fat source and xylanase supplementation on weight gain (g/bird), feed intake (g/bird) and feed per gain (g feed/g gain) of broiler starters fed wheat-based diets, 1-21 days post-hatch[1]

Fat source	Xylanase	Weight gain	Feed intake	Feed per gain
Soybean oil	-	880	1118	1.262
	+	920	1117	1.221
Tallow	-	819	1093	1.336
	+	858	1126	1.312
SEM[2]		14.0	12.5	0.010
Main effects				
Fat source				
Soybean oil		900[a]	1118	1.242[b]
Tallow		838[b]	1110	1.324[a]
Xylanase				
-		850[b]	1106	1.299[a]
+		889[a]	1122	1.267[b]
Probabilities, P ≤				
Fat source		***	NS	***
Xylanase		**	NS	**
Fat source x Xylanase		NS	NS	NS

NS, not significant; ** $P < 0.01$; *** $P < 0.001$.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.
[a,b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

The main effects of fat source ($P < 0.001$) and supplemental xylanase ($P < 0.01$) were significant for feed per gain. Birds fed tallow diets had higher feed per gain compared to those fed soybean oil supplemented diets. Feed per gain was lower in birds fed diets supplemented with xylanase. No interaction ($P > 0.05$) was observed between the fat source and xylanase for feed per gain.

6.4.4. AME and total tract retention and ileal digestibility of fat

An interaction ($P < 0.01$) between the fat source and xylanase was observed for the AME (Table 6.8). In soybean oil supplemented diets, AME was not affected by xylanase addition, whereas xylanase addition resulted in higher AME in tallow supplemented diets.

The main effect of fat source was significant ($P < 0.001$) for fat retention. Birds fed soybean oil supplemented diets showed higher ($P < 0.001$) fat retention coefficient compared to those fed tallow supplemented diets. However, there was a tendency ($P = 0.058$) for fat source x xylanase interaction. In soybean oil supplemented diets, xylanase improved the retention, whereas in tallow supplemented diets, no effect was observed.

The main effect of fat source was significant ($P < 0.001$) for ileal fat digestibility. Birds fed soybean oil supplemented diets had higher ileal fat digestibility than those fed tallow supplemented diets. Neither the main effect of xylanase nor the interaction between fat source and xylanase was significant ($P > 0.05$) for ileal fat digestibility.

Table 6.8. Influence of fat source and xylanase supplementation on the AME (MJ/kg DM) and, total tract fat retention and ileal fat digestibility of broiler starters fed wheat-based diets[1]

Fat source	Xylanase	AME	Total tract fat retention	Ileal fat digestibility
Soybean oil	-	14.37[c]	0.819	0.877
	+	14.50[bc]	0.835	0.880
Tallow	-	14.62[b]	0.672	0.717
	+	15.07[a]	0.671	0.696
SEM[2]		0.04	0.004	0.015
Main effects				
Fat source				
Soybean oil		14.43	0.827[a]	0.879[a]
Tallow		14.84	0.672[b]	0.706[b]
Xylanase				
-		14.49	0.746	0.797
+		14.78	0.753	0.788
Probabilities, P ≤				
Fat source		***	***	***
Xylanase		***	NS	NS
Fat source x Xylanase		**	0.058	NS

NS, not significant; **P < 0.01; *** P < 0.001.

[1] Each value represents the mean of six replicates.

[2] Pooled standard error of mean.

[a,b,c] Means in a column not sharing a common superscript are significantly different (P < 0.05).

6.4.5. Caecal microflora counts

The influence of fat source and xylanase on a number of selected bacteria in the caecal contents is summarised in Table 6.9. A fat source x xylanase interaction ($P < 0.01$) was observed for lactobacilli. In tallow supplemented diets, supplemental xylanase decreased the numbers of lactobacilli compared to diets without xylanase, whereas no effect was observed in soybean oil diets.

Xylanase supplementation influenced ($P < 0.01$) the numbers of *Clostridium* species, with numbers being greater in birds fed xylanase-supplemented diets compared to those fed diets without xylanase. Neither the main effect of fat source nor the interaction between fat source and xylanase was significant ($P > 0.05$) for the numbers of *Clostridium* species.

The numbers of *Bifidobacterium* and *Bacteroides* species were not affected ($P > 0.05$) by dietary treatments.

Table 6.9. Influence of fat source and xylanase supplementation on bacterial counts (log 10 cells/g caecal content) in the caeca of broiler starters[1]

	Xylanase	*Lactobacillus*	*Bifidobacterium*	*Clostridium*	*Bacteroides*
Soybean oil	-	7.19[b]	7.24	7.09	6.72
	+	7.12[b]	7.22	7.22	6.48
Tallow	-	7.80[a]	7.39	7.13	6.79
	+	7.28[b]	7.33	7.36	6.76
SEM[2]		0.076	0.085	0.054	0.102
Main effects					
Fat source					
Soybean oil		7.15	7.23	7.16	6.60
Tallow		7.54	7.36	7.24	6.77
Xylanase					
-		7.50	7.32	7.11[b]	6.75
+		7.20	7.28	7.29[a]	6.62
Probabilities, P ≤					
Fat source		***	NS	NS	NS
Xylanase		***	NS	**	NS
Fat source x Xylanase		**	NS	NS	NS

NS, not significant; ** $P < 0.01$; *** $P < 0.001$.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.
[a,b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$).

6.5. Discussion

Cereal type x fat source interactions

The present study was conducted to investigate whether the performance of broiler starters fed diets based on different cereals was influenced by different fat sources. An interaction between cereal type and fat source was observed for weight gain. Birds fed wheat- and maize-based diets with tallow had lower weight gain than those fed diets with soybean oil. However, weight gain of birds fed sorghum-based diets was not influenced by fat source. The observed depression in weight gain of birds tallow-supplemented diets based on viscous cereals, such as wheat, is in agreement with published data (Danicke *et al*., 1999a) and has been attributed to high intestinal viscosity caused by soluble NSP. The interaction observed in maize-based diets may also be explained by high concentrations of soluble NSP, which originate from the relatively high inclusion levels of soybean meal used in the diet formulation (Table 6.1).

Feed intake of wheat-based diets was higher than those of maize- and sorghum-based diets. Annison (1993) stated that birds fed diets containing high concentrations of NSP may have higher feed intake because of the inhibition of the assimilation of nutrients and the need of the birds to consume more feed to compensate the poor absorption of nutrients.

Fat source influenced the feed per gain, with birds fed tallow diets having higher feed per gain than those fed soybean oil diets. Similar findings have been reported by Smulikowska and Mieczkowska (1996) and Danicke *et al*. (1999a). Higher feed per gain in birds fed tallow supplemented diets indicate that the utilisation of tallow was poorer than soybean oil and this was confirmed by the lower total tract fat retention and ileal fat digestibility of tallow in the current study.

The AME of wheat based- and maize-based diets were determined to be similar, and lower than that of sorghum-based diets. Lower AME in birds fed wheat- and maize-based diets may be attributed to the higher NSP concentrations in these diets

Tallow supplemented diets had higher AME than soybean oil supplemented diets. This finding is in contrast with those of Smulikowska and Mieczkowska (1996) and Preston *et al.* (2001) who reported that the AME of soybean oil diets was higher than tallow diets. Higher AME values of diets supplemented with tallow in the present study was unexpected and, contrary to othe results obtained such as the better retention and digestibility of fat in soybean oil supplemented diets. One possible explanation is

that this may be related to the level of NSP in these diets. As shown by the analysis (Table 6.4), soybean oil supplemented diets had consistently higher insoluble and total NSP compared to tallow supplemented diets. The addition of cellulose to the soybean oil supplemented diets to achieve AME contents similar to that of tallow supplemented diets resulted in higher insoluble and total NSP contents which may have lowered the AME values.

Birds fed wheat-based diets had lower total tract fat retention than those fed maize- and sorghum-based diets. Although total NSP of wheat-based diets was almost similar to maize-based diets, wheat-based diets had slightly higher soluble NSP than maize-based diets. Soluble NSP not only increase digesta viscosity, but also stimulate microbial growth in the gastrointestinal tract (Smulikowska and Mieczkowska, 1996; Choct, 1997). Annison and Choct (1991) stated that when diets with high levels of NSP are ingested, NSP are solubilised in the anterior gastrointestinal tract of chickens. In the hindgut, solubilised NSP can be a fermentable carbohydrate source, which aids the proliferation of anaerobe bacteria. Increasing bacterial populations in the intestinal tract may have a systemic effect on gut secretions, intestinal morphology and increase the deconjugation of bile acids, causing the lower retention of fat.

Birds fed diets supplemented with soybean oil showed higher retention and ileal digestibility of fat compared to those fed tallow supplemented diets. These results are consistent with the findings of several researchers who reported that the tallow had lower digestibility compared to soybean oil (Smulikowska and Mieczkowska, 1996; Danicke *et al.*, 1997b; 1999a). It is well documented that fat utilisation in birds is correlated with the degree of saturation. Fats rich in unsaturated fatty acids are better digested and absorbed than those containing high proportions of saturated fatty acids (Sklan, 1979; Danicke, 2001). Moreover, the absorption of long chain saturated fatty acids is limited by their incorporation rate into micelles (Friedman and Nylund, 1980). Saturated fatty acids are less rapidly incorporated into micelles than polyunsaturated fatty acids because of their non-polarity, which makes them reliant on an adequate presence of bile salts for efficient emulsification (Polin *et al.*, 1980; Danicke, 2001). Higher total tract fat retention and ileal fat digestibility of soybean oil can be attributed to higher proportions of unsaturated fatty acids in soybean oil which are absorbed easily, whereas saturated fatty acids in tallow need to be emulsified prior to absorption (Krogdahl, 1985).

Fat source x xylanase interaction in wheat-based diets

As expected, xylanase supplementation of wheat-based diets increased weight gain and improved feed per gain. These results are consistent with the findings of other authors (Bedford and Classen, 1992; Bedford 1995, 1996; Danicke *et al*., 1997a; Choct *et al*., 2004; Wu *et al*., 2004). Xylanase addition has been shown to degrade soluble arabinoxylans and to reduce the digesta viscosity in the intestinal tract (Bedford and Classen, 1992; Choct, 1997; Danicke *et al*., 1997a; Preston *et al*., 2001), resulting in improved nutrient digestibility and performance.

Xylanase supplementation increased the AME of tallow supplemented diets, but had no effect on soybean oil supplemented diets. A similar finding was reported by Langhout *et al*. (1997) who found that the birds fed diets containing animal fats supplemented with enzyme showed greater improvement in AME than those fed diets containting soybean oil supplemented with enzyme. Enzyme supplementation of rye or wheat-based diets containing animal fats resulted in greater response than those containing vegetable oil (Antoniou and Marquardt, 1982; Smulikowska and Mieczkowska, 1996; Danicke *et al*., 1997b; 1999a). It is well documented that NSP in viscous cereals increase the digesta viscosity which may slow down the rate of diffusion of substrates, decrease digestive enzyme activity, decrease the formation of mixed micelles and depress digestion of fat and other nutrients (Choct, 1997; Smulikowska, 1998). Addition of xylanase to soybean oil supplemented diets had no effect on the AME possibly due to the fact that soybean oil contains high amounts of unsaturated fatty acids which have been shown to be highly digestible even in high viscous chyme conditions (Danicke *et al*., 1997b).

The number of lactobacilli decreased in birds fed tallow diets supplemented with xylanase, but no effect of xylanase on number of lactobacilli was observed in birds fed soybean oil diets. The present results are in contrast with those of Nian *et al*. (2011) who reported that birds fed soybean oil diets supplemented with xylanase had higher caecal lactobacilli counts than those fed diets without xylanase. High concentration of NSP in wheat when combined with tallow, a saturated fat, may result in higher digesta viscosity compared to soybean oil (Danicke *et al*., 1999a), which may increase the population of gut microflora. The addition of xylanase may be expected to diminish the combined negative effects of saturated fats and wheat NSP, leading to the greater

reduction of lactobacilli compared to tallow diets without xylanase. However, this was not the case and these results are difficult to explain.

Supplemental xylanase increased the numbers of clostridia in the caeca of broilers. These findings are in contrast with those of Choct *et al*. (2006) who reported that enzyme supplementation of wheat-based diets reduced the numbers of *C. perfingens* in caecal contents. Higher numbers of clostridia in xylanase supplemented diets in this study was unexpected. However, it should be noted that the increasing clostridia numbers in the present study did not have any negative effect on the performance of broilers.

6.6. Conclusions

In conclusion, the effect of fat source differed depending on cereal type; with tallow supplemented diets resulting in lower weight gain than soybean oil in wheat- and maize-based diets. Fat source had no effect on the weight gain of birds fed sorghum-based diets. Birds fed soybean oil supplemented diets had lower feed per gain, higher fat retention and ileal fat digestibility compared to those fed tallow supplemented diets due to higher unsaturated to saturated ratios in soybean oil compared to tallow. Addition of xylanase to wheat-based diets increased weight gain and lowered feed per gain of broilers. The benefit of supplemental xylanase on AME was more pronounced in tallow diets than in soybean oil diets. Contrary to expectations, supplemental xylanase lowered lactobacilli counts and increased clostridia counts in the caecal contents. Overall, the present results suggest that soybean oil is a better source of fat for broiler starters compared to tallow in terms of growth rate (only wheat- and maize-based diets), feed efficiency, total tract retention and ileal digestibility of fat in all three cereals tested. Addition of xylanase improved growth rate and feed efficiency of broiler starters regardless of the fat source.

Cereal type is a major diet-related factor influencing the digestion of lipids. However, factors such as dietary calcium level may also have an influence on the digestion of fat. Therefore, future studies are warranted to evaluate the influence of fat and calcium concentrations on performance, and energy utilisation and nutrient digestibility in broiler starters.

CHAPTER 7

Influence of tallow and calcium concentrations on the apparent metabolisable energy and nutrient utilisation in broiler starters fed maize-based diets

7.1. Abstract

The influence of tallow and calcium concentrations on the performance, AME, total tract retention and ileal digestibility of nitrogen, calcium and phosphorus in broiler starters fed maize-based diets was examined. The experimental design was a 3 × 3 factorial arrangement of treatments evaluating three inclusion levels of tallow (0, 40 and 80 g/kg) and three concentrations of calcium (11, 13 and 16 g/kg). Nine treatment diets were formulated to meet the requirements for major nutrients for broiler starters, except for AME and calcium concentrations. The results showed that increasing tallow inclusion increased ($P < 0.001$) weight gain and decreased ($P < 0.001$) feed per gain. Increasing calcium concentration decreased ($P < 0.001$) weight gain. Birds fed diets containing 11 g/kg calcium had similar ($P > 0.05$) feed per gain to 13 g/kg calcium but lower ($P < 0.05$) than that of 16 g/kg calcium. In diets with no tallow, increasing calcium concentration decreased ($P < 0.05$) feed intake, whereas in diets with 40 and 80 g/kg tallow containing 16 g/kg calcium showed lower ($P < 0.05$) fed intake than that of 11 and 13 g/kg calcium. Diets supplemented with 40 and 80 g/kg tallow containing 11 g/kg calcium showed the lowest ($P < 0.05$) excreta soap. Total tract retention of fat was found to be higher ($P < 0.001$) in diets with 40 g/kg tallow. Birds fed diets containing 11 g/kg calcium had similar ($P > 0.05$) fat retention to that of 13 g/kg calcium, but higher ($P < 0.05$) than that of 16 g/kg calcium. Calcium retention decreased ($P < 0.001$) with increasing calcium concentrations. Diets containing 16 g/kg calcium had the lowest ($P < 0.01$) phosphorus retention. Diets with no inclusion of tallow containing 11 g/kg calcium had higher ($P < 0.05$) nitrogen retention than that of 16 g/kg calcium, but similar to 13 g/kg calcium, whereas in 40 g/kg tallow diets, 11 g/kg calcium had the highest ($P < 0.05$) nitrogen retention. Increasing fat inclusion increased ($P < 0.001$) digesta soap. Diets with 40 g/kg tallow had the highest ileal digestibility of fat ($P < 0.001$) and nitrogen ($P < 0.01$). Increasing inclusion of fat decreased ($P < 0.001$) ileal calcium digestibility. Diets containing 11 g/kg calcium showed the highest ileal digestibility of fat ($P < 0.01$), nitrogen ($P < 0.01$) and phosphorus ($P < 0.001$). Birds fed diets containing 16 g/kg calcium had higher ileal digestibility of calcium ($P < 0.001$) and ash ($P < 0.001$) than those fed 11 and

13 g/kg calcium diets. Overall, the current results suggest that supplementation of 40 g/kg of tallow showed higher total tract retention and ileal digestibility of fat than supplementation of 0 and 80 g/kg tallow. High dietary calcium concentrations adversely affected the performance and, energy and mineral utilisation in broiler starters.

7.2. Introduction

Lipids, which contain at least twice the available energy of of carbohydrates and protein, are widely used in poultry diets to meet the energy requirements of fast growing modern broilers. Tallow is commonly used as a feed ingredient in countries with large red meat industries because of its relatively low cost. Tallow consists of a high proportion of saturated fatty acids, particularly long chain saturated fatty acids such as palmitic and stearic acids, which are nonpolar and difficult to be digested and absorbed by poultry (Krogdahl, 1985).

Calcium plays an important role in the growth, skeletal development and biological functions of the animal. Low dietary calcium concentrations result in poor bone development and reduce growth rate, and deficiency of calcium in layer diets results in soft-shelled eggs (Tisch, 2006). On the other hand, high dietary calcium has also been linked to negative performance in broilers (Sebastian *et al*., 1996; Rama Rao *et al*., 2006) and layer hens (Hurwitz *et al*., 1969). Studies have shown that high calcium concentrations may decrease the digestibility of nutrients, especially fat, in broilers (Atteh and Leeson, 1983; 1984). During the digestion of fat, two of the fatty acids from the triglyceride are released (Krogdahl, 1985). These free fatty acids have the potential to bind with other nutrients such as calcium and magnesium and form soluble or insoluble soap in the gut lumen. If insoluble soaps are formed, there is the possibility that both the fatty acid and the mineral will be unavailable and excreted. Formation of excreta soap has been shown to increase with increasing dietary calcium concentrations. Moreover, excreta soap formation was found to be higher in birds fed diets supplemented with saturated fatty acids than those fed diets containing unsaturated fatty acids (Atteh and Leeson, 1983; 1984), suggesting that the energy derived from lipids may be limited by metabolic soaps, particularly in broilers fed saturated fats. When supplied in the form of limestone, high dietary calcium also increases the pH in the anterior gastrointestinal tract, leading to the reduction in bioavailability of other minerals, including phosphorus, zinc, and magnesium (Shafey and Mcdonald, 1991; Sebastian *et al*., 1996; Tamim *et al*., 2004). The adverse effect of high concentrations of

calcium is more pronounced on phosphorus utilisation due to the ability of calcium to bind with phytate and form insoluble complexes (Qian *et al*., 1997; Tamin *et al*., 2004).

Dietary calcium concentration is an important practical issue because of possible adverse effects on the digestion of lipids and other nutrients. Therefore, the present study was undertaken to investigate the influence of different dietary concentrations of tallow and calcium on the performance, apparent metabolisable energy (AME) and, total tract retention and ileal digestibility of fat and minerals in broiler starters. It was hypothesised that birds fed diets containing different tallow and calcium concentrations will show different patterns of growth responses, utilisation of fat, energy and mineral.

7.3. Materials and methods

7.3.1. Birds and Housing

Two hundred and eighty eight day-old male broilers (Ross 308) were obtained from a commercial hatchery, individually weighed and allocated on the basis of body weight to 36 cages (8 birds per cage) in electrically heated battery brooders. Diets, in mash form, were offered *ad libitum* from day 1 to 21 post hatch. Water was freely available at all times. Housing conditions have been described in Chapter 3, section 3.1.

7.3.2. Diets

Nine experimental diets, based on the maize and soybean meal, containing three inclusion levels of tallow (0, 40 and 80 g/kg) and three concentrations of calcium (7, 10 and 13 g/kg), were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters, except for AME and calcium concentrations (Table 7.1).

7.3.3. Performance

Performance data were recorded as described in Chapter 3, section 3.1.

7.3.4. AME determination

Total collection of excreta was carried out between days 17-20 post-hatch for the determination of AME, as described in Chapter 3, section 3.2.

Table 7.1. The composition and calculated analysis of the experimental diets (g/kg as fed)

Ingredient	T[1] 0 Ca[2] 7	T 0 Ca 10	T 0 Ca 13	T 40 Ca 7	T 40 Ca 10	T 40 Ca 13	T 80 Ca 7	T 80 Ca 10	T 80 Ca 13
Maize	502.1	502.1	502.1	526.4	526.4	526.4	460.1	460.1	460.1
Soybean meal, 480g/kg	380.2	380.2	380.2	375.7	375.7	375.7	387.9	387.9	387.9
Tallow[3]	0	0	0	40.0	40.0	40.0	80.0	80.0	80.0
Maize starch	60.0	60.0	60.0	0	0	0	0	0	0
Sand	15.9	8.0	0.1	15.9	8.0	0.1	30.2	22.3	14.4
Dicalcium phosphate	20.1	20.1	20.1	20.1	20.1	20.1	20.2	20.2	20.2
Limestone	3.8	11.7	19.6	3.8	11.7	19.6	3.7	11.6	19.5
DL-methionine	3.6	3.6	3.6	3.6	3.6	3.6	3.7	3.7	3.7
Titanium dioxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sodium bicarbonate	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Trace mineral premix[4]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lysine. HCl	2.2	2.2	2.2	2.3	2.3	2.3	2.1	2.1	2.1
L Threonine	0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.8	0.8
Vitamin premix[5]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Calculated									
Metabolisable energy (MJ/kg)	11.9	11.9	11.9	12.4	12.4	12.4	12.7	12.7	12.7
Crude protein	230	230	230	230	230	230	230	230	230
Calcium	7.0	10.0	13.0	7.0	10.0	13.0	7.0	10.0	13.0
Total phosphorus	7.5	7.5	7.5	7.6	7.6	7.6	7.5	7.5	7.5
Available Phosphorus	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Methionine + cystine	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7
Lysine	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3
Threonine	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4
Analysed (as fed)									
Crude protein (N x 6.25)	220	220	225	212	197	206	199	215	201
Fat	29.1	29.1	29.0	68.9	68.6	70.4	101.8	106.1	109.5
Calcium	9.8	12.6	16.5	10.8	13.4	16.5	11.0	12.7	15.8
Phosphorus	6.9	6.7	6.8	6.9	7.0	6.9	7.0	6.9	6.7

[1]Tallow.
[2]Calcium.
[3]ME content in tallow was assumed to be 27.0 MJ/kg.
[4]Supplied per kilogram of diet: Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1.0 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.
[5]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4.0 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg.

7.3.5. Total tract retention measurements

Excreta collected between days 17-20 post hatch were processed and analysed for fat, nitrogen, calcium and phosphorus contents as described in Chapter 3, section 3.4 and total tract retention coefficients of fat, nitrogen, calcium and phosphorus were calculated.

7.3.6. Ileal digestibility and toe ash determination

On day 21, four birds from each cage were euthanised by intravenous injection of sodium pentobarbitone and the toes from both legs of four birds per replicate were collected. Ileal digesta were collected and processed as described in Chapter 3, section 3.3. Diet and digesta samples were analysed for fat, nitrogen, ash, calcium and phosphorus contents. Toe samples were analysed for ash.

7.3.7. Gizzard pH determination

On day 21, two birds from each cage were euthanised by intravenous injection of sodium pentobarbitone and the gizzards were collected. To measure gizzard pH, the procedure reported by Pang and Applegate (2007) was used. In brief, the gizzard digesta was immediately collected. Nine-fold of distilled deionised water of the digesta weight (weight/volume) was added to beaker and stirred for 5 min. The pH of the solution was then measured by a pH meter (ISFET pH meter IQ 120, Shindengen, Japan) and assumed as the pH of the gizzard contents.

7.3.8. Chemical analysis

Dry matter, nitrogen, gross energy, calcium, phosphorus, titanium and fat contents were determined as described in Chapter 3, section 3.4.

Ash was determined by standard procedures (method 942.05; AOAC, 2005). In brief, samples were weighed into dried and pre-weighed beakers. Beakers were placed into the oven at 105 C for 16 hours, re-weighed and then beaker was placed into the muffle furnace at 550°C for 16 hours. After cooling in a desiccator, the weight of the beakers was recorded and ash content was calculated.

To measure the proportion of fat and fatty acids presented as soap in excreta and digesta, the procedure reported by Atteh and Leeson (1984) was used. In brief, the samples were subjected to two stages of petroleum ether extraction. The first extraction

removed neutral fat and free fatty acids which were not presented as soaps. The residue from the first extraction was then placed in 25% hydrochloric acid for about 1 hour at 100°C to liberate the fatty acids that were presented as soap. The samples were then extracted with diethyl ether and petroleum ether. These extracts were considered to represent the fat and fatty acids that were presented as soap.

7.3.9. Calculations

The AME and, apparent ileal digestibility and total tract retention coefficients of fat, nitrogen, calcium, phosphorus and ash were calculated using the formula described in Chapter 3, section 3.6.

7.3.10. Data analysis

The data were analysed as a two way factorial arrangement of treatments, as described in Chapter 3, section 3.7.

7.4. Results

Analysed crude protein, fat, calcium and phosphorus contents of the experimental diets are shown in Table 7.1. Analysis showed that diets with no tallow inclusion contained 29 g/kg of fat, while diets with 40 and 80 g/kg tallow contained 69 and 106 g/kg of fat. Analysed values of calcium in diets were found to be higher than the calculated values. The diet with 7 g/kg calcium contained 11 g/kg of calcium, whereas diet with 10 and 13 g/kg calcium contained 13 and 16 g/kg of calcium, respectively. Therefore, the analysed values are used in the presentation and discussion of the results.

7.4.1. Performance

Mortality during the experiment was negligible. Only eight out of 288 birds died and the deaths were not related to any specific treatment. The influence of the dietary treatments on the performance of broilers is summarised in Table 7.2. The main effects of tallow inclusion and calcium concentration were significant ($P < 0.001$) for weight gain. Birds fed diets supplemented with 80 g/kg of tallow showed higher weight gain than those fed 0 and 40 g/kg supplemented tallow, whereas birds fed diets with 40 g/kg supplemented fat had higher weight gain compared to 0 g/kg tallow. Increasing calcium concentration decreased ($P < 0.001$) weight gain. Birds fed diets contained 16 g/kg calcium had lower

weight gain compared to those fed diets contained 11 and 13 g/kg calcium and birds fed diets contained 13 g/kg calcium had lower weight gain than those fed diets contained 11 g/kg calcium. No interaction between tallow inclusion and calcium concentration was observed (P > 0.05) for weight gain.

Table 7.2. Influence of calcium concentration on the weight gain (g/bird), feed intake (g/bird) and feed per gain (g feed/g gain) of broiler starters fed diets containing different inclusions of tallow, 1-21 d post-hatch[1]

Tallow, (g/kg)	Ca g/kg	Weight gain	Feed intake	Feed per gain
0	11	755	1004^{bc}	1.347
0	13	684	919^{d}	1.391
0	16	569	769^{e}	1.421
40	11	807	1050^{b}	1.330
40	13	789	1029^{b}	1.352
40	16	705	954^{cd}	1.359
80	11	915	1195^{a}	1.284
80	13	887	1139^{a}	1.295
80	16	747	988^{bc}	1.340
SEM[1]		22.5	22.5	0.0175
Main effects				
Tallow inclusion				
0		669^{c}	897	1.386^{a}
40		767^{b}	1011	1.347^{b}
80		850^{a}	1107	1.306^{c}
Ca concentration				
11		826^{a}	1083	1.320^{b}
13		786^{b}	1029	1.346^{ab}
16		674^{c}	903	1.373^{a}
Probabilities, P ≤				
Tallow inclusion		***	***	***
Ca concentration		***	***	***
Tallow inclusion x Ca concentration		NS	*	NS

NS, not significant; *, P<0.05; *** P < 0.001.

[1] Each value represents the mean of four replicates.

[2] Pooled standard error of mean.

[a-e] Means in a column not sharing a common superscript are significantly different (P < 0.05)

There was an interaction ($P < 0.05$) between tallow and calcium concentrations for feed intake. In diets containing 0 g/kg tallow, increasing calcium concentration decreased the feed intake, whereas in diets containing 40 and 80 g/kg supplemented fat, 11 and 13 g/kg calcium resulted in similar feed intake but higher than 16 g/kg calcium.

Inclusion of tallow had a significant effect ($P < 0.001$) on feed per gain. Increasing inclusion of tallow decreased feed per gain, with birds fed diets supplemented with 80 g/kg of tallow having the lowest feed per gain compared to those fed diets supplemented with 0 and 40 g/kg of fat. The main effect of calcium concentration was significant ($P < 0.001$) for feed per gain. Birds fed diets containing 11 g/kg calcium had similar feed per gain to 13 g/kg calcium but lower than birds fed 16 g/kg calcium. The interaction between tallow and calcium concentrations was not significant ($P > 0.05$) for feed per gain.

7.4.2. Excreta soap, AME and mineral retention

The influence of the dietary treatments on excreta soap, AME, total tract fat and mineral retention in broiler starters is presented in Table 7.3. An interaction between tallow inclusion and calcium concentration was observed ($P < 0.01$) for excreta soap. In diet with no tallow, calcium concentration had no effect on excreta soap, whereas in diets with 40 g/kg tallow, 11 g/kg calcium had similar excreta soap to 13 g/kg calcium, but lower than 16 g/kg calcium and in diets with 80 g/kg tallow, 13 and 16 g/kg calcium resulted in higher excreta soap compared to 11 g/kg calcium.

As expected, the main effect of tallow inclusion was significant ($P < 0.001$) for AME, with diets containing no tallow showing the lowest AME. There was a tendency ($P = 0.06$) for calcium concentration to affect the AME. Diets containing 11 and 13 g/kg calcium tended to have similar AME, but higher than those containing 16 g/kg calcium. The interaction between tallow and calcium concentrations was not significant ($P > 0.05$) for AME.

Inclusion of tallow significantly ($P < 0.001$) affected the total tract retention of fat, with higher fat retention in birds fed diets supplemented with 40 g/kg tallow. Fat retention was also affected ($P < 0.05$) by the dietary concentration of calcium. Birds fed diets containing 11 g/kg calcium had fat retention similar to those fed diets containing 13 g/kg

calcium, but higher than those fed diets with 16 g/kg calcium. The interaction between tallow and calcium concentrations was not significant (P > 0.05) for fat retention.

Table 7.3. Influence of tallow inclusion and calcium concentration on the excreta soap (g/100 g excreta, DM basis), AME (MJ/kg DM), total tract retention coefficients of fat, calcium (Ca), phosphorus (P) and nitrogen (N) in broiler starters[1]

Tallow, g/kg	Ca, g/kg	Excreta soap	AME	Total tract retention			
				Fat	Ca	P	N
0	11	2.34^{c}	12.78	0.552	0.489	0.469	0.615^{a}
0	13	2.39^{c}	12.78	0.552	0.399	0.434	0.597^{abc}
0	16	2.46^{c}	12.74	0.544	0.401	0.429	0.585^{c}
40	11	2.49^{c}	13.42	0.625	0.509	0.449	0.620^{a}
40	13	3.01^{bc}	13.33	0.589	0.435	0.479	0.586^{c}
40	16	3.67^{b}	12.96	0.556	0.368	0.418	0.588^{bc}
80	11	3.51^{b}	13.38	0.572	0.496	0.467	0.588^{bc}
80	13	6.11^{a}	13.38	0.566	0.388	0.463	0.613^{ab}
80	16	6.14^{a}	13.26	0.558	0.344	0.436	0.599^{abc}
SEM[2]		0.309	0.111	0.0116	0.0153	0.0124	0.0091
Main effects							
Tallow inclusion							
0		2.40	12.77^{b}	0.550^{b}	0.429	0.444	0.600
40		3.06	13.24^{a}	0.595^{a}	0.437	0.449	0.599
80		5.25	13.34^{a}	0.565^{b}	0.409	0.455	0.598
Ca concentration							
11		2.78	13.19	0.583^{a}	0.498^{a}	0.461^{a}	0.607
13		3.84	13.16	0.569^{ab}	0.407^{b}	0.459^{a}	0.598
16		4.09	12.99	0.553^{b}	0.371^{c}	0.428^{b}	0.590
Probabilities, P ≤							
Tallow inclusion		***	***	***	0.08	NS	NS
Ca concentration		***	0.06	*	***	**	0.08
Tallow inclusion x Ca concentration		**	NS	NS	NS	NS	*

NS, not significant; * P < 0.05; ** P < 0.01; *** P < 0.001.

[1] Each value represents the mean of four replicates.

[2] Pooled standard error of mean.

[a,b,c] Means in a column not sharing a common superscript are significantly different (P < 0.05).

Dietary calcium concentration had a significant (P < 0.001) effect on calcium retention, with increasing calcium concentration resulting in lower calcium retention. There was a tendency (P = 0.08) for tallow inclusion to influence calcium retention. Birds fed diets supplemented with 40 g/kg tallow tended to have calcium retention similar to

those fed 0 g/kg tallow, but higher than those fed 80 g/kg tallow. No interaction between tallow and calcium concentrations was observed ($P > 0.05$) for calcium retention.

Calcium concentration had a significant ($P < 0.01$) effect on the retention of phosphorus. Birds fed diets containing 16 g/kg calcium had lower phosphorus retention than those fed diets containing 11 and 13 g/kg calcium. Neither the main effect of tallow inclusion nor the interaction between tallow and calcium concentrations was significant ($P > 0.05$) for phosphorus retention.

A tallow inclusion x calcium concentration interaction ($P < 0.05$) was observed for nitrogen retention. In diets with no tallow, 11 g/kg calcium resulted in nitrogen retention similar to that of 13 g/kg calcium but higher than that of 16 g/kg calcium. In diets with 40 g/kg tallow, 11 g/kg calcium resulted in higher nitrogen retention compared to 13 and 16 g/kg calcium and in diets with 80 g/kg tallow; calcium concentration had no effect on nitrogen retention.

7.4.3. Digesta soap and digestibility of fat and minerals

The influence of tallow inclusion and calcium concentration on soap in ileal digesta and, ileal digestibility of fat, calcium, phosphorus, nitrogen and ash is summarised in Table 7.4. Inclusion of tallow influenced ($P < 0.001$) the digesta soap, with soap formation increasing with increasing inclusion of tallow. Neither the main effect of calcium concentration nor the interaction between inclusion of tallow and calcium concentration was significant ($P > 0.05$) for soap formation.

The main effect of tallow inclusion was significant ($P < 0.001$) for ileal fat digestibility. The digestibility of fat in birds fed diets supplemented with 40 g/kg tallow was higher than those fed diets with 0 and 80 g/kg tallow. Calcium concentration significantly ($P < 0.01$) affected ileal fat digestibility. Birds fed diets containing 11 g/kg calcium had higher fat digestibility compared to those fed diets containing 13 and 16 g/kg calcium. No interaction ($P > 0.05$) was observed between tallow and calcium concentrations for ileal fat digestibility.

Tallow inclusion influenced ($P < 0.001$) the ileal digestibility of calcium. Increasing the inclusion of tallow resulted in lower digestibility of calcium. The main effect of calcium concentration was significant ($P < 0.001$) for calcium digestibility. Birds fed diets containing 16 g/kg calcium had the highest ileal calcium digestibility. Birds fed diets containing 11 g/kg calcium had higher calcium digestibility than those fed 13 g/kg

calcium. No interaction between tallow and calcium concentrations was observed (P > 0.05) for calcium digestibility.

Table 7.4. Influence of tallow inclusion and calcium concentration on the digesta soap (g/100 g digesta, DM basis), and apparent ileal digestibility coefficients of fat, calcium, phosphorus and nitrogen in broiler starters [1]

Tallow, g/kg	Ca, g/kg	Digesta soap	Ileal digestibility				
			Fat	Ca	P	N	Ash
0	11	2.85	0.633	0.531	0.537	0.835	0.464
0	13	2.86	0.503	0.460	0.385	0.785	0.373
0	16	3.03	0.501	0.570	0.408	0.778	0.505
40	11	5.56	0.688	0.472	0.495	0.853	0.418
40	13	5.76	0.628	0.424	0.436	0.830	0.410
40	16	6.88	0.613	0.546	0.475	0.819	0.520
80	11	8.67	0.565	0.468	0.519	0.818	0.364
80	13	9.01	0.556	0.324	0.426	0.815	0.304
80	16	9.20	0.543	0.473	0.436	0.802	0.445
SEM[2]		0.495	0.0274	0.0202	0.0201	0.0119	0.0341
Main effects							
Tallow inclusion							
0		2.91[c]	0.546[b]	0.520[a]	0.448	0.799[b]	0.448[a]
40		6.06[b]	0.643[a]	0.481[b]	0.469	0.834[a]	0.449[a]
80		8.96[a]	0.554[b]	0.422[c]	0.460	0.812[b]	0.371[b]
Ca concentration							
11		5.69	0.628[a]	0.490[b]	0.517[a]	0.835[a]	0.415[b]
13		5.88	0.562[b]	0.402[c]	0.440[b]	0.810[b]	0.362[b]
16		6.37	0.552[b]	0.530[a]	0.416[b]	0.799[b]	0.490[a]
Probabilities, P ≤							
Tallow inclusion		***	***	***	***	NS	**	*
Ca concentration		NS	**	***	***	**	***
Tallow inclusion x Ca concentration		NS	NS	NS	NS	NS	NS

NS, not significant; * P < 0.05; ** P < 0.01; *** P < 0.001.

[1] Each value represents the mean of four replicates.

[2] Pooled standard error of mean.

[a,b,c] Means in a column not sharing a common superscript are significantly different (P < 0.05).

Ileal digestibility of phosphorus was significantly (P < 0.001) affected by calcium concentration. Birds fed diets with 11 g/kg calcium had higher digestibility than those fed diets with 13 and 16 g/kg calcium. Neither the main effect of tallow inclusion nor the interaction between tallow and calcium concentrations was significant (P > 0.05) for phosphorus digestibility.

Ileal nitrogen digestibility was influenced ($P < 0.01$) by the inclusion of tallow. Birds fed diets supplemented with 40 g/kg tallow showed higher nitrogen digestibility than those fed diets supplemented with 0 and 80 g/kg tallow. Ileal nitrogen digestibility was also affected ($P < 0.01$) by calcium concentration. Birds fed diets containing 11 g/kg calcium showed higher nitrogen digestibility than those fed diets containing 13 and 16 g/kg calcium. The interaction between tallow inclusion and calcium concentration was not significant ($P > 0.05$) for nitrogen digestibility.

The main effect of tallow inclusion had a significant ($P < 0.05$) effect on ileal ash digestibility. Birds fed diets supplemented with 80 g/kg tallow had lower ileal ash digestibility than those fed diets supplemented with 0 and 40 g/kg tallow. The main effect of calcium concentration was a significant ($P < 0.001$) for ileal ash digestibility. Birds fed diets containing 16 g/kg calcium had higher ash digestibility than those fed diets containing 11 and 13 g/kg calcium. The interaction between tallow and calcium concentration was not significant ($P > 0.05$) for ash digestibility.

7.4.4. Toe ash content and gizzard pH

Tallow inclusion and calcium concentration had no effect ($P > 0.05$) on the toe ash content and gizzard pH (Table 7.5). No significant ($P > 0.05$) interaction was observed between tallow inclusion and calcium concentration for these parameters.

Table 7.5. Influence of tallow inclusion and calcium concentration on toe ash (% dry matter) and gizzard pH of broiler starters [1]

Tallow, g/kg	Ca, g/kg	Toe ash	Gizzard pH
0	11	13.34	3.25
0	13	13.10	2.93
0	16	12.40	3.18
40	11	12.81	3.25
40	13	12.74	3.06
40	16	12.64	3.22
80	11	12.65	3.16
80	13	12.76	3.38
80	16	12.55	3.21
SEM[2]		0.254	0.160
Main effects			
Tallow inclusion			
0		12.94	3.12
40		12.73	3.17
80		12.65	3.25
Ca concentration			
11		12.93	3.22
13		12.87	3.12
16		12.53	3.20
Probabilities, P ≤			
Tallow inclusion		NS	NS
Ca concentration		NS	NS
Tallow inclusion x Ca concentration		NS	NS

NS, not significant.
[1] Each value represents the mean of four replicates.
[2] Pooled standard error of mean.

7.5. Discussion

Analysed dietary concentrations of calcium were determined to be 35% higher than the calculated values. It is possible that the assumed calcium content of soybean meal was lower. The calcium content of soybean meal is often variable as limestone is sometimes added to soybean meal at the end of processing to prevent caking of the warm meal (Tamim *et al*, 2004).

The present study showed that the feed intake of broiler starts fed diets containing different tallow levels is influenced by calcium concentration. In birds fed the diet with no tallow, increasing calcium concentrations decreased feed intake. However, feed intake of

diets with 40 and 80 g/kg of tallow supplemented with 11 and 13 g/kg of calcium were similar but higher than 16 g/kg of calcium. Negative effects of high dietary calcium concentrations on feed intake in broilers have been reported by Sebastian *et al*. (1996) and Wilkinson *et al*. (2012). Wilkinson *et al*. (2012) suggested suggested that modern commercial broilers have the ability to monitor body calcium concentrations and adjust dietary intake to meet perceived deficiency, which may account for the lower feed intake in birds fed diets containing high calcium concentrations. Lower feed intake in diets with no tallow may also be attributed to the high dustiness leading to poor palatability.

High calcium concentrations had negative effects on weight gain, and feed per gain of broilers. These findings are in general agreement with previous reports (Shafey and McDonald, 1991; Sebastian *et al*., 1996; Rama Rao *et al*., 2006; Wilkinson *et al*., 2012). One possible explanation for the negative effects of high calcium on the performance may be the increase in pH in the gastrointestinal tract. Limestone is the major source of calcium in poultry diets. Increasing the dietary calcium concentration by the use of limestone tends to increase gastrointestinal pH due to it's extremely high acid binding capacity (Selle *et al*., 2009). Shafey *et al*. (1991) suggested that increased intestinal pH may reduce the soluble fraction of minerals and limit their absorption. In the present study, however, increasing calcium concentrations had no effect on gizzard pH which is consistent to those of Shafey *et al*. (1991). In contrast, Walk *et al*. (2012) reported that the pH in the gizzard and distal ileum was increased with increasing concentrations of calcium.

The differences observed in the weight gain and AME of diets containing different inclusion levels of tallow were as expected. These three basal diets were formulated to contain different AME contents. The energy contain in diets supplemented with no tallow inclusion was lower than diets contain 40 and 80 g/kg tallow. The present results also showed that birds fed diets with no tallow inclusion had higher feed per gain compared to those fed 40 and 80 g/kg of tallow. Higher feed per gain in diets with no tallow can be attributed to low palatability of diets which resulted in lower weight gain and increasing feed per gain.

Increasing dietary calcium concentration tended to decrease the AME, which is in agreement with published reports (Atteh and Leeson, 1983; 1985; Shafey and McDonald, 1991).The negative effects of high calcium concentrations on the AME may be attributed, to a large extent, to the formation of insoluble soaps.

Increasing inclusion of tallow increased the soap formation in digesta and increasing inclusion of tallow to 80 g/kg with high calcium concentrations resulted in increasing soap in excreta. These results are in agreement with the findings of Atteh and Leeson (1984). These researchers fed birds with diet supplemented with 80 g/kg palmitic acid containing 8, 12 or 16 g/kg calcium and found that increasing calcium concentrations increased excreta soap contents. Similarly, Al-Marzooqi and Leeson (1999) found that birds fed diets supplemented with 80 g/kg animal-vegetable fat containing 9 g/kg calcium had higher excreta soap than those fed diets with 40 g/kg fat containing 9 g/kg calcium. These soaps limit the utilisation of energy derived from lipids, particularly saturated fats, in broiler diets. Soap formation is a spontaneous process occurring during fat digestion. Free fatty acids, particularly saturated fatty acids, have the potential to bind with calcium-phytate complexes and form soluble or insoluble soap in the gut lumen, thereby reducing digestibility of fat and calcium (Leeson, 1993). Lower fat retention and lower ileal digestibility of fat, calcium and ash in diets with 80 g/kg tallow may be attributed to soap formation in the digesta and excreta of birds. Furthermore, current results indicate that the inclusion of tallow at 40 g/kg may be an appropriate amount for use in broiler starter diets, as fat retention and ileal fat digestibility at this inclusion level were higher than those at 80 g/kg tallow. Lower fat retention and fat digestibility in birds fed 80 g/kg tallow may indicate that there may be a threshold for effective fat emulsification by the bile and this may have been reached at 40 g/kg tallow inclusion.

Diets containing 13 g/kg calcium had lower ileal calcium digestibility than those containing 11 and 16 g/kg calcium. No plausible explanation can be provided for this apparent anomaly. Increased calcium digestibility with increasing calcium concentrations has been reported by Tamim *et al*. (2004). These researchers fed broilers with diets containing calcium concentrations from 2 to 7 g/kg and observed improvements in ileal calcium digestibility. Similarly, Walk *et al*. (2012) reported significant increases in ileal calcium digestibility when the concentration of calcium was increased from 6.4 to 10.3 g/kg. Interestingly, calcium retention decreased with increasing dietary calcium concentrations. Increasing the calcium concentration from 11 to 13 and 16 g/kg reduced the calcium retention by 18% and 26%, respectively. These findings are in accordance with those of Sebastian *et al*. (1996) who fed birds with diets containing 6, 10 and 12.5 g/kg calcium and reported that calcium retention decreased with increasing calcium

concentrations. Atteh and Leeson (1985) also evaluated the effect of dietary calcium concentration on the calcium retention of broilers and reported that increasing the dietary calcium from 8 to 12 g/kg lowered calcium retention by 6%. Two possible reasons may be provided for the higher digestibility and lower retention of calcium at high dietary calcium concentrations. First, birds fed high dietary calcium may have higher rates of intestinal calcium absorption. High plasma concentrations of calcium, however, may cause hypercalcaemia, resulting in higher urinary calcium excretion (Guo *et al*., 2005). High urinary calcium will increase the concentration of calcium in excreta and lower the total tract retention of calcium. Second, high calcium concentration may increase the formation of insoluble calcium soaps, which are poorly utilised and will be excreted. The negative effect of excreta soap formation on total tract calcium retention has been previously reported (Griffith *et al*., 1961; Atteh and Leeson, 1985; Lin and Chiang, 2010).

Increasing dietary calcium concentration from 11 to 16 g/kg resulted in 7% reduction in phosphorus retention and 20% reduction in ileal phosphorus digestibility. These findings are consistent with previous published data (Sebastian *et al*., 1996; Tamim *et al*., 2004; Plumstead *et al*., 2008). The study of Sebastian *et al*. (1996) demonstrated that high calcium concentration (12.5 g/kg) decreased the availability of phytate phosphorus, resulting in 6% lower phosphorus retention compared to diets containing 10 g/kg calcium. Tamim *et al*. (2004) reported that the apparent ileal digestibility of phytate-phosphorus and total phosphorus in birds fed diets containing 2 g/kg calcium were higher than those fed diets with 7 g/kg calcium. Similarly, Walk *et al*. (2012) reported that reducing the dietary calcium concentration from 10.3 g/kg to 6.4 g/kg improved the apparent ileal digestibility of phosphorus by 7%. Several explanations may be provided for the adverse effects of high dietary calcium levels on the digestibility and retention of phosphorus: i) increasing calcium concentration may reduce the solubility of minerals (Shafey *et al*., 1991) and possibly increase the formation of insoluble calcium-phytate complexes; ii) high dietary calcium concentrations are known to reduces the intestinal activities of phytase and alkaline phosphatase by competing for the active sites of the enzyme (McCuaig *et al*., 1972), iii) the ability of calcium to react with dietary inorganic phosphorus to form insoluble calcium orthophosphate [$(Ca_3(PO_4)_2$] (Hurwitz and Bar, 1971; Plumstead *et al*., 2008), which may lead to lower absorption of inorganic phosphorus.

Tallow inclusion and calcium concentration had no effect on bone mineralization, a finding which is in agreement to those of Wilkinson *et al*. (2012). However, the highest level of tallow inclusion (80g/kg) and the highest calcium concentration (16 g/kg) increased the ileal digestibility of ash, a pattern that paralleled that of ileal digestibility of calcium.

The negative effect of increasing calcium concentrations on the retention (except in 80 g/kg tallow diet) and ileal digestibility of nitrogen observed in this experiment is similar to finding of Shafey and McDonald (1991). These researchers showed that high dietary calcium concentration depressed the efficiency of nitrogen digestion by reducing the digesta transit time and increasing the microorganism population in gastrointestinal tract. The latter can result in the irritation of the intestinal mucosa, increased intestinal wall thickness and impaired the absorption of nutrients (Shafey and McDonald, 1991).

The inclusion of tallow influenced the digestibility of nitrogen. Addition of fat to a diet slows the transit time of the digesta in the gastrointestinal tract, resulting in better digestion and absorption of all nutrients (Mateos *et al*., 1982). It is possible that transit time may be faster with diets containing no tallow with the resultant low nitrogen digestibility. However, lower nitrogen digestibility in a diet with 80 g/kg tallow cannot be attributed to the rate of feed passage. One possible explanation may be that the amount of bile is insufficient to emulsify the high level of tallow. Consequently, the partial non-emulsification of fat might impede the solubilisation and absorption of other nutrients such as nitrogen.

7.6. Conclusions

In conclusion, the present experiment demonstrated that high calcium concentrations in broiler diets, regardless of tallow inclusion levels, generally result in negative effects on bird performance. Diets containing lowest concentration of calcium (11 g/kg) resulted in higher weight gain, feed intake and lower feed per gain. Calcium concentration had no effect on the excreta soap at 0 g/kg of tallow inclusion, but higher calcium concentrations resulted in higher excreta soap when tallow inclusion was increased, an effect which was more pronounced at 80 g/kg of tallow inclusion. Total tract retention of fat, calcium and phosphorus decreased with increasing the calcium concentration. Ileal digestibility of fat, nitrogen and phosphorus were higher at the lower calcium concentration. Results of the current study revealed that diets with tallow at 40 g/kg had similar AME value to those containing 80 g/kg, but higher fat retention and lower

digesta soap formation. The present data suggested that the appropriate calcium concentration in broiler starter diets should be considered due to the adverse effects of high calcium on the performance and nutrient utilisation of the birds.

Among the number of factors that influence fat digestion, the effects of unsaturated to saturated fatty acid ratio have not been well investigated. Studies are warranted to evaluate the influence of the ratio of unsaturated to saturated fatty acids on performance, total tract retention and digestibility of fat of broilers.

CHAPTER 8

Influence of ratio of unsaturated to saturated fatty acids on performance, total tract retention of fat, apparent metabolisable energy, carcass characteristics and gut microflora counts in broilers fed wheat-based diets

8.1. Abstract

An experiment was conducted to determine the influence of unsaturated to saturated fatty acids ratio (U:S ratio) on the performance, total tract retention of fat, AME, gut microflora counts and carcass characteristics of broilers fed wheat-based diets. An additional aim was to examine the relationship between the U:S ratio and AME of fat. Six wheat-based diets were formulated and supplemented with varying proportions of animal fat and soybean oil (animal fat: soybean oil, 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100, respectively) which corresponded to dietary U:S ratios of 1.30, 1.64, 2.10, 2.74, 3.73 and 5.43, respectively. Each diet was fed to six replications of 8 birds each from day 1 to 35 post hatching. During the starter period (d1 to 21), weight gain ($P < 0.01$) and feed intake ($P < 0.001$) were linearly decreased with increasing U:S ratios. Feed per gain decreased quadratically ($P < 0.01$) with increasing U:S ratios. Over the whole trial period (d1 to 35), weight gain and feed per gain were unaffected ($P > 0.05$) by dietary treatments. Feed intake tended ($P = 0.053$) to decrease linearly with increasing U:S ratios. The AME of the diet decreased linearly ($P < 0.001$) as the U:S ratio increased. Total tract fat retention increased quadratically ($P < 0.05$) with increasing U:S ratios. Fat retention increased at the ratio of 1.64, and reached a plateau up to 2.74 and then increased with further increase in the ratio. Carcass recovery was not affected ($P > 0.05$) by dietary treatments. Breast meat yield showed a quadratic ($P < 0.05$) response with increasing U:S ratio. Abdominal fat weights decreased with increasing U:S ratio up to 2.74 and reached a plateau with further increase. A linear ($P < 0.05$) increase was observed in the numbers of bifidobacteria with increasing U:S ratios. The numbers of clostridia tended ($P = 0.07$) to decrease quadratically with increasing U:S ratios. The numbers of lactobacilli, *Bacteroides* species and *Campylobacter* species were unaffected ($P > 0.05$) by dietary treatments. A positive correlation ($R^2=0.63$; $P < 0.001$) was observed between the U:S ratio and AME of fat and, a regression equation to predict the AME of fat blends was developed. Overall, the present data suggested increasing the U:S ratio through the blending of animal fat with soybean oil improved the total tract retention and AME of fat.

8.2. Introduction

Fats and oils are widely used in poultry diets as energy sources. However, it is known that the efficiency of utilisation of fats varies depending on the source of fat. Results of the study reported in Chapter 4 demonstrated that beef tallow was poorly utilised by broilers compared to soybean oil. Data reported in Chapters 5 and 6 showed that the digestibility of tallow was lower than that of soybean oil due to the high concentration of long chain saturated fatty acids, which are nonpolar and thus difficult to digest and absorb (Krogdahl, 1985).

It is well documented that the utilisation of saturated fats can be improved by the addition of small amounts of unsaturated fat (Garrett and Young, 1975; Muztar *et al*., 1981). Data from the study reported in Chapter 5 also showed that blending of tallow with soybean oil improved the AME and digestibility of fat. Changes in the unsaturated to saturated fatty acid ratio (U: S ratio) through the blending of saturated fats with unsaturated fats has been shown to increase the digestibility and AME of blended fats (Wiseman and Lessire, 1987; Ketels and De Groote, 1989), which sometimes may be synergistic (Sibbald *et al*., 1962; Lall and Slinger, 1973; Muztar *et al*., 1981).

Dietary addition of fats may also affect the carcass characteristics of broilers. It has been reported that dietary fatty acids are incorporated with little change in body fat (Sanz *et al*., 1999), and the deposition of the abdominal fat pad was related to the concentration of unsaturated fatty acids (Pinchasov and Nir, 1992; Sanz *et al*., 1999; Crespo and Esteve-Garcia, 2001; 2002). Broilers fed diets containing tallow and lard had heavier abdominal fat pads than those fed the diet containing sunflower oil (Sanz *et al*., 1999). Crespo and Esteve-Garcia (2001) studied the effect of dietary fat on the breast meat yield and abdominal fat pad weights. These researchers reported that fat source had no effect on breast meat yield, but broilers fed tallow diets had higher abdominal fat pad weights than those fed sunflower oil or linseed oil diets.

Manipulation of U:S ratios, through blending of saturated and unsaturated fats, may be a potential strategy to improve the utilisation of fat in broiler diets. However, most studies evaluating the effect of U:S ratios have used wheat-maize-based (Wiseman and Lessire, 1987) or rye-based (Danicke *et al.*, 2000) diets. These researchers reported that increasing U:S ratios improved the digestibility of fat. No studies to date have examined the effect of U:S ratios in birds fed wheat-based diets. Hence, the objective of the present study was to investigate the effect of U:S ratios on the performance, AME,

fat retention, gut microflora counts and carcass characteristic of broilers fed wheat-based diets. An additional aim was to examine the relationship between the U:S ratio and the AME of fat in wheat-based diets.

8.3. Materials and methods

8.3.1. Birds and Housing

Day-old male broilers (Ross 308) were obtained from a commercial hatchery, individually weighed and allocated on the basis of body weight to 36 cages (8 birds per cage) in electrically heated battery brooders. Each of the six dietary treatments was randomly assigned to six cages. Feed, in mash form, was offered *ad libitum* from day 1 to 35 post hatch. Water was freely available at all times. Housing conditions were as described in Chapter 3, section 3.1.

8.3.2. Diets

Six diets, based on wheat and soybean meal, were formulated to meet the Ross 308 strain recommendations for the major nutrients (Ross, 2007; Table 8.1). The experimental diets were formulated to contain 60 g/kg fat, using different proportions of animal fat and soybean oil (animal fat: soybean oil, 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100, respectively) which corresponded to dietary U:S ratios of 1.30, 1.64, 2.10, 2.74, 3.73 and 5.43, respectively. The animal fat used in this experiment was a mixture of 850 g/kg sheep and beef tallow with 150 g/kg lard. All diets were supplemented with a commercial xylanase (Avizyme®, Danisco Animal Nutrition, Marlborough, UK) as per standard commercial practice. All diets were formulated to be isocaloric and isonitrogenous. The calculated differences in the AME between animal fat and soybean oil were overcome by the inclusion of cellulose and maize starch.

8.3.3. Performance data

Performance data were recorded as describe in Chapter 3, section 3.1.

8.3.4. AME determination

Total collection of excreta was carried out between days 17-20 post-hatch for the determination of AME, as described in Chapter 3, section 3.2.

Table 8.1. Composition and calculated analysis (g/kg as fed) of the experimental diets

Ingredient	Animal fat : Soybean oil (w/w)					
	100:0	80:20	60:40	40:60	20:80	0:100
Wheat	600	600	600	600	600	600
Soybean meal, 480 g/kg	190	190	190	190	190	190
Soy protein isolate	55	55	55	55	55	55
Animal fat[1]	60	48	36	24	12	0
Soybean oil[2]	0	12	24	36	48	60
Salt	2.6	2.6	2.6	2.6	2.6	2.6
DL-methionine	3.1	3.1	3.1	3.1	3.1	3.1
Lysine. HCl	3.4	3.4	3.4	3.4	3.4	3.4
L Threonine	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	20.8	20.8	20.8	20.8	20.8	20.8
Limestone	12.3	12.3	12.3	12.3	12.3	12.3
Trace mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[4]	0.6	0.6	0.6	0.6	0.6	0.6
Maize starch	42.5	26.0	25.5	17.0	8.5	0
Cellulose	6.2	14.7	23.2	31.7	40.2	48.7
Enzyme[5]	0.5	0.5	0.5	0.5	0.5	0.5
Provision						
Metabolisable energy (MJ/kg)	12.9	12.9	12.9	12.9	12.9	12.9
Crude protein	210	210	210	210	210	210
Calcium	10.2	10.2	10.2	10.2	10.2	10.2
Available phosphorus	5	5	5	5	5	5
Methionine	5	5	5	5	5	5
Methionine + cysteine	9.1	9.1	9.1	9.1	9.1	9.1
Lysine	13.1	13.1	13.1	13.1	13.1	13.1
Threonine	8.2	8.2	8.2	8.2	8.2	8.2
Tryptophan	2.5	2.5	2.5	2.5	2.5	2.5
U:S ratio	5.43	3.73	2.74	2.10	1.64	1.30

[1]ME content in animal fat was assumed to be 27.0 MJ/kg.
[2]ME content in soybean oil was assumed to be 38.0 MJ/kg.
[3]Supplied per kilogram of diet: Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.
[4]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg.
[5]Avizyme®, Danisco Animal Nutrition, Marlborough, UK.

8.3.5. Fat retention measurements

Excreta samples, from the collection between days 17-20 post-hatch, were analysed for fat content as described in Chapter 3, section 3.4 and total tract fat retention was calculated.

8.3.6. Determination of microflora

On day 35, two birds from each cage were euthanised by intravenous injection of sodium pentobarbitone. Three group of dietary treatments which had the U:S ratio of 1.30, 2.47 and 5.43 were chosen to see an extremely effect of the U:S ratio on ceacal microflora. Caecal contents were collected and stored at -20°C to determine the counts of *Lactobacillus*, *Bifidobacterium, Clostridium*, *Bacteroides* and *Campylobacter* species by fluorescent in situ hybridisation (FISH) method as described in Chapter 3, section 3.5.

8.3.7. Carcass measurements

On day 35, two more birds from each cage were weighed and killed by exsanguination. After removal of the neck, shanks, feathers and viscera, the weight of carcass, abdominal fat and breast meat (without skin) were individually recorded. The data were expressed as gram per kilogram of live weight.

8.3.8. Chemical analysis

The fatty acid profile of the animal fat and soybean oil, and dry matter, gross energy and fat contents were determined as described in Chapter 3, section 3.4.

8.3.9. Calculations

The AME of the diets and total tract retention of fat were calculated using the formula described in Chapter 3, section 3.6. The AME of fat was calculated by multiplying the total tract fat retention by the gross energy of the fat.

8.3.10. Data analysis

Data were analysed by orthogonal polynomials contrasts as described in Chapter 3, section 3.7 to examine whether the effect of the increasing ratio of unsaturated to saturated fatty acids was linear or quadratic (SAS, 2004). The following linear regression model was used to examine the relationship between the U:S ratio and the AME of fat (SAS, 2004).

$$Y = a + bX$$

Where, Y = apparent metabolisable energy of fat;

a = intercept of the equation

b = regression coefficient

X = unsaturated to saturated fatty acid ratio

8.4. Results

The fatty acid composition of the soybean oil and animal fat is presented in Tables 8.2. Linoleic and oleic acids were the major unsaturated fatty acids in soybean oil, whereas oleic acid was the major unsaturated fatty acid in animal fat. The animal fat also contained high concentrations of palmitic and stearic acids.

Table 8.2. Fatty acid composition of soybean oil and animal fat (g/kg)

Fatty acid	Soybean oil	Animal fat
Saturated fatty acids		
C8:0 Caprylic	-	0.3
C10:0 Capric	-	0.6
C12:0 Lauric	0.2	1.4
C14:0 Myristic	0.7	18.0
C16:0 Palmitic	97.6	205.8
C17:0 Margaric	2.0	9.4
C18:0 Stearic	37.3	128.6
C20:0 Arachidic	2.8	1.2
C21:0 Heneicosanoic	-	2.4
C22:0 Behenic	3.2	-
Unsaturated fatty acids		
C14:1 Myristoleic	-	2.6
C16:1 Palmitoleic	0.8	35.6
C18:1 Elaidic	-	3.7
C18:1 Vaccenic	10.9	27.7
C18:1 Oleic	189.9	334.9
C18:2 Linoleic	509.1	56.4
C18:3 Linolenic	67.9	7.6
C20:1 Eicosenoic	2.2	4.0
C20:2 Eicosadienoic	-	1.3
C20:3 Eicosatrienoic	-	0.5
C20:5 Timnodonic	-	1.2
C22:5 Clupanodonic	-	1.4
C22:6 Cervonic	-	2.0
Total fatty acids (g/kg)	924.6	846.6

The fatty acid profile of the soybean oil and animal fat is shown in Table 8.3. Soybean oil consisted of high concentration of unsaturated fatty acids, whereas animal fat contained high concentration of saturated fatty acids. The U:S ratio of soybean oil and animal fat were determined to be 5.43 and 1.30, respectively.

Table 8.3. Fatty acid profile of soybean oil and animal fat (g/kg fat)

Fatty acid	Soybean oil	Animal fat
Saturated fatty acids		
C8:0 Caprylic	-	0.4
C10:0 Capric	-	0.7
C12:0 Lauric	0.3	1.6
C14:0 Myristic	0.8	21.3
C16:0 Palmitic	105.6	243.0
C17:0 Margaric	2.1	11.1
C18:0 Stearic	40.4	151.9
C20:0 Arachidic	3.0	1.4
C21:0 Heneicosanoic	-	2.8
C22:0 Behenic	3.5	-
Unsaturated fatty acids		
C14:1 Myristoleic	-	3.1
C16:1 Palmitoleic	0.9	42.1
C18:1 Elaidic	-	4.3
C18:1 Vaccenic	11.8	32.8
C18:1 Oleic	205.3	395.6
C18:2 Linoleic	550.6	66.6
C18:3 Linolenic	73.4	8.9
C20:1 Eicosenoic	2.3	4.7
C20:2 Eicosadienoic	-	1.6
C20:3 Eicosatrienoic	-	0.6
C20:5 Timnodonic	-	1.5
C22:5 Clupanodonic	-	1.6
C22:6 Cervonic	-	2.4
Saturated fatty acids (g/kg)	155.7	434.2
Unsaturated fatty acids(g/kg)	844.3	565.8
Unsaturated to saturated ratio	5.43	1.30

8.4.1. Performance

Mortality during the experiment was negligible. Only eight out of 288 birds died and the deaths were not related to any specific treatment.

During the starter period (d1 to 21), weight gain ($P < 0.01$) and feed intake ($P < 0.001$) were linearly decreased with increasing U:S ratios (Table 8.4). A quadratic effect ($P < 0.01$) was observed for feed per gain, which was due primarily to the high feed per gain at the U:S ratios of 1.30 and 3.73.

Over the whole trial period (d1 to 35), weight gain and feed per gain were unaffected ($P > 0.05$) by the U:S ratio. Feed intake tended ($P = 0.053$) to decrease linearly with increasing U:S ratio.

8.4.2. AME and total tract retention of fat

The influence of U:S ratio on the AME and total tract retention of fat is summarised in Table 8.5. The AME decreased linearly ($P < 0.001$) as the U:S ratio increased. Total tract fat retention showed a quadratic ($P < 0.05$) response, with retention increasing when the U:S ratio increased from1.30 to 1.64, then plateauing with increases up to 2.74 and then increasing with further increase.

8.4.3. Carcass characteristics

The influence of U:S ratio on the carcass characteristics is presented in Table 8.6. Carcass recovery was unaffected ($P > 0.05$) by U:S ratio. A quadratic response ($P < 0.05$) was observed in breast meat yield. Breast meat yield increased with increasing U:S ratios up to 2.10 and then decreased with further increase in the ratio. Relative abdominal fat weight was also affected quadratically ($P < 0.05$) with increasing U:S ratio. Abdominal fat weights decreased with increasing the ratio up to 2.74 and reached a plateau with further increase.

Table 8.4. Influence of U:S ratio on the weight gain (g/bird), feed intake (g/bird) and feed per gain (g feed /g gain) of broilers during starter (d 1 to 21) and over the whole trial periods (d 1 to 35)[1]

U:S ratio	Starter (d 1 to 21)			Whole trial period (d 1 to 35)		
	Weight gain	Feed intake	Feed per gain	Weight gain	Feed intake	Feed per gain
1.30	969	1255	1.304	2409	3483	1.464
1.64	955	1229	1.286	2486	3587	1.447
2.10	910	1158	1.272	2407	3373	1.432
2.74	927	1165	1.257	2424	3465	1.429
3.73	928	1162	1.297	2414	3399	1.444
5.43	913	1138	1.271	2425	3420	1.436
SEM[2]	14.1	18.0	0.0057	37.7	46.9	0.0109
Probability						
Linear	**	***	**	NS	0.053	NS
Quadratic	NS	NS	**	NS	NS	NS

NS, not significant; ** P < 0.01; *** P < 0.001.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

Table 8.5. Influence of U:S ratio on AME of diet (MJ/kg DM) and total tract fat retention coefficient of broilers[1]

U:S ratio	AME of diet	Total tract fat retention
1.30	14.33	0.607
1.64	14.32	0.630
2.10	14.06	0.632
2.74	13.94	0.645
3.73	14.11	0.708
5.43	13.90	0.744
SEM[2]	0.084	0.0152
Probability		
Linear	***	***
Quadratic	NS	*

NS, not significant; * P < 0.05; *** P < 0.001.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

Table 8.6. Influence of U:S ratio on the carcass characteristics (g/kg of live weight) of broilers[1]

U:S ratio	Carcass recovery	Breast meat yield	Abdominal fat
1.30	720.1	190.9	11.3
1.64	726.2	192.0	11.0
2.10	727.5	197.4	8.2
2.74	720.5	195.5	6.8
3.73	730.3	188.9	8.8
5.43	721.8	183.7	8.8
SEM[2]	4.14	4.10	0.96
Probability			
Linear	NS	NS	*
Quadratic	NS	*	*

NS, not significant; * $P < 0.05$.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

8.4.4. Caecal microflora counts

A linear increase ($P < 0.05$) was observed in the numbers of *Bifidobacterium* species as the U:S ratio increased (Table 8.7). The numbers of Clostridium species tended ($P = 0.07$) to decrease quadratically with increasing U:S ratios. The counts of *Lactobacillus*, *Bacteroides* and *Campylobacter* species were not influenced ($P > 0.05$) by U:S ratios.

Table 8.7. Influence of the U:S ratio on bacterial count (Log10 cells/g of caecal content) in caeca of broiler chickens[1]

U:S ratio	*Lactobacillus*	*Bifidobacterium*	*Clostridium*	*Bacteroides*	*Campylobacter*
1.30	7.83	7.66	6.84	6.78	7.22
2.74	7.95	7.78	6.98	6.98	7.17
5.43	7.99	8.01	6.70	6.99	7.00
SEM[2]	0.102	0.112	0.088	0.118	0.122
Probability					
Linear	NS	*	NS	NS	NS
Quadratic	NS	NS	0.07	NS	NS

NS, not significant; * $P < 0.05$.
[1] Each value represents the mean of six replicates.
[2] Pooled standard error of mean.

8.4.5. Relationship between U:S ratio and AME of fat blends

The AME of fat blends with varying U:S ratios, calculated by multiplying total tract fat retention by the gross energy content of fat, are presented in Table 8.8. The AME of fat increased with increasing U:S ratios.

The relationship between the U:S ratio and AME of fat is shown in Figure 8.1. The AME of fat was positively correlated with the U:S ratio ($R^2 = 0.63$; $P < 0.001$).

Table 8.8. Influence of the U:S ratio on apparent metabolisable energy of the fat[1] (MJ/kg)

U:S ratios	Gross energy	AME of fat[2]
1.30	39.17	23.77
1.64	39.12	24.67
2.10	39.07	24.69
2.74	39.02	25.21
3.73	38.97	27.60
5.43	38.92	28.98

[1] Calculated by multiplying total tract retention of fat with gross energy of fat (MJ/kg).
[2] Each value represents the mean of six replicates.

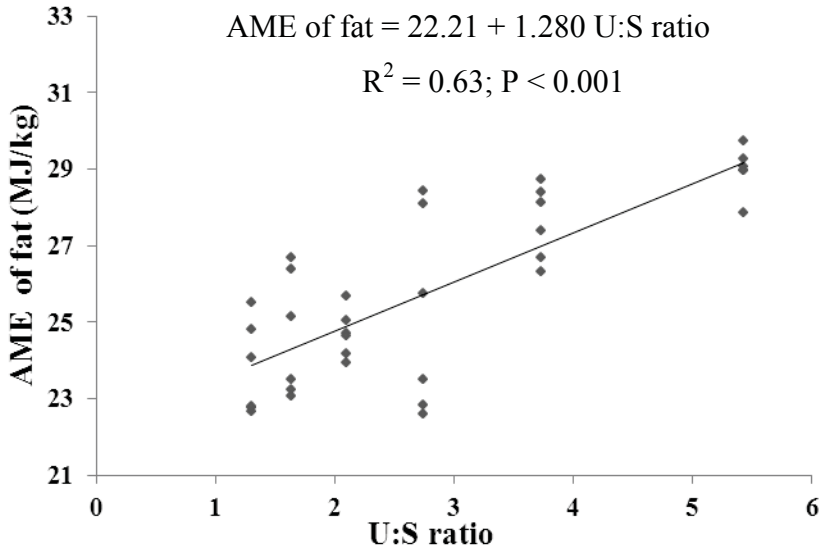

Figure 8.1. Relationship between unsaturated to saturated fatty acid ratio (U:S ratio) and apparent metabolisable energy of fat blends.

8.5. Discussion

Fatty acid composition of the animal fat (blend of sheep and beef tallow with lard) used in the present study differed from that of beef tallow used in previous trials reported in Chapters 5 and 6. The fatty acid analysis showed that the animal fat in the current study also contained small concentrations of eicosadienoic (20:2), eicosatrienoic (20:3), timnodonic (20:5), clupanodonic (22:5) and cervonic (22:6) which were not found in beef tallow. These fatty acids are found in oils from marine sources (Sauvant *et al*., 2004) and their presence suggests minor contamination with marine oils. Furthermore, the animal fat used had relatively lower concentration of stearic acid, and higher concentrations of unsaturated fatty acids than beef tallow. The U:S ratio of the animal fat used was higher (1.30) than that of beef tallow (0.63 to 0.80) used in trials reported in Chapters 5 and 6. These observations highlight the variable quality of animal fats, which may vary widely depending on the type of animal fat included in the blend.

During the starter period, increasing the U:S ratio decreased the weight gain of broilers and this was associated with decreased feed intake. Increasing U:S ratios, however, resulted in decreased feed per gain. These findings are in contrast with those of Danicke *et al.* (2000) who examined the effect of different U:S ratios by the use of blends of soybean oil and tallow in broilers fed rye-based diets. These researchers reported that increasing U:S ratio increased weight gain and decreased feed per gain of broilers. In the present study, higher feed intake in diets containing lower U:S ratios may be attributed to the palatability of diets. It is possible that diets containing high proportions of animal fat in the blend may have better palatability. Similarly, Park *et al*. (2009) reported higher feed intake in pigs fed diets containing tallow compared to those fed diets with soybean oil. Higher feed per gain in broilers fed diets containing high proportions of animal fat can be attributed to the physiological limitation to digest fat in young birds. As shown in Chapter 5, the utilisation of fat in young birds was poor, especially in birds fed diets with tallow. Over the whole trial period, weight gain and feed per gain were unaffected by the U:S ratio. These findings are in accordance with those of Wongsuthavas *et al*. (2007a) who fed broilers diets with different U:S ratio using blends of tallow with soybean oil. These researchers reported that weight gain, feed intake and feed per gain of broilers were unaffected by dietary treatments. Based on these observations, it may be concluded that the U:S ratios influence the performance

of broilers during the starter period, but has no effect during the grower period as the physiological ability of broilers to utilise fat is fully developed.

The U:S ratio influenced the AME of diets, with the AME decreasing with increasing U:S ratios. It has been reported that the AME and utilisation of saturated fats can be improved by using them in combination with unsaturated fats (Lall and Slinger, 1973; Sibbald, 1978; Muztar *et al*., 1981). However, the present finding are in contrast to those of Danicke *et al*. (2000) who reported that increasing U:S ratios increased the AME. Lower AME with increasing U:S ratios in the present study may be related, in part, to the dietary concentrations of NSP. The addition of cellulose in the current study to diets with soybean oil to achieve similar AME contents may have increased dietary NSP contents, and lowered the AME.

The present results showed that increasing the U:S ratio increased total tract retention of fat. Increasing the U:S ratio from 1.30 to 1.64, 2.10, 2.74, 3.73 improved the retention by 3.8, 4.1, 6.3 and 16.7%, respectively. The observed improvements in the total tract fat retention can be explained by the increasing concentrations of unsaturated fatty acids in the blend. It is known that the utilisation of saturated fatty acids is improved in the presence of high concentrations of unsaturated fatty acids (Garrett and Young 1975; Leeson and Summers, 1976). Similarly, Danicke *et al.* (2000) reported that total tract retention of blends of tallow and soybean oil increased with increasing proportions of soybean oil. The absorption of long chain saturated fatty acids is limited by their incorporation rate into micelles (Friedman and Nylund, 1980). Saturated fatty acids are also less rapidly incorporated into micelles because of their non-polarity, which makes them rely on the presence of adequate amount of bile salts and unsaturated fatty acids for efficient emulsification (Polin *et al*., 1980; Danicke, 2001). Increasing concentration of unsaturated fatty acids will increase the formation of mixed micelles and absorption of saturated fatty acids (Scheele *et al*., 1997).

Carcass recovery was unaffected by the U:S ratio, but increasing U:S ratios had quadratic effect on the breast meat yield. The effect of addition of animal fat or vegetable oil on breast meat yield has been studied. Danicke *et al*. (1999a) reported no significant differences in the percentage of breast meat in broilers fed diets containing soybean oil or tallow. Sanz *et al*. (1999) fed broilers with diets supplemented with different fat sources (tallow, lard and sunflower oil) and reported no significant effect of fat source on breast meat yield. Similar results have been observed by Crespo and Esteve-Garcia (2001).

In the present study, broilers fed diets containing U:S ratio of 2.10 and 2.74 had higher breast meat yield than those fed diets containing the other ratios. This finding is difficult to explain, but it may be speculated that these ratios are the best to achieve high breast meat yield.

Increasing the U:S ratio in diets decreased the relative weights of abdominal fat. The effect of blends of vegetable oil and animal fat on abdominal fat has been reported by Pinchasov and Nir (1992). These researchers fed broilers with diets containing five blends of vegetable oil (1:1 mixture of soybean oil and safflower oil) with tallow and found no effect on abdominal fat weight. On the contrary, Wongsuthavas *et al*. (2007a) fed broilers diets with five different ratios of tallow and soybean oil, and reported that percentage of abdominal fat pad decreased when 75% of tallow was replaced by soybean oil. Wongsuthavas *et al*. (2008) fed broilers with five different ratios of tallow with soybean oil at three fat inclusion levels (30, 60 and 90 g/kg) and noted that the percentage of abdominal fat increased with increasing proportions of tallow. Reductions in abdominal fat at higher U:S ratios in the present study may be explained by the increase in the concentration of unsaturated fatty acids in diets. Several studies have shown that relative abdominal fat pad weights increase when fed diets containing high saturated fat, and lower in broilers fed diets rich in unsaturated fatty acids (Vila and Esteve-Garcia, 1996a; Zollitsch *et al*., 1997; Sanz *et al*., 1999; Crespo and Esteve-Garcia, 2001; Villaverde *et al*., 2005). These variable effects on abdominal fat pad weight can be attributed to difference in the metabolic use of difference fat sources. Sanz *et al*. (2000) suggested that unsaturated fatty acids are oxidised as fuel sources more rapidly than saturated fatty acids. Energy from saturated fats is easily deposited as abdominal fat pad and around the internal organ than that from unsaturated fatty acids (Zollitsch *et al*., 1997).

To the author's knowledge, there are no previous reports on the effect of U:S ratio on caecal microflora. Under current experimental conditions, the results showed that bifidobacteria were the only caecal bacterial populations to be affected by dietary treatments. Increasing the U:S ratio increased the population size of *Bifidobacterium* species. Bifidobacteria have a beneficial effect on growth performance (Geier *et al*., 2009) and immune modulation (Lan *et al*., 2005). The increase in bifidobacteria counts is difficult to explain. It is unclear whether this increase was related to the changes in U:S ratios or to reduce by other microflora. However, it should be noted that the performance of broilers was unaffected by increasing bifidobacteria numbers in the present study.

In the current study, a linear positive correlation was observed between U:S ratio and AME of fat in birds fed wheat-based diets, with the AME of fat increasing as the U:S ratio increased. Similarly, data reported in Chapter 5 also showed that increasing the U:S ratio by the blending of tallow with soybean oil increased the AME of fat in birds fed maize-based diet. Linear regression equations predicting the AME of fat have been determined by several researchers (Wiseman and Lessire, 1987; Huyghebaert *et al.*, 1988; Scheele *et al*., 1997), but only the study of Wiseman and Lessire (1987) evaluated the linear regression for blends two fat sources. These researchers observed improvements in the AME of fat when broilers were fed wheat-maize-based diets supplemented with five ratios of tallow and rape oil (100:0, 95:5, 90:10, 80:20 and 0:100). Although the fat sources and proportion of blending of fat were different, the regression equation obtained in the present study showed similar trends as that reported by Wiseman and Lessire (1987). The regression equation generated in the present study can be used, based on the U:S ratio, to predict the AME of blends of animal fat and soybean oil in wheat-based diets. However, it must be noted that the equation reported in the current study may not be applicable to predict the AME of blends of fat sources other than animal fat and soybean oil. Factors such as content of free fatty acids, chain length, position of fatty acids, quality of fat and age of birds should also be considered to accurately predict the AME of fat blends.

8.6. Conclusions

In conclusion, the present experiment demonstrated that the U:S ratio influenced the performance of broilers during the starter period (1 to 21 day), but had no effect on the performance over the whole trial period (1 to 35 day). Increasing the U:S ratio decreased the AME of diet and increased the total tract retention of fat. Carcass recovery was unaffected by the U:S ratio, but increasing the U:S ratio affected breast meat yield and abdominal fat weight. Increasing the U:S ratio had no influence on caecal microbial counts, except on *Bifidobacterium* species which were increased. There was a positive linear correlation between U:S ratio and the AME of fat blends, indicating that the AME can be improved by manipulating the U:S ratio. The regression equation developed in the present study can be used to predict the AME of blends of animal fat and soybean oil in wheat based-diet by using the U:S ratio.

Overall, the present data suggest that the manipulation of the U:S ratio, through blending of fat sources, may be a potential strategy to improve the utilisation of fat.

CHAPTER 9

General discussion

9.1. Introduction

Energy content is a major aspect considered by nutritionists when formulating poultry diets as poultry are known to eat to meet their energy requirement. Moreover, genetic development of modern broiler strains has increased their growth potential and the need for higher intake of energy necessitates feeding of high energy diets. For this reason, lipids (fats and oils), the energy value of which is at least twice as those of carbohydrates and protein (NRC, 1994), are commonly added to poultry diets. Dietary addition of fats and oils is also beneficial to reduce the dustiness, increase palatability and improve the texture of diets (Leeson, 1993). Cost of energy sources represents the major cost in feed formulations. Due to recent increases in the price of cereal grains, there is renewed interest in exploring ways to improve the energy value of fats. Therefore, there is a need for greater understanding of the digestion and absorption of fats in poultry, factors affecting fat digestion and strategies to improve fat digestion in poultry. These aspects were the focus of this thesis research.

The general findings of this thesis confirm that unsaturated fatty acids are better to utilised by poultry than saturated fatty acids. Numbers of factors were found to influence fat utilisation and these included age of birds, dietary calcium concentration, cereal type and the ratio between unsaturated and saturated fatty acids. The data also showed that blending of vegetable oils and animal fats, by changing unsaturated to saturated fatty acid ratios, may also improve fat utilisation.

9.2. Site of digestion and absorption of fat and fatty acids

Identification of the intestinal sites of fatty acid absorption is critical to understand the dynamics of fat digestion. Data reported in Chapter 4 demonstrated that jejunum was the predominant site of fat and fatty acid absorption in broiler chickens. Fat was rapidly digested and absorbed in the jejunum, and the digestion and/or absorption continued in the upper ileum. The digestibility of unsaturated fatty acids (oleic and linoleic acids) were determined to be higher than those of saturated fatty acids (palmitic and stearic acids). It was clearly shown that soybean oil was better digested than tallow because of its higher content of unsaturated fatty acids. Importantly, the data implied that lipids

with high unsaturated to saturated fatty acid ratio can improve the digestion and absorption of saturated fatty acids.

9.3. Fat content and fatty acid composition of the chicken bile

Bile is the main source of endogenous fat and fatty acids, and also plays an important role in the digestion and absorption of fat. No published data are available on the fatty acid composition of bile. Data from Chapter 4 showed that bile contained 2.4 g fat/kg DM and 64% of bile consisted of fatty acids. Moreover, the present data confirmed that fatty acids in the bile are closely associated with phospholipids.

9.4. Endogenous losses of fat

Significant losses of nutrients occur during the digestion and absorption along the intestinal tract. Estimations for ileal endogenous losses of protein and amino acids in poultry have been published (Ravindran and Bryden, 1999; Lemme *et al*., 2004; Cowieson *et al*., 2009), but reports on endogenous fatty acid losses are scant. Ajuyah *et al*. (1996) conducted a study to evaluate endogenous losses of fat in broilers, but these researchers fed broilers with semi-purified diets containing fat which could have influenced the determined values. Endogenous losses of fat presented in Chapter 4 are more accurate than that reported by Ajuyah *et al*. (1996) due to the birds in the current study were fed a fat-free purified diet. The findings in Chapter 4 showed that the fatty acid profile of endogenous fat corresponded closely to that of the bile, indicating that the flow of ileal endogenous fat may originate primarily from unabsorbed bile. The present results also showed that only 48% of the endogenous fat was of fatty acid origin. The balance 52% was accounted by non-fatty acid sources and these may include cholesterol, phospholipids in the bile, desquamated intestinal epithelial cells and fats of microbial origin.

9.5. Influence of age of birds on AME and total tract digestibility of fats

Data reported in Chapter 5 demonstrated that the AME and digestibility of fat sources are influenced by the age of bids. These results confirm the physiological limitation in newly hatched chicks to digest and/or absorb fats (Carew *et al*., 1972). An important implication of these findings is that lower AME values must be assigned for fats in the formulation of pre-starter diets.

It was also found that AME and total tract digestibility of tallow were lower than other tested fat sources (soybean oil, palm oil, poultry fat and, blend of tallow and

soybean oil), particularly during the first week of life. Based on the present data, it is recommended that tallow be avoided in diets for young birds and instead vegetable oils be used in pre-starter diets.

9.6. Fat x cereal type interaction

The interaction between dietary fat sources (tallow and soybean oil) and cereal types (maize, sorghum and wheat) was reported in Chapter 6. The data showed that, in wheat- and maize-based diets, birds fed soybean oil grew faster than those fed the tallow. Irrespective of the cereal type, feed per gain of birds fed tallow supplemented diets was poorer than those fed soybean oil diets. In all three cereals, total tract retention and ileal digestibility coefficient of fat in soybean oil were considerably higher than those in tallow.

A major limitation in a design examining the effects of different cereals and fat sources was the challenge of formulating iso-caloric diets. In the current study, despite maize being a non-viscous cereal, maize-based diet contained NSP content similar to that of wheat-based diet because cellose had to be added to maize-based diets to achieve iso-caloric diets. Another contributing factor to the NSP levels was the relatively high inclusion levels of soybean meal in the maize-based diet compared to the wheat-bassed diet. Both these factors could have confounding effects on the observed influence of different cereals on the parameters studied.

9.7. Xylanase x fat source interaction

Wheat contains high concentrations of NSP which exhibit anti-nutritive activity in poultry diets (Annison, 1993). Nowadays, it has become a common practice to supplement exogenous xylanases in wheat-based diets to overcome the problem of high digesta viscosity and, to improve nutrient digestibility and bird performance. Data reported in Chapter 6 showed that, irrespective of the fat source (tallow or soybean oil), supplemental xylanase increased the weight gain and lowered feed per gain of young broilers. Supplementatal xylanase improved the AME of tallow supplemented diets, but this effect was not observed on soybean oil supplemented diets. These data suggest that the use of exogenous xylanases is beneficial to improve the digestion and energy utilisation of saturated fats.

9.8. Dietary calcium level x tallow inclusion interaction

Results of the study reported in Chapter 7 highlighted the adverse effects of high dietary calcium concentrations on the performance and nutrient utilisation in broilers, irrespective of tallow inclusion levels. Increasing calcium concentrations lowered the total tract retention and ileal digestibility of fat, and this was associated with increased formation of excreta soap. The formation of excreta soap was also found to increase with increasing inclusion levels of tallow and the effect calcium on excreta soap formation was more pronounced at the highest inclusion of tallow.

In the current study, analysed dietary concentrations of calcium were determined to be 35 % higher than the calculated values. This observation underlines the need to analyse for calcium in feed ingredients before formulating diet for use in studies examining the influence of dietary calcium concentrations. It is known that calcium concentrations in feedstuffs are more variable than phosphorus values. In particular, calcium concentration in soybean meal can vary widely because limestone is added at the end of processing in some processing plants to prevent caking of the warm meal (Tamim *et al.*, 2004).

9.9. Value of different fats as energy sources

A range of fat sources are available for use in poultry diets, with variable effects on bird performance, AME and fat digestibility. Data reported on Chapter 5 showed that soybean oil, poultry fat and palm oil are useful energy sources due to their high AME and digestibility of fat compared to tallow. Soybean oil is a good source of lipid for broilers because it contains high concentration of unsaturated fatty acids. The major unsaturated fatty acids in soybean oil were linoleic and oleic acids, whereas palmitic acid was the major saturated fatty acid. The fatty acid profile of soybean oil used in Chapters 5, 6 and 8 was almost similar and the unsaturated fatty acids to saturated fatty acid ratio (U:S ratio) ranged between 4.8 and 5.4. These observations are suggestive of the uniformity of the product, in contrast to that of animal fats.

Poultry fat is the rendering by product from poultry processing plants. Data from Chapter 5 showed that poultry fat is a good energy source for broilers due to its high concentration of unsaturated fatty acids, mainly oleic and linoleic acids. The major saturated fatty acid in the poultry fat was palmitic acid. The U:S ratio of poultry fat was 2.07. Despite the relative high U:S ratio, AME and fat digestibility of poultry fat were similar to that of soybean oil and can be a valuable energy source in poultry diets. However, Leeson and Summer (2005) suggested that feeding of poultry fat continuously

may increase fat soluble contaminants in poultry meat. This problem can be resolved by breaking the feeding cycle for one or two bird cycles.

Data from Chapter 5 showed that the AME of palm oil was comparable to those of soybean oil and poultry fat. However, the digestibility of fat in palm oil was found to be lower than that of soybean oil. Palm oil used in this study had a U:S ratio similar to that of tallow(0.93 vs. 0.80), but the AME and fat digestibility were higher than those of tallow. It is noteworthy that the AME of palm oil determined in the present study was higher than those reported by previous researchers (Wiseman and Salvador, 1991; Scheele *et al*., 1997; Zumbado *et al*., 1999; Pesti *et al*., 2002). A number of palm oil products are normally used in broiler diets and these include crude palm oil, refined palm oil (refined, bleached, deodorised) palm olein and palm acid oils (Walker, 2011). Palm oil type used in different AME studies has not been adequately described. In the study reported in Chapter 5, refined palm oil was evaluated. The wide range of AME values reported for palm oil in the literature clearly relate to the different types of palm oil used and further research is needed to evaluate the AME of different palm oil products.

Beef tallow was evaluated as an energy source in the studies reported in Chapters 4, 5 and 6 and, the results clearly demonstrated that tallow was poorly digested and utilised compared to soybean oil. Beef tallow is the animal fat which is commonly used as an ingredient in countries with large red meat industries because of its relatively low cost. However, the work reported in this thesis showed that the utilisation of beef tallow was lower than the other fat sources, especially in young broiler chickens.

The fatty acid profile of animal fat (blend of sheep and beef tallow with lard) in Chapter 8 was found to be different from that of beef tallow used in Chapters 5 and 6. The animal fat blend had a higher U:S ratio (1.30) compared to beef tallow (0.63-0.80). These observations highlight the variability in the fatty acid profile of animal fats, which will vary widely depending on the fats included in the blend. The present data suggests that the variability in the composition of animal fats is an important issue to be considered.

9.10. Influence of U:S ratio on fat digestion and carcass characteristics

Data reported in Chapter 5 showed that blend of tallow and soybean oil improved the AME and digestibility of fat. Increasing U:S ratios also increased the total tract fat retention and AME of fat (Chapter 8). Therefore, blends of two fat sources can be used as

a strategy to improve the utilisation of fat and may have the synergistic effect on the utilisation and digestibility of fat.

Although carcass recovery was unaffected by the U:S ratio, the breast meat yield and abdominal fat were affected by the U:S ratio. Broilers fed diet containing U:S ratio of 2.10 and 2.74 had higher breast meat yields and lower abdominal fat than the U:S ratio of 1.30, 1.64, 3.73 and 5.43. Therefore, it can be suggested that blends of animal fat and soybean oil containing a U:S ratio between 2.10 and 2.74 may be considered in formulations to achieve the highest breast meat yield in broiler chickens.

9.11. Relationship between U:S ratio and AME of fat blends

In the study reported in Chapter 8, the relationship between U:S ratio and the AME of fat was examined. A linear positive correlation ($R^2 = 0.63$; $P < 0.001$) was observed between the U:S ratio and AME of fat, with increasing the U:S ratios resulting in increased AME of fat. Data from Chapter 8 suggested that increasing U:S ratio through blending of animal fat with soybean oil is a potential strategy to increase the AME of blends fat. The regression equation reported in Chapter 8 will be useful to predict the AME of blends of animal fat and soybean oil, by using U:S ratio, in wheat-based diets. However, other factors such as content of free fatty acids, chain length, position of fatty acids, quality of fat and age of birds should also be considered to accurately predict the AME of fat blends.

9.12. Influence of xylanase and blending of two fat sources on caecal microflora

Data from Chapter 6 revealed that supplemental xylanase lowered lactobacilli counts and increased clostridia counts in caecal contents. Data presented in Chapter 8 showed that increasing U:S ratio increased the numbers of bifidobacteria in caecal contents. The observed effects of xylanase and blending of two fat sources on the microflora are contrary to expectations and in contrast with those reported by other researchers (Choct *et al*., 2006; Nian *et al*., 2011). The discrepancy between these studies may be explained by the different methods employed to determine the bacterial counts. Conventional culture methods are normally used to study the gut microflora. In this thesis, counts of selected bacteria in caecal contents were determined by the fluorescent *in situ* hybridisation (FISH) method. This method uses fluorescent probes to bind parts of the chromosome to detect bacteria and, is rapid and more accurate than conventional culture methods (Kempf *et al*., 2000; Gharibi *et al*., 2010). The probes used in this method

measured the genus of the bacteria, whereas the above-mentioned studies have focussed on specific bacterial species. The present data showed that xylanase increased the number of clostridia, but it must be noted that not all clostridial strains are detrimental to the bird. Rather, most clostridial strains are commensal and only a narrow range of clostridia are pathogenic. Therefore, in future studies, it would be meaningful to use probes which are specific to species to determine bacterial counts.

9.13. Suggestions for future research

The study presented in this thesis identified several factors that influence fat digestion. Age of bird showed significant effect on fat utilisation (Chapter 5). Lower fat utilisation in young birds is possibly due to the insufficient bile salts and lipase (Krogdahl, 1985, Noy and Sklan, 1995) which may result in lower emulsification of fat. Addition of lipase, bile salts or synthetic emulsifiers to diet to improve fat utilisation in young birds has been evaluated by several researchers (Polin *et al*., 1980; Al-Marzooqi and Leeson, 1999). Although increasing the emulsification of fat may lead to an improved fat digestion (Zhang *et al*., 2011), underdeveloped gastrointestinal tract may not accomplish the absorption of fat in young birds. It has been reported that feeding diets with structural components such as oat hulls, whole wheat or wood shaving to broilers resulted in rapid development of gastrointestinal tract by increasing size and weight of gizzard and improved starch and nitrogen utilisation (Amerah *et al*., 2009; Svihus, 2011). It is possible that feeding diet with structural components may also improve the digestion and absorption of fat in young birds. However, the information on type and optimal amount of structural components to improve fat digestion is limited and need to be investigated.

With regard to meeting the energy requirements of birds, the amount of available energy to the bird is more important than the calculated energy content of the diet. Although fat, due to high energy content, provides the opportunity to formulate high energy diets; this advantage may not be applicable for newly hatched chicks when the fat digestibility taken into account. Due to the very low fat utilisation during the first week of age, it might be useful to investigate possibility of using highly digestible fats or replacing fat sources with highly digestible ingredients in pre-starter diets to meet the energy requirements.

Data reported in Chapter 7 indicated that high calcium concentrations adversely influenced on the performance and nutrient utilisation in broilers, especially of fat. On the other hand, reducing dietary calcium concentrations will affect bone development

and reduce growth rate (Tisch, 2006). Based on the study presented in Chapter 7, it could be suggested that calcium concentrations in broiler diets should be kept low as realistically as possible to enhance the utilisation of fat. However, the information on the minimum calcium requirement to optimise fat digestion is scant and further research is warranted to investigate this issue.

9.14. Summary and main conclusions

The work reported in this thesis reported findings on the site of fat digestion, and endogenous losses of fat and fatty acids. Factors affecting fat digestion including age of birds, cereal type and concentration of calcium were also examined. Increasing the U:S ratio as a possible strategy to improve fat digestion was also studied.

Overall, the work reported in this thesis demonstrated that the digestion of fat in poultry can be affected by both bird-related and diet-related factors. Age of birds had a marked impact on the utilisation of fat, but this issue can be largely overcome by assigning lower AME values for fat sources when formulating diets for young birds. Cereal type may be another factor that needs to be considered when selecting the fat source in feed formulation. High dietary calcium concentrations had negative impact on the fat digestibility. To optimise fat digestion, calcium concentrations should maintained low as realistically as possible especially when the diets contain high levels of saturated fats. The findings of this thesis also showed that fat sources with high U:S ratio or blends of fat sources to increase U:S ratio should be considered in feed formulations to improve the utilisation of fat.

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