ASPECTS OF LIPID UTILIZATION IN THE NEONATAL CHICKEN

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SUMMARY

Lipids play an important role throughout the life of the birds but particularly during embryonic and neonatal periods. Following hatching the lipids are derived from two sources, the residual yolk sac material and the diet. In modern broiler production much emphasis is placed upon the diet, thus providing the chick with a high lipid oriented environment very early in the post-hatch period. This is normally aimed at enhancing rapid growth in birds. However, a combination of exogenous and endogenous lipid sources to the chick during this period is likely to exert enormous pressure on the physiological processes most of which are still in the developmental stage. Thus, the main objectives of the present studies were to investigate the relative roles of the yolk lipids versus dietary lipids during the neonatal period on lipid and fatty acid compositional changes of the residual yolk sac material, liver, gall bladder bile and gastrointestinal tract. In addition the effects of exogenous and endogenous dietary lipids on the establishment of the gastrointestinal tract microflora, lipid and fatty acid digestibilities were studied. During the study the effect of post-hatching age on all the mentioned parameters was also assessed.

Three dietary treatments were used. Diet 1 was a complete diet from a commercial company or a diet compounded using a commercially available fat source. This diet contained high levels of unsaturated fatty acids and free fatty acid. Diet 2 was based on tallow oil as the major fat source and thus contained high levels of saturated fatty acids. Diet 3 was based on soyabean oil as the major fat source and contained high levels of unsaturated fatty acids. The diets were formulated in such a way as to provide adequate levels of other nutrients. Standard procedures for chick rearing and feeding were followed. Established analytical methods for lipid and fatty acids were used throughout.

Experiment 1 involved a study on the effects of post-hatching age and dietary fat sources on the lipid and fatty acid compositional changes of the residual yolk sac material, liver and gall bladder bile during the first 12 post-hatch days. The results obtained showed that both the weight and lipid compositions of the residual yolk sac material and the liver were significantly affected by post-hatching age. The changes included significant decline in the weight of residual yolk sac material from about 11 percent of the total weight of the chick immediately post-hatch to less than 0.1 percent of the chick's body weight by day 12 post-hatch. Between day 1 to 9 post-hatch the rate of decline was higher in chicks receiving the commercial diet and the tallow oil based diet, but no further differences were observed after day 9. Significant increases in the proportion of cholesterol esters and decreases in triglyceride and phospholipid fractions with post-hatching age were observed for all dietary treatments. Dietary effects were also noted, for cholesterol ester and triglyceride, proportions being significantly higher and lower, respectively, in chicks receiving the tallow oil based diet.

In common with the residual yolk sac material, the proportion of liver tissue to the chick's body weight was significantly affected by age, particularly between days 1 and 3 post-hatch during which a 50 percent increase was observed in all dietary treatments. After day 3 post-hatch increases were observed only in chicks receiving the commercial diet and the soyabean oil based diet, whilst, decreases occurred in chicks receiving the tallow oil based diet. Liver lipid compositional changes with post-hatching age were characterised by significant decreases of cholesterol esters from 77 to less than 2 percent of total lipid present on day 1 and day 12 post-hatch, respectively. Concomitantly there were significant increases in the triglyceride and phospholipid proportions, whilst, minor changes occurred in the other lipid fractions. Dietary effects on cholesterol esters and triglyceride levels

were also observed, levels being higher and lower in chicks receiving the soyabean oil based diet. The lipid composition of the gall bladder bile was significantly affected by post-hatching age in particular the increase in triglyceride levels. In all the tissues studied the effect of age and dietary fat source on fatty acid composition was relatively small.

In experiment 2, the influences of the post-hatching age and dietary fat source on the lipid and fatty acid compositional changes in relation to lipid digestion and absorption within the different sections of the gastrointestinal tract were studied. The major feature observed throughout the 12 day post-hatch period was the high phospholipid levels (more than 60 percent of total lipid present) within the duodenal contents irrespective of the dietary treatment. At day 3 post-hatch and in all subsequent days high proportions of triglyceride and free fatty acid were observed in GIT sections beyond the duodenum. It was clear from this study that most of the processes of lipid digestion occurred beyond the duodenum. The proportion of free fatty acid remained high along the remaining sections of the gastrointestinal tract in all the dietary treatments, particularly in chicks receiving the tallow oil based diet. As in experiment 1, the effects of dietary fat source on the fatty acid compositional changes were relatively small.

Experiment 3 describes the effect of post-hatching age and dietary fat sources on the intake and excretion of fat during the first 21 days post-hatch. Increases in fat intake, lipid and fatty acid digestibilities with post-hatching age were observed. The apparent digestibilities for long chain saturated fatty acids were lower when compared with the long chain unsaturated ones. Differences in fatty acid digestibilities were also observed between the dietary treatments, digestibilities being higher with increased dietary unsaturation. Dietary differences also occurred in fat excretion, excretion being higher in chicks receiving the tallow oil based diet.

Free fatty acid was the major lipid component of the faecal material in all dietary treatments. The fatty acid composition of the faecal material was a reflection of the dietary fatty acid composition.

Experiment 4 describes the establishment of various species of micro-organisms in the gastrointestinal tract and the possible influences of the post-hatching age and dietary fat sources during the first 4 weeks post-hatch. The gastrointestinal tract was divided into 3 sections, the duodenum, ileum and the caecum. Most of the micro-organisms studied were not detected within the duodenal contents on day 1 post-hatch and on day 26 post-hatch only Clostridial and Streptococcal species were detected. In both the ileum and the caecum substantial numbers of Lactobacilli, Clostridial, Streptocccal species were present at day 1 post-hatch and during the subsequent days. A decline in most of the bacterial species with post-hatching age was observed in all dietary treatments. The concentration of the different species were generally higher in chicks receiving the tallow oil based diet.

In all the experiments the body weight of the chicks was measured throughout. With the exception of experiment 1 body weights were lower in chicks receiving the tallow oil based diet.

It was clear from the present investigations that differing lipid constituents in diets fed early in the post-hatched chick might influence the normal developmental changes occurring in the gastrointestinal tract and other tissues related to lipid metabolism. It is therefore suggested that since most of the developmental processes, both physiologically and anatomically occur during the first 2 weeks after hatching, due attention must be paid in dietary formulation to the enhancement of these processes.

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DEDICATION

I dedicate this work to my

sons AMON MUJUNI and MUGISHA MUGYABUSO

my FATHER and MOTHER

DECLARATION

The contents of this thesis are the work of the author. The thesis has not been submitted previously for the award of a degree to any University.

Signature... Date...10.3.93

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CHAPTER 1

BACKGROUND

The use of animal products in human diets is normally determined by the wealth of individuals or a country as a whole. Studies carried out in United Kingdom and other countries have shown that the trend of food consumption has changed considerably since the post-war period. The most obvious change has been the increase in the consumption of animal origin foods which are associated to some extent with increase in wealth. Indeed, with the economies of many countries improving, an increased demand for animal products is likely to continue (Summers and Leeson, 1985). Due to the ever increasing demand for animal products, attempts are continually being made to improve animal production systems; the improvements by increasing the production size and most importantly improving the productivity per animal.

Poultry production as a source of animal protein is one of the areas which has received much attention for sometime now. This is because poultry production requires relatively low economic inputs when compared to large animals and their products, particularly meat are widely accepted by consumers (Summers and Leeson, 1985). As a result of the above, it is anticipated that there will be a large increase in poultry consumption in the immediate future. The poultry industry, especially broiler production has made tremendous progress during the past decade through manipulation of genetics, nutrition and management. Rapid improvement through genetical selection in particular has come about because the chicken has a short generation interval and a high production turnover compared to other animal species.

Over the last 20-30 years selection of broiler birds has been aimed at producing fast growing and heavy strains. Thus the time required to produce a 2.0 kg broiler bird has been reduced from 15 weeks in 1960 to about 6 weeks in 1985 (Gyles, 1989). Feed efficiency is another parameter which has been improved

through genetical selection; thus the feed required for a 2.0 kg liveweight bird has been reduced from 7.3 kg to about 3.5-4.0 kg during the 1960-1985 period (Summers and Leeson, 1985). However, the success of genetical selection has to be accompanied by improved nutrition and management practices if production advantages are to be maximised. In most of the large scale production systems, poultry has moved away from extensive and semi-intensive to wholly intensive systems. Under an intensive system it is possible to provide the birds with optimal environmental conditions e.g. temperature, relative humidity, ventilation etc, coupled with emphasis on disease control. As a result large numbers of chickens per unit area are kept under this system in comparison with other systems.

Egg incubation effectively marks the beginning of the productive capability of birds. Lipids play a prime role during embryonic development. During the development of the chick embryo it is estimated that more than 90 percent of the total energy requirement is derived from the oxidation of yolk lipid fatty acid (Romanoff, 1967; Freeman and Vince, 1974). As a result developing embryos are exposed to a highly lipid oriented environment with about 1 gram of lipid per day being absorbed from the yolk over the last seven days of incubation (Noble, 1987). Despite this extensive mobilization, a large proportion of yolk lipid remains unabsorbed at hatching. The amount of the unabsorbed yolk material accounts for about 18 percent of the total weight of the hatched chick and contains about 1.7 gram of lipids (Noble and Ogunyemi, 1989). This remnant yolk material is sufficient to supply the nutrient needs of the emerged chick under normal circumstances for up to 3-4 days post-hatching (Nir *et al.*, 1988). Hence, the presence of such yolk material provides an important continuation of nutrient supply during the critical transition period from embryonic to free living stage. Grossly, it contributes about 50 and 43 percent, respectively, of the total energy and protein supplies (from both

feed and yolk) to the chick on day 1 post-hatch; 2 and 6 percent, respectively, on day 4 post-hatch and negligible thereafter (Murakami *et al.*, 1988; Nir *et al.*, 1988). Although much is known about the contribution of the remnant yolk material to nutrient supply of the newly hatched chick, little is known about the mechanisms as to how this is accomplished. An equal divergence of opinion exists on whether there is a continuation of embryonic processes of the residual yolk uptake (Romanoff, 1960; Noble, 1987) or whether there is a continuation of the expulsion of yolk material into the gastrointestinal tract (GIT) via the vitelline diverticulum similar to that observed during the final days of incubation (Esteban *et al.*, 1991).

Nutrition is an important factor in any production system and is estimated to account for about 60-70 percent of total production costs (Neishem *et al.*, 1979a). The major purpose of the producer must therefore be to provide a well balanced diet to the birds at a reasonable cost in order to maximise the bird's genetic potential. Nevertheless, improper feeding does occur especially by overfeeding the broilers during the earlier stages of life. Furthermore, due to the ever changing patterns in consumer preference mainly geared to better carcass quality, constant changes in feeding regimes are essential so as to produce broilers which suit the existing markets. Hence, the viability of any broiler production system depends on the speed with which changes to the system can be implemented.

The market age of broilers is currently about six to seven weeks. For the past 40 years the market age of the broiler bird has been reduced by about one day per year (Gyles, 1989). At present, almost 25 percent of broiler market weight is attained during the first two weeks post-hatching (Bjornhag, 1979). Such a trend emphasises the importance of growth during the first week of life since this covers almost 16 percent of the life span of the broiler and this proportion might increase in the near future (Gyles, 1989). As a result of these changes and achievements, much

attention has recently been given to the feeding regimes during the early post-hatch period so as to maximize the bird's genetical potential. However, the advantages observed during the immediate post-hatch period can only be achieved if there is very early transition of the digestive system from embryonic to a normal monogastric system (Murakami *et al.*, 1988). This mainly includes the rapid growth rate of various organs associated with digestion and improvements of the enzymatic systems (Lilja, 1983; Nir *et al.*, 1988; Sell *et al.*, 1991). These changes are governed by the bird's genetic potential as well as the nature of diet supplied. An important relationship exists between the level of feeding of broilers and the development of the GIT and its associated enzymes (Plavnik and Hurwitz, 1985; Pinchasov *et al.*, 1985). The slow growth and failure to attain the market weight at a normal age demonstrated in the young chick on a depressed feed intake is sometimes a result of reduced development of the GIT (Pinchasov *et al.*, 1985).

The growth rate in broilers is rapid during the early post-hatch period and reaches a peak at about two weeks. The maximal growth is usually accompanied by large increases in the weights and bulk of most of the important organs such as liver, pancreas and small intestine (Dror *et al.*, 1977). The duodenum displays the most rapid development, achieving a five to seven fold increase in size over the first 2 weeks post-hatch period. However, little changes are observed in the size of lower segments of the GIT particularly the colon. Developmental changes of the duodenum are more pronounced in broilers than in layers. This difference is mainly thought to be due to an adaptation to realise the high growth potential of the broilers through an increased digestive capacity (Dror *et al.*, 1977; Bjornhag, 1979). Owing to the continual need of maximising the growth potential of the broiler birds, it is deemed by the producer to add high levels of lipid in the diet from as early neonatal age as possible (Ferket, 1991). The feeding of such lipid oriented diet in conjuction

with the presence of large amounts of residual yolk lipid, demands for an extensive ability of the chick at hatching to digest lipids. Various propositions put forward, sometimes indicate that the situation posed by exposure to such large concentration of lipid within the GIT during the early stages of growth may not entirely be beneficial to the subsequent development and viability of the broiler bird.

It was therefore the objective of the present study to investigate the following with respect to the role of lipids during the early post-hatch period in broiler chicks:

(i) The role of remnant yolk lipids and their possible implication on GIT lipid concentration.

(ii) Gross lipid changes in the GIT, liver and yolk sac remnants, during the early post-hatch period.

(iii) Qualitative and quantitative changes of the GIT lipids when different dietary fat sources are fed.

(iv) Effect of different dietary fat sources on growth of the broiler bird and lipid digestibility.

(v) The effect of different dietary fat sources on the establishment of GIT flora.

CHAPTER 2

LITERATURE REVIEW

2.1 LIPIDS

The recent definition of lipids encompasses fatty acids, their derivatives and substances related biosynthetically or functionally to these compounds (Christie, 1989). Most of the lipids are soluble in organic solvents such as chloroform, diethylether, hexane, benzene etc, but insoluble in water although a few exceptions have been reported e.g gangliosides (Christie, 1973; 1989). Lipids are divided into two major groups depending on the type of products produced upon hydrolysis. Simple lipids are those which yield at most two types of hydrolysis products namely fatty acids and glycerol per mole. This group mainly includes triglycerides and cholesterol esters the former being more abundant (Noble, 1987; Christie, 1989). Complex lipids yield three or more primary products per mole. Examples of this group are phospholipids namely, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline and sphingomyelin which also contain phosphoric acid and nitrogen and glycolipids which contain carbohydrates.

2.1.1 Simple and Complex Lipids

The triglycerides are the predominant group of the simple lipids. Nearly all the commercially important fats and oils of plant and animal origin contain high levels of triglycerides (McDonald *et al.*, 1988; Enser, 1984; Gurr, 1984; Noble, 1987). The fatty acid composition of triglycerides is very variable with acids being arranged on different positions of the glycerol backbone. In seed oils the C18 unsaturated fatty acids tend to dominate, whereas in animal fats especially adipose tissue, the composition tends to reflect dietary fat composition (Gurr, 1984). Fish triglycerides and those of marine mammals differ from other sources because of their high proportion of polyunsaturated fatty acids (Christie, 1989). Triglycerides are the major sources of chemical energy and act as thermal insulators and

protective cushions for organs (Wiseman, 1990). Upon hydrolysis, triglycerides give rise to mono- and di-glycerides which contain respectively, one mole and two moles of fatty acids (Hokin, 1985). These are collectively known as partial glycerides and are not abundant in nature (Hokin, 1985). When a triglyceride contains more than one type of fatty acid in its molecule it is known as a mixed triglyceride; such triglycerides normally form a large proportion in most of the natural fats.

The complex lipids are dominated by phospholipids. These moieties form major constituents of membranes in animal tissues (Enser,1984; Christie, 1989; Wiseman, 1990). Due to the presence of the phosphate group in their structure, phospholipids are more polar than triglycerides (Christie, 1989). Each phospholipid subclass in tissues has a distinctive fatty acid composition, normally related to its function. Phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl choline are important constituents of cell membranes and sphingomyelin of nerve cells (McDonald *et al.*, 1988). Most of the phospholipids in animal tissues contain high levels of polyunsaturated fatty acids.

Free fatty acids are the hydrolytic products of both the simple and complex lipids. Free fatty acids are rarely found in nature and high quantities of free fatty acids indicate increased hydrolytic breakdown or contamination (Noble, 1984; Christie, 1989; Wiseman, 1990). Free fatty acids form a major variable of the chemical composition of fats included in diets and their presence greatly influences the digestibility of feeds (Wiseman, 1990).

Steroids include biologically important compounds such as cholesterol, derived sterols, bile acids, adrenalin and sex hormones. Cholesterol is by far the most common member of this group and it exists both in a free state, where it has a vital role of maintaining membrane fluidity and in an esterified form (McDonald *et al.*, 1981). Cholesterol is an important constituent of the brain lipids forming up to

17 percent of the total dry matter. Bile acids are structually related to cholesterol but differ in their functions and occurrence (McDonald *et al.*, 1981; Griminger, 1986). The liver synthesizes and secretes bile acids which have several digestive functions. They aid in the emulsification of fats in the duodenum and in the activation of enzymes such as amylase and lipase (McDonald *et al.*, 1981). The lipid composition of the avian bile is slightly different from other monogastric animals since it contains high levels of triglycerides in contrast to the high phospholipids levels found in other animals (Griminger, 1986; Noble *et al.*, 1988).

2.1.2 Fatty Acids

The principal classes of all lipids contain fatty acids, which are normally long chain monocarboxylic acid moieties, linked by an ester bond to an alcohol; they may also be linked to other substances such as phosphoric acid, organic bases and sugars (Christie, 1973; Enser, 1984). Fatty acids are the major determinants of the physical and chemical characteristics of lipids (Enser, 1984; Christie, 1989). They are synthesized in nature via condesation of malonyl coenzyme A units by a fatty acid synthetase complex (Abraham, 1970). The fatty acids of plant, animal and microbial origin generally contain even numbers of carbon atoms in straight chains (Enser, 1984; Noble and Connor, 1984; Christie, 1989). In animal tissues the common fatty acids vary in chain length from 14-22 carbon atoms, but those with carbon atoms as low as 2 or higher than 22 are not uncommon. The carbon atoms are covalently linked and may have one to six double bonds. Due to this they are classified as saturated or unsaturated (Christie, 1973; Enser, 1984). A saturated fatty acid contains no double bond between the carbon atoms, whilst one or more pairs of double bonds exist in an unsaturated fatty acid carbon chain. The unsaturated fatty acids are further subdivided depending on the number of double bonds present into

monoenoic containing one double bond and polyunsaturated fatty acids containing more than one but with a maximum of six double bonds (Enser, 1984). Although these fatty acids predominantly exist in the *cis* form, occasionally they display the *trans* configuration. The presence of an unsaturated structure allows the fatty acid molecule to combine with oxygen and other chemical elements and to be more chemically active than the saturated fatty acid equivalent. This property is of vital significance in lipid utilization (Enser, 1984; Gurr, 1984). The degree of fluidity of lipids and lipid containing structures in the animal is of major importance and is to a large extent determined by the type of fatty acids present (McDonald *et al.*, 1988; Enser, 1984). The melting point of saturated straight chain fatty acids increases with increasing chain length. Unsaturated fatty acids have lower melting points than the saturated fatty acids of the same length. Most of the natural lipids such as triglycerides have a wide range of melting points, because different tissues contain varying ratios of the saturated and unsaturated fatty acids. Lipids with high concentrations of saturated fatty acids are solids at room temperature, whereas high concentrations of unsaturated fatty acids in a lipid renders it to be a liquid at room temperature. The two different types are commonly known as fats and oils, respectively (McDonald *et al.*, 1988).

Palmitic and stearic acids are the commonest saturated fatty acids in nature. Palmitic is found abundantly in lipids of all plants and animals (Christie, 1973; Enser, 1984; Noble and Connor, 1984). Stearic acid is also relatively common and on some occasions its concentration may surpass palmitic acid, particularly in many complex lipids (Gillespie, 1987). Longer chain saturated fatty acids occur less frequently but often form a large proportion of waxes. Amongst the unsaturated fatty acids oleic acid is the most common widely distributed and forms more than half of the fatty acids present in many fats (Gillespie, 1987). Some polyunsaturated

fatty acids including linoleic and arachidonic are also widely distributed and have essential roles in the animal's body. Some of the polyunsaturated fatty acids such as oleic and linoleic are known as essential fatty acids since they cannot be synthesised in the animal's body and have to be supplied in the diet in order to facilitate a selection of specific metabolic functions (McDonald *et al.*, 1988). Linoleic acid is required for normal growth, reproduction and healthy development whereas arachidonic acid is an essential component of the membrane and is a precursor of prostaglandins. Likewise linolenic, docosapentaenoic and docosahexaenoic acids also play important roles in the growth and normal functioning of the animal (Enser, 1984; Gillespie, 1987; Noble, 1987; McDonald *et al.*, 1988).

2.2 LIPID METABOLISM IN THE NEONATAL AND EARLY POST-NATAL STAGES OF CHICKS

2.2.1 Egg Lipid Composition

An average egg weighing 60 grams contains approximately 6 grams of lipid which is virtually confined to the yolk with only an extremely small amount associated with the shell cuticle (Noble and Cocchi, 1990). The onset of egg laying in the hen is characterised by an exceptionally increased demand for nutrients, particularly lipids which are essential in yolk formation (Gilbert, 1971). In some cases the lipid demand for daily egg production exceeds normal supply. Compensation in such cases is achieved by increased liver and adipose tissue lipid mobilization with egg production and lipid composition being affected under most severe circumstances (Husbands and Brown, 1965).

The egg yolk can simplistically be divided into two parts, the white and yellow components. The white yolk, which is associated with the germinal disc is deposited during early ovum maturation and constitutes less than 3 percent of total

yolk mass (Noble and Cocchi, 1990). The majority of yolk material i.e the yellow component consists an oil-water phase in the form of large floating spheres (25-150 μm diameter) and contain most of the yolk lipids (Gilbert, 1971; Noble *et al.*, 1990).

Extractable lipid account for about 33 percent of the total weight of the yolk and 60-65 percent of its dry matter content (Noble, 1987). Triglycerides form the major portion (approximately 60 percent) followed by phospholipids (25-30 percent). Both cholesterol esters and free cholesterol constitute less than 5 percent. Levels of free fatty acids found in the yolk are negligible under normal circumstances. Table 2.1 shows the distribution of the major lipid moieties in the egg yolk and their fatty acid compositions. C16 to C22 fatty acids predominate, with high levels of polyunsaturated fatty acids in the phospholipid fraction (Noble, 1987). The fatty acid composition of the yolk is affected by various factors, nutritional and environmental features being the most important (Edwards, 1964; Washburn, 1979).

2.2.2 Lipid metabolism during the embryonic stage

The developing embryo derives its nutrients from the yolk via a highly specialised yolk sac membrane (Romanoff, 1960; Noble and Moore, 1964). The yolk sac is essentially a thick and opaque double membrane which is well vascularized. Its endoderm is continuous with the embryo gut via a connecting yolk stalk. Despite this connection, no yolk ordinarily passes into the intestine during the incubation period, although studies by Esteban *et al.* (1991) indicated that emptying of the yolk material into the GIT occurs during the last days of incubation. Once

Table 2.1: Yolk lipid distribution and fatty acid composition of the major lipid fration (weight percent of total present)

	CE	TG	PL	FC
total lipid:	1.3	63.1	29.7	4.9
free fatty acid:				
16:0*	29.1	24.8	28.4	-
16:1	1.0	6.6	1.9	-
18:0	9.5	6.4	14.9	-
18:1	40.1	46.2	29.5	-
18:2	18.0	14.7	13.8	-
18:3	0.3	1.1	0.3	-
20:4	0.9	0.2	6.2	-
22:6	0.5	0.2	4.1	-

Source: Noble *et al.* (1990)

* The common names of the fatty acids as per description in Table 3.1

Table 2.2: The influence of egg weight on the weight of the chick, yolk sac and carcass in Bobwhite quail (gm)

	1	2	3
weight (gm) of:			
egg	9.6	10.4	11.2
chick	6.8	7.4	8.1
yolk sac	0.8	0.9	1.08
carcass	6.0	6.4	7.0
proportions (%)			
yolk sac wt/egg wt	8.0	9.1	9.6
chick wt/egg wt	71.1	70.8	72.2
yolk sac wt/chick wt	11.5	12.9	13.3
yolk sac wt/carcass wt	13.2	15.1	15.6

Source: Skewes *et al.* (1988)

within the yolk sac membrane and following a limited amount of hydrolysis, the lipid constituents are transferred from the endodermal cells to the embryo via the blood vessels of the yolk sac (Noble and Cocchi, 1990).

The precise mechanism by which the yolk lipids are assimilated by the developing embryo is still unclear since the yolk sac membrane has no lymphatic system. Whereas a few studies support the view that extensive hydrolysis of lipids occurs with the aid of enzymes produced by the yolk and yolk sac membrane, the vast majority have concluded that almost all the yolk lipid is taken up by yolk sac membrane by engulfment, which involves very little hydrolysis. Nevertheless, where hydrolysis does take place the products are rapidly mobilized and utilized by the developing embryo since only extremely small concentration of hydrolytic products, free fatty acids, mono- and di-glycerides can be detected in the lipids of the yolk and yolk sac membrane (Noble and Moore, 1964). Mobilization of yolk lipids is greatly enhanced during the last week of embryo development. Most of the lipid is mobilized and absorbed into the embryonic tissues over this time. The process coincides with rapid growth and maturation of the embryo (Romanoff, 1960; Noble and Moore, 1964; 1966; Freeman and Vince, 1974). This leads to a dramatic decrease in yolk lipid weight and total dry matter.

The yolk sac and yolk contents are under a continuous state of change throughout the incubation period, characterised by progressive and marked histological changes in the yolk sac membrane, altered blood supply and a large decrease in the volume of the yolk sac. Such changes continue during the early post-hatch period (Romanoff, 1960). Changes in biochemical content and composition of the yolk lipids in the developing chick embryo have been reviewed by Noble (1987a). During incubation small increases in triglycerides and decreases in phospholipids of the yolk lipid occurs between day 17 and 21. However,

significant changes in the levels of free cholesterol and cholesterol esters, in particular the increase in the proportion of cholesterol esters occurs as incubation proceeds. The yolk sac membrane contains lower levels of free cholesterol and higher levels of cholesterol esters than those found in the yolk. Changes in free fatty acids of both the yolk and the yolk sac membrane during incubation are minimal. In the organs of the chick embryo, the liver becomes exceedingly rich in cholesterol esters during the last week of incubation. About 70 percent of the total lipids present in the liver, by day 19 of incubation is composed of cholesterol esters, in contrast to the yolk whose major lipid moiety is triglycerides (Moore and Doran, 1962; Noble and Moore, 1964). An increase in the level of liver cholesterol esters, was found to coincide with a period of intense lipid uptake by the chick embryo thereby prompting suggestions that cholesterol esters might be essential in the transfer of yolk lipids to other parts of the body (Entenman *et al.*, 1940; Noble and Moore, 1964; Noble, 1987).

2.2.3 Post-natal lipid metabolism

In spite of intensive metabolism during the last week of incubation, the chick emerges with a substantial amount of yolk reserves (Noble and Ogunyemi, 1989). The amount of unassimilated yolk reserves in the egg of the chicken varies from 5 - 12 g and may constitutes up to 18 percent of the total weight of the hatched chick (Romanoff, 1960; Ogunyemi, 1987). The amount of lipid in the yolk reserves has been reported to be about 1 - 2 g. Various factors such as egg size, parental age and breed influence the amount of yolk reserves at hatching; of these egg size being the most important (Romanoff, 1960; Pinchasov, 1991; Wilson, 1991). Further studies by Skewes *et al.* (1988) have shown that chick weight is significantly and positively correlated to egg and yolk sac weight, since chicks hatched from large eggs are

heavy and normally have large yolk sacs (see Table 2.2). However, the chick carcass and yolk sac weights are negatively correlated, indicating that efficient utilization of yolk materials during embryonic growth give rise to less yolk reserves and more tissue. Differences in the proportion of the yolk sac reserves in relation to the weight of the newly hatched chick are greater among species than within species, for example the residual yolk sac materials constitutes about 34 percent in the Kiwi Calder (1979), 13 percent in the Bobquail Skewes *et al.* (1988) and 18-29 percent in chicken (Romanoff, 1960; Ogunyemi, 1987). Table 2.3 Romanoff (1960) and Ogunyemi (1987) shows the changes that occur in the lipid weight of the residual yolk of the chick following hatching. Almost all the residual yolk disappears during the first five to seven days after hatching. Thus the rate of yolk assimilation during the post-hatch period is faster than during the embryonic period. There is a considerable variation between birds in the rate of assimilation of unabsorbed yolk. However, the process of yolk assimilation appears to be unaffected by feed and water intake or extreme brooding temperatures (Romanoff, 1960). Lack of absorption of the yolk reserves in chicks although rare is sometimes observed. This is due to bacterial infection and may result to high mortalities (Bains, 1979).

The precise mechanism by which the residual yolk sac contents are assimilated during the post-hatch period is still equivocal. Romanoff (1960) reported that the yolk sac undergoes involution leading to almost completely yolk lipid absorption by day 5 post-hatch. Thereafter, the yolk sac membrane regresses through phagocytic processes. The yolk sac involution is less dependent on chick age but is closely related to the rate of exhaustion of the yolk. When additional yolk was injected into the yolk sac cavity, yolk sac immediately retained its structural integrity for a considerable time (Romanoff 1960). Esteban *et al.* (1991) alternatively suggested that the residual yolk sac contents were pushed into the

Table 2.3: The weight of the yolk reserves, lipid content and the distribution of the major lipid fractions during the early post-hatch days

	age (days)				
	1	2	3	4	5
chick wt (gm)	41.4	54.4	63.0	84.5	84.9
yolk sac wt (gm)	8.1	1.4	1.1	0.4	0.2
yolk sac wt/chick wt (%)	12.3	9.3	5.1	3.7	0.4
total lipid in yolk sac (g)	1.7	0.8	0.2	0.1	0.1
lipid group (%):					
cholesterol ester	19.6	33.8	52.3	54.9	66.7
triglyceride	60.2	50.2	33.9	29.6	17.6
phospholipid	10.4	6.2	4.1	6.2	4.2
free cholesterol	6.3	6.5	6.5	5.7	6.5

Source: Noble and Ogunyemi (1989)

intestine through the yolk stalk and then digested. In contrast Romanoff (1960) showed that the yolk material could be traced no further than the distal end of the yolk stalk. Though the weight of the yolk sac declines rapidly during the first week after hatching as shown in Table 2.3 the changes may proceed at highly variable rates and therefore periods of absorption as long as 2-4 weeks in the chicken have been observed (Romanoff, 1960).

The various constituents of the yolk are not removed at the same rate. Proteins and fatty substances are both utilized rapidly (Romanoff, 1960). The lipids continue to be deposited in the liver from which they are distributed throughout the body (Svanberg, 1971). The disappearance of triglycerides is very rapid during the first week when compared to the phospholipids. The decline of free cholesterol is much slower (Ogunyemi, 1987). The changes which occur in the fatty acid compositions of the major lipid moieties of the residual yolk are not very drastic, hence they continue to resemble the compositions observed during the embryonic period (Romanoff, 1960; Ogunyemi, 1987; Noble and Ogunyemi, 1989). Primarily, the lipids of the residual yolk are used as sources of oxidative energy and serve as transitional nutrient sources during the immediate period of change over from an embryonic to free living existence and before the extensive use of exogenous nutrients (Bjornhag, 1979; Calder, 1979; O'Connor, 1984; Skewes *et al.*, 1988).

2.3 FATS IN POULTRY NUTRITION AND UTILIZATION

2.3.1 Fats in poultry diets

The importance of fats in poultry nutrition has been extensively reviewed (Ewing, 1963; Brue and Latshaw, 1985; Griminger, 1986; Watkins, 1987; Phelps, 1989; Ketels and De Groote, 1989; Keren-Zvi *et al.*, 1990). It is widely accepted that addition of fat to the diet improves it both physically and nutritionally. Physically

there is a reduction in the dustiness of the ration, improvement in pelleting procedures and pellet forms and in some cases better floor litter conditions, leading to improved palatability, feed efficiency and eventually growth. Nutritionally, the major contribution of fat to the diet is through its supply of energy which increases the energy density of the diet, since the gross energy of fat is 39.2 kJ/kg, which is 2.25 and 2.29 times that of starch and protein respectively (Scott *et al.*, 1982; Maiorino *et al.*, 1986). The incorporation of fat in broiler diets enables a sufficient elevation of the energy density of the diet, which in turn helps in meeting the high energy demands brought upon by rapid growth. Feed intake by chickens is inversely related to the energy concentration of the diet, being lower for high energy diets and vice versa (McDonald *et al.*, 1988). Since fat is a high energy concentrate, lower feed intake and higher feed efficiency are normally observed in birds when fat is added to the diet (McDonald *et al.*, 1988). Fat also supplies essential fatty acids, non identifiable growth factors and acts as a carriage of fat soluble vitamins in the blood system of the animal. The low solubility of lipids also makes them useful components of the membrane dividers, separating cells and myriad subcellular micro-compartments essential to the existence of multicellular animals (Senior, 1964).

Although it is generally accepted that the addition of fat improves feed efficiency (March and Bailey, 1963; Vermeersch and Vanschoubroek, 1968; Mateos and Sell, 1981; Phelps, 1989) there are still conflicting reports as to how this is achieved. One theory is that fat supplementation slows down the rate of feed passage through the digestive tract, therefore increasing the time over which the nutrients are exposed to enzymatic actions resulting in better feed absorption and utilization. Mateos *et al.* (1982) observed an increase in the transit time from 193-270 minutes when the level of fat in the diet was increased from 0 to 30 percent

respectively. This was in agreement with other earlier reports (Mateos and Sell, 1981; Rosebrough *et al.*, 1981). The improvement in nutrient absorption and utilization, in turn improves energy utilization because the efficiency of energy use is highly influenced by other nutrients in the diet (see Table 2.4). This phenomenon is termed as 'extrametabolic' or 'extra caloric effect' and is more pronounced in older birds. Sunde (1956) and Golian and Polin (1984) observed no increase in the transit retention time, when fat was supplemented to chick diets and the average rate of passage was lower (with an average of only 126 minutes) than that observed in adult birds. However, Golian and Polin (1984) later observed an increase in the transit retention time as chicks grew older. It was therefore suggested that shorter transit retention times observed in the chick reflect to some extent the immaturity of the physiological and the enzymic systems during the early post-hatch period (Sunde, 1956; Renner and Hill, 1960; Jensen *et al.*, 1970; Golian and Polin, 1984; Brue and Latshaw, 1985; Ketels *et al.*, 1986[a]; Ketels and De Groote, 1989). Hence, the improved growth performance observed when fat is supplemented to broiler diets particularly during the early stages does not merely depend on improved feed efficiency and utilization.

Fat digestibility and utilization is influenced by both intrinsic properties of fat itself and a selection of animal-related factors. The animal factors which influences fat utilization are mainly age of the bird, physiological and production status, breed and species (review by Krogdahl, 1985). The intrinsic factors include fatty acid chain length, the degree of their unsaturation and the extent of their esterification. All these features are normally used as a measure of fat quality. Fat synthesis in the chicken mainly occurs in the liver in contrast to the majority of animals in which the adipose tissue comprises the major site for fat synthesis (Leveille *et al.*, 1975). The presence of other nutrients such as carbohydrates also

Table 2.4: The apparent digestibility of lipids of different diets fed to chickens and the effect of adding bile acids on the performance of chicks fed tallow based diet

	[1]total lipid intake (gm/5 days)	apparent lipid digestibilty (%)
diet:		
starch no fat	6.8 ± 0.8	65.0 ± 2.2
sucrose no fat	5.0 ± 1.6	58.5 ± 3.0
starch added fat	28.9 ± 7.4	90.6 ± 2.8
sucrose added fat	37.0 ± 5.5	87.6 ± 3.0

	[2]weight gain/ bird/day (gm)		feed intake bird/day (gm)	
age (days)	0 - 7	0 - 21	0 - 7	0 - 21
Bile acid in diet				
none (tallow diet0	2.51	7.73	6.4	15.2
0.04% cholic acid	2.63	7.89	6.16	14.6
0.04% sodium taurocholate	2.19	7.43	6.03	14.2
0.04% chenodeoxycholic acid	2.67	7.91	6.31	12.9
0.04% deoxycholic acid	2.52	7.84	6.20	15.4
0.04% dehydrocholic acid	2.58	8.02	6.28	13.2

Sources: [1]Mateos and Sell, (1981) [2]Polin *et al.* (1980)

Table 2.5: Duodenal secretion and overall absorption of fatty acids in the intestines of laying hens

	Net duodenal secretion (mg/day)	Net absorption percentage of intake
fatty acid:		
16:0*	1130	81.9 ± 2.6
18:0	760	85.7 ± 2.8
18:1	440	82.8 ± 3.7
18:2	3960	87.2 ± 1.2
18:3	30	93.7 ± 1.6

Source: Hurwitz *et al.* (1973)

affects fat utilization in the chicken, by altering the lipogenic activities (Whitehead, 1973; Griminger, 1986; Watkins, 1987; Ketels and De Groote, 1989; Phelps, 1989). Enhanced hepatic lipogenesis was observed in chickens when the level of carbohydrates was increased in the diet (Leveille *et al.*, 1975; Pearce, 1971). Contrary to the effects of carbohydrates, high levels of fat in the diet reduces hepatic lipogenesis, as a result of decreased concentrations of hepatic coenzyme A and increases in plasma free fatty acids (Griminger, 1976). However, some studies have shown that certain types of fats can be used as the main source of energy and in some cases replace carbohydrates completely without having adverse effects on growth rate in chicks (Brambilla and Hill, 1966; Hillard *et al.*, 1980). Although normal growth is observed when chicks are fed carbohydrate free diets, conditions such as dermatitis and beak deformities are a common feature (Pearce, 1971).

The advantages of dietary fat supplementation can only be achieved if the above factors are carefully taken into consideration during feed formulation; failure to do so in most cases results in poor performance, characterised by reduced growth, egg production and sometimes *in extremis* body deformities (Phelps, 1989). The effects of unbalanced diets are more pronounced in chicks than in adult birds probably due to immature physiological and enzymic systems. To minimise such effects it was suggested (Ewing, 1963) that the following factors should be taken into account when adding fat to chick diets:

(i) their low requirements of fat during the early stages of growth

(ii) the low tolerance of chicks to high fat diets and

(iii) the quality of added fat

2.3.2 Digestion and absorption by the chicken

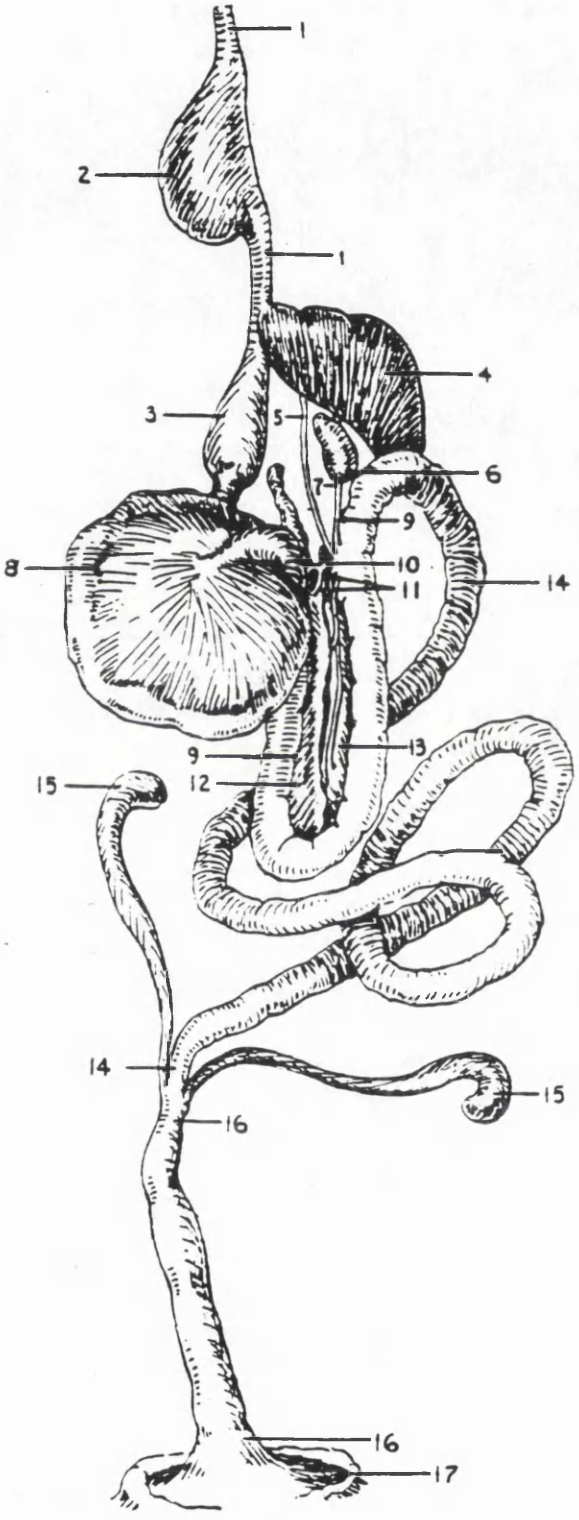
Many of the major components of food (i.e carbohydrates, proteins, fats, vitamins and minerals) are in the form of large insoluble molecules which have to be broken down into simpler compounds before they are distributed and used by the body (Sturkie, 1986; Gillespie, 1987; McDonald *et al.*, 1988). The raw materials are processed (digested and metabolized) and passed through the mucous membrane (absorbed) to other parts of the body. Carbohydrates are converted into simple sugars by amylases, lactase and maltase before absorption; fats are hydrolysed to fatty acids and glycerol by pancreatic lipases and proteins are hydrolysed to amino acids. The processes of digestion and absorption in the chicken occur in distinct anatomically divided sections of the alimentary tract (see Figure 2.1). These sections have differing specific functions during the processes of digestion and absorption (Sturkie, 1986; McDonald *et al.*, 1988). Of all the sections the small intestine is by far the most important site for digestion and absorption processes in the chicken.

The small intestine of avian species consists of the duodenum, jejunum and ileum. The remnant of the attachment of the yolk stalk (Meckel's diverticulum) is found midway along the small intestine and marks the boundary between the jejunum and ileum (Sturkie, 1986). In relation to body size the intestines of birds are relatively shorter than those of mammals. Studies by Dror *et al.* (1977), Akiba *et al.* (1988) and Sell *et al.* (1991) have shown that the development of the digestive tract measured by length, size and weight was rapid during the early stages of the chick's life. The development of the digestive tract to some extent mirrors the overall growth of the bird; thus differences in digestive tract development between breeds are the norm, since different breeds differ in their growth patterns. The growth of the digestive tract is higher in broiler chicks when compared to layer chicks reflecting the fast growth of broiler and their adaptation to high food intakes required to

Figure 2.1: Digestive tract of the chicken

Source: Sturkie (1986)

Alimentary Canal



Digestive tract of the chicken.

1 and 2, esophagus and crop; 3, proventriculus; 4, liver; 5, hepatic duct; 6, gall badder; 7, cystic duct or duct from gall bladder; 8, gizzard; 9, duodenum; 10, pancreatic ducts from dorsal lobe; 11, pancreatic ducts from ventral lobe; 12, dorsal lobe of pancreas; 13, ventral lobe of pancreas; 14, upper and lower segments of small intestine; 15, ceca; 16, large intestine or rectum; 17, cloaca.

achieve fast growth rates (Bjornhag, 1979). The presence of micro-organisms also has an effect on the growth of the digestive tract and the bird as a whole (Boyd and Edwards, 1967; Eyssen and De Somer, 1967). Birds reared conventionally normally have heavy intestines and thicker intestinal walls while germ free birds have lighter intestines and thinner intestinal walls (Furuse and Yokota, 1984a). The rate of nutrient passage through the intestinal walls is affected amongst other factors by the thickness of the wall, being faster and slower for the thin and the thick walls respectively (Harrison and Coates, 1972). As a result lower and higher nutrient digestibility in conventional and germ free birds respectively, have been observed (Furuse and Yokota, 1984; Mead *et al.*, 1984a).

The small intestine is also the principal site of chemical digestion and nutrient absorption, the processes involving enzymes of both intestinal and pancreatic origin (McDonald *et al.*, 1988). Intestinal secretions are capable of digesting starch, sucrose, fats and proteins (Lepkovsky and Furuta, 1970). The small intestine also produces hormones which are primarily involved in the regulation of gastric secretions. Nutrients are absorbed from the small intestine by any of the three mechanisms namely:

(i) passive transport which involves simple diffusion

(ii) active transport involving the use of a specific carrier

(iii) pinocytosis whereby cells have the capacity to engulf large molecules present in solution or suspension (Sturkie, 1986; McDonald *et al.*, 1988). The efficiency of absorption processes in the small intestine is influenced by the type of diet, age of the bird and to some extent the microbial population (McDonald *et al.*, 1988).

2.3.3 Lipid Digestion in Chickens

As already mentioned, lipids mainly in the form of triglycerides are an important source of energy and fat soluble vitamins in chicken rations. The triglycerides are natural components of feed ingredients from both plant and animal sources whilst some are deliberately added to the diet in the form of supplements (Phelps, 1989). Other lipid groups such as phospholipids and cholesterol are also naturally present in substantial amounts. Fat digestion in the chicken is characterised by a selection of both physical and chemical events which transform lipids to chemically active substances (Senior, 1964). Figures 2.2 and 2.3 show the phases of fat digestion in the small intestine. The chemical events include the enzymatic hydrolysis of ester bonds to form a range of lipolytic products whilst a selection of physical events are responsible for changing the products, from a predominantly oil phase to that of an aqueous phase and the formation of micelles (Hofmann, 1970).

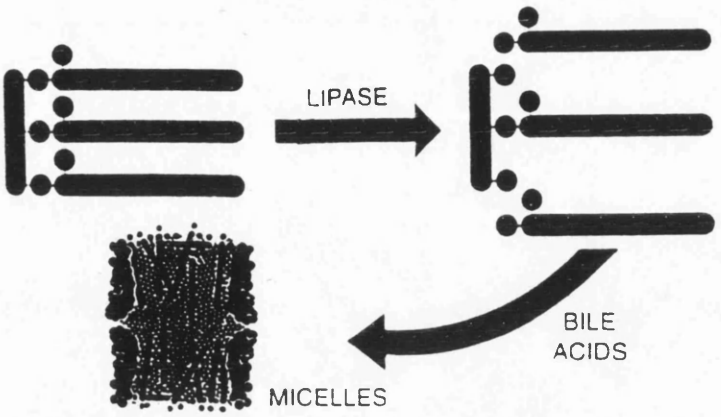
Lipids ingested by chickens are most frequently in the physical form of oil droplets of various sizes ranging from visible to finely emulsified droplets (Hofmann, 1970). Once ingested, the lipid undergoes various stages of intestinal emulsification, hydrolysis, micellar formation and solubilization, cell membrane permeation and intracellular esterification before any distribution into the body is possible (Senior, 1964; Hofmann, 1970; Freeman, 1984; Krogdahl, 1985). It is generally accepted that the processes of fat digestion and absorption in the chicken do not differ very much from other monogastric animals, since the chemical composition of dietary fat is almost identical (Senior, 1964; Pearce, 1974; Butler, 1975; Freeman, 1976). Nevertheless differences have been reported, particularly with respect to the site at which both the bile and pancreatic ducts enter the duodenum. In the chicken these ducts empty their secretions at the distal end of duodenum in contrast to other monogastric species, in which the ducts enter the

Figure

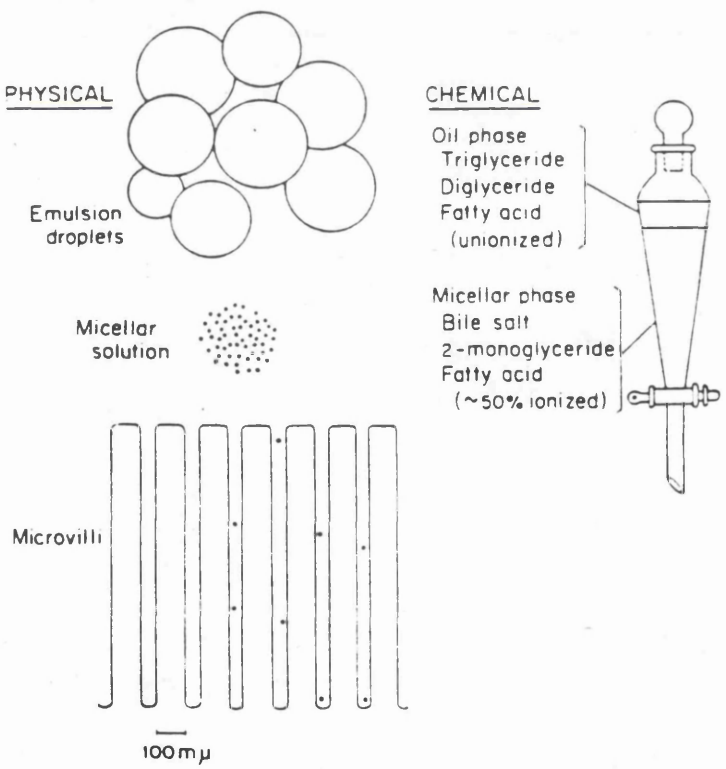
Figure 2.2(i): Schematic representation of the chemical and physical events in fat digestion

Figure 2.2(ii): Physical state of lipids in intesinal content during fat absorption

Source: Hoffman (1970)



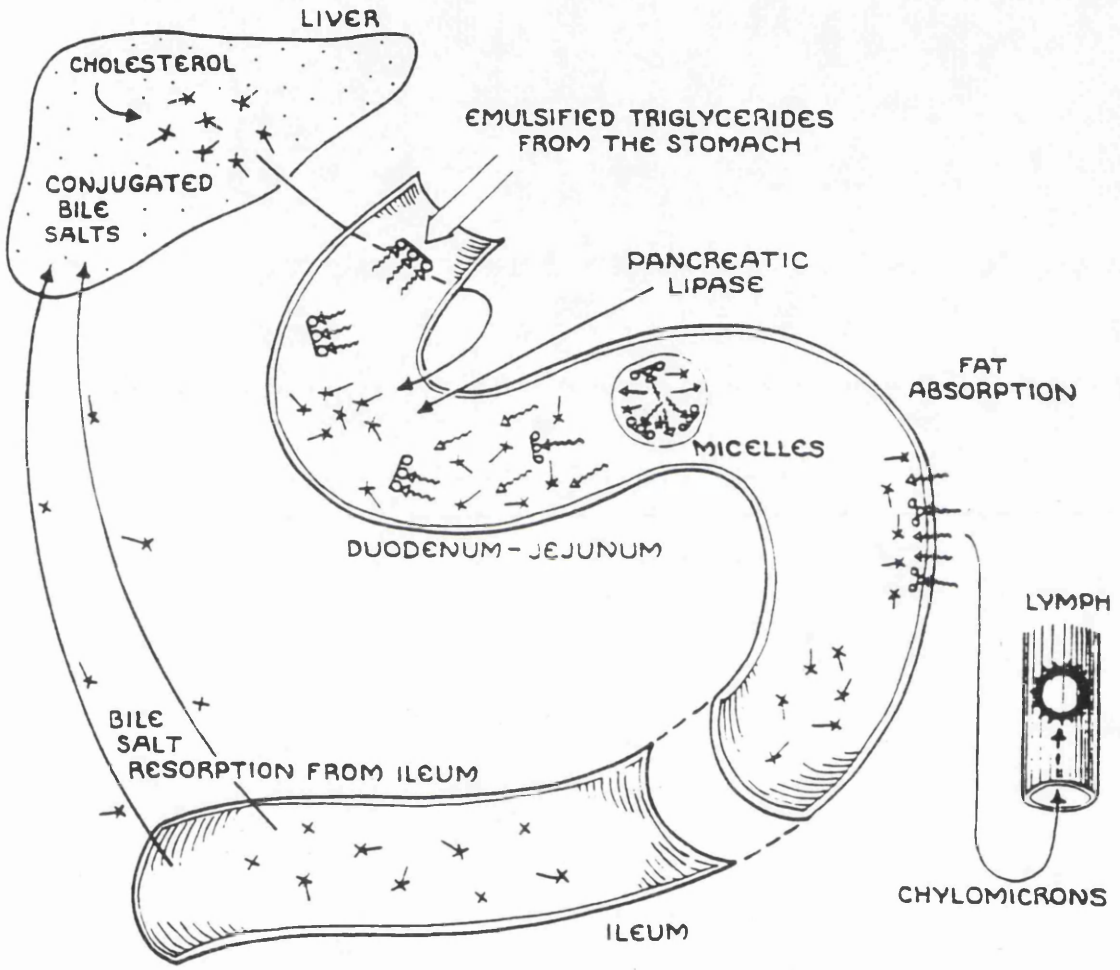
Schematic representation of the chemical and physical events in fat digestion. Pancreatic lipase cleaves the 1-ester bonds of triglyceride to form 2-monoglyceride and fatty acid . These polar, but poorly soluble lipids are dispersed in the aqueous phase of small intestinal content by forming mixed micelles with the bile acids. In the model of the micelle shown the bile acid molecules coat a bimolecular leaflet of fatty acid and monoglyceride. The micelle is considered to have a liquid hydrocarbon center in which other molecules such as cholesterol can dissolve. The knobs on the surface of the bile acid molecules are hydrophilic groups such as hydroxyl groups.



Physical state of lipids in intestinal content during fat absorption. A micellar phase containing bile acids, fatty acids (partially ionized), and monoglyceride is in equilibrium with an emulsified phase containing higher glycerides and nonionized fatty acid . Other lipids such as cholesterol or the fat-soluble vitamins are partitioned between the two phases. The present view is that the micelle serves as the final common path for virtually all water-insoluble molecules which are absorbed; absorption from the oil phase is considered to be insignificant from a quantitative standpoint.

Figure 2.3: A scheme of intraluminal micelle formation, fat and bile salt absorption

Source: Senior (1964)



A scheme of intraluminal micelle formation, fat and bile salt absorption: x–, conjugated bile salt; x, unconjugated bile salt; ◁~~, free fatty acid; OOO, free glycerol.

duodenum at its middle part (Hurwitz *et al.*, 1973; Griminger, 1976). As a result compared to other species lipids passing through the duodenum in the chicken are likely to be hydrolysed and emulsified for absorption at a more distal location (Griminger, 1976). The major activities associated with fat digestion and absorption in the chicken are confined to the small intestine in particular the upper parts (Krogdahl, 1985; Sturkie, 1986). Neishem et al. (1962) and Sklan et al. (1973) observed rapid secretion and absorption of fat between the duodenum and the jejunum. The amount of fat associated with the different sections of the small intestine shows a wide variation. The duodenum contains the highest total lipid content approximately (85 mg/ml), followed by the jejunum approximately (34.2 mg/ml) while the ileum contains the least amount of fat approximately (13.1 mg/ml) (Freeman, 1976). Most of the lipid within the duodenum is of dietary origin (Sklan *et al.*, 1973) and is combined with high levels of lipids of biliary secretion which sometimes account for up to 8 percent of the total dry matter intake (Hurwitz *et al.*, 1973). The high levels of linoleic and palmitic acids, with slightly lower concentrations of stearic and oleic acids within lipids of the duodenum are a reflection of a substantial contribution from the bile lipids as illustrated in Table 2.5 (Senior, 1964; Hurwitz *et al.*, 1973; Freeman, 1976).

Following ingestion, the various fats of the diet are converted to a coarse emulsion within the crop by means of churning, kneading and squirting movements brought about by gastric motility (Senior, 1964). A limited hydrolysis of dietary triglycerides can be observed in the gizzard because of the presence of both bile and pancreatic lipases arising from reversed peristalsis; the extent of the bile and pancreatic lipase activities in the gizzard can be up to 10-20 percent of the level found in the duodenum (Annison, 1971). Emulsification is the first most important step in fat digestion because it increases the surface area upon which the enzymes

can act and reduces the size of the fat particles. The emulsion thus formed is then acted upon by lipase at the oil water interface whereby free fatty acids, 1,2-di-glycerides, 2 monoglycerides and to a limited extent free glycerol are formed. The efficiency of emulsification and subsequent digestion is controlled by biliary secretions which are to some extent influenced by the type of dietary fat (Hurwitz *et al.*, 1973; Freeman, 1976; Sklan, 1979). Diets containing high levels of triglycerides enhance biliary secretions, whereas, high levels of free fatty acids reduce them (Sklan, 1979).

The combination of emulsification, enzyme hydrolysis and the presence of bile salts give rise to micelles. The combination of micelles and conjugated bile salts, give rise to very fine particles with a diameter of about 40-100 A^{O} which is less than 1/100 of the size of the course emulsion delivered into the proximal duodenum by the crop. The bile lipolytic products are involved in the transport of lipids up to the surfaces of the absorbing cells (Hofmann, 1970). For a given amount of lipid the micelles have a surface area 100 times that of the crop's coarse emulsion. The ability of the micelles to increase surface area and to provide a high concentration of lipid in the layer adjacent to the mucosal cells comprises their most important functional role in lipid absorption (Senior, 1964; Hofmann, 1970). Bile salt micelles also have the ability to dissolve considerable amounts of non-polar lipid such as palmitic and stearic acids in aqueous solutions (Garret and Young, 1975). However, both polar and non polar lipids exhibit low solubilities in the absence of bile salts stressing the importance of bile salts in the overall process of micellar formation and lipid absorption (Hurwitz *et al.*, 1973; Garret and Young, 1975).

Although the mechanism by which hydrolysis products in the micelles are transported to the interior of mucosal cells is unclear, some suggestions put forward include the disruption of micelles and the separation of bile acids from lipids (Butler, 1975; Krogdahl, 1985). Various reports (Senior (1964), Hofmann (1970), Freeman (1976) and Krogdahl (1985)) showed that conjugated bile salts were not absorbed in the proximal part of the small intestine where the majority of the fat portion was absorbed. Furthermore, most of the long and medium chain unsaturated fatty acids are absorbed from the jejunum, whilst the ileum is important in the absorption of palmitic and stearic acids which are major constituents of the bile salts. Since there are different sites of absorption for the different fatty acids it would appear that the micelles are not absorbed in an intact form (Hurwitz *et al.*, 1973). This feature has also been observed in a range of monogastric species (Annison, 1971; Whitehead and Fisher, 1975; Freeman, 1976; Krogdahl, 1985). It is also thought that some of the bile salt is circulated back to the duodenum for reutilization. There is no fat absorption beyond the ileum which means that most of the unabsorbed fat associated with the large intestine is subsequently excreted in the faeces. The caeca in the chicken seems to have no important role in the digestion and absorption of fat (Griminger, 1986).

2.4 FACTORS AFFECTING LIPID DIGESTION IN THE CHICKEN

2.4.1 Chemical composition of the diet

Nutritional experiments involving growth rate and digestibility that have been performed to assess the nutritive value of feeds and to compare differences between feeds in chickens are extensive (Brambilla and Hill, 1966; Sibbald and Krammer, 1978; Mateos and Sell, 1981; Brue and Latshaw, 1985; Laurin *et al.*, 1985; Teeter and Smith, 1985). The investigations have involved both vegetable and

animal fats. The two groups of fats differ mainly in their fatty acid composition whereby high unsaturated and saturated fatty acids are found as major components in vegetable and animal fat, respectively (Enser, 1984; Gurr, 1984). The fatty acid compositional differences between the two groups is thought to be a major cause of the variations observed in aspects of lipid metabolism and the performance of birds fed differing diets. Ketels and De Groote (1989) showed that about 75 percent of the variation in fat utilization and apparent metabolizable energy was due to the chemical composition of the fat fraction in the diet as measured by the ratio between saturated and unsaturated fatty acids. Long chain saturated fatty acids are poorly absorbed when compared to both short chain and unsaturated fatty acids (Renner and Hill, 1961; Ketels and De Groote, 1989). The poor absorption of long chain saturated fatty acids might be due to their low polarity see Table 2.6 (Freeman, 1969; Annison, 1983) and the difficulty in forming emulsions prior to micelle formation (Ketels and De Groote, 1989). In contrast most of the unsaturated fatty acids can be absorbed directly without being emulsified (Freeman, 1976). The investigations by Franzen and May (1968) showed that different types of fatty acids have varying emulsifying capacities. Thus the short chain fatty acids from coconut oil and the unsaturated fatty acids from corn oil had greater emulsifying capacities than the highly saturated fatty acids from lard. However, the emulsifying capacity in both cases was greatly improved by the addition of phospholipids.

Fat utilization by the chicken is also affected by the form in which fatty acids are fed. Esterified fatty acids are efficiently utilized, whereas completely hydrolysed fats, hydrogenated and acidified soapstock are poorly utilized due to the presence of high free fatty acid levels which hinders the process of micelle formation (Brambilla and Hill, 1966; Ketels and De Groote, 1989). Sklan (1979) observed higher lipid digestibilities when birds were fed diets based on triglycerides than when receiving

Table 2.6: Influence of chain length on absorbability of fatty acids and absorbability of palmitic acid as influenced by the presence of oleic and linoleic acids in young chicks

	[1]absorbability (%)		[2]absorbabilty of palmitic acid (%)	
fatty acid:		ratio	16:0 to 18:1*	16:0 to 18:2
12:0	65	1:0.26	15.0	9.1
14:0	25	1:0.43	25.6	20.2
16:0	2	1:0.69	35.8	20.5
18:0	-2			

Sources: [1]Renner and Hill (1961) [2]Young and Garret (1963)

* The common names of the fatty acids as per description in Table 3.1

free fatty acids or acidified soapstocks. The composition of fatty acids found in the intestines, however, showed extensive similarity irrespective of the differences in the dietary fat sources, with the exception of the duodenal fatty acids from triglycerides fed chicks which showed higher proportions of stearic and arachidonic acids than chicks receiving free fatty acids and acidified soapstock based diets. The high levels of stearic and arachidonic acids was thought to be the result of the biliary secretions into the duodenum, the secretion of which is generally increased by high levels of dietary triglycerides and decreased by high levels of free fatty acids (Sklan, 1979). It has also been reported that fats and fatty acids from the basic endogenous ingredients of feeds are more highly digested than supplementarily added fats (Hakansson, 1974). It was suggested that the differences in fatty utilization observed between fat sources might be due to the respective amounts of triglycerides and esterified fatty acids present (Hakansson, 1974; Sklan, 1979). Depressed growth rates, beak deformities and dermatitis were observed in chicks when fatty acids from soyabean oil were added to the diet as sources of fat, but exhibited normal growth when complete soyabean oil was added (Young and Chang, 1964; Brambilla and Hill, 1966). In practical terms most diets contain both the saturated and unsaturated fatty acids, a result of which is that the absorption of the saturated fatty acids is improved by the presence of unsaturated fatty acids (see Table 2.6) (Annison, 1983). This effect is known as synergism and is important, since it improves the utilization of long chain saturated fatty acids. Ketels and De Groote (1989) observed a steep increase in fat utilization, when the ratios between the unsaturated:saturated components were increased from 0-4 and no further improvement was observed beyond an unsaturated:saturated ratio of 4. It was also pointed out that most of the basic dietary ingredients have an unsaturated:saturated ratio of about 4 or slightly more, hence, addition of unsaturated fatty acids to the diet in some cases do not

improve fat utilization very much (K:tels and De Groote, 1989). The presence in the diet of high levels of short chain fatty acids results in poor growth rates and a range of body deformities in spite of a high absorption rate. The probability is that the abnormalities might result from the deficiency of essential fatty acids which occurs when short chain fatty acids are fed (Menge, 1971).

The effect of different dietary fat sources on the performance of chicks and chickens has been extensively studied. A range of variations in the performance of chicks fed diets containing different sources of fat have been observed. Fedde *et al.* (1960), Fuller and Rendon (1979) and Lopez *et al.* (1982) found no significant differences in growth rates when birds were fed diets containing different fat sources, whereas, Sim *et al.* (1985) observed differences in growth rates when different dietary fat sources were added to the diet. Fuller and Rendon (1979) failed to obtain differences in apparent metabolizable energy of fat when various fat sources were used. However, other studies by Brue and Latshaw (1985), Sibbald and Krammer (1980) and Wiseman and Salvador (1991) showed significant differences in apparent metabolizable energy of fat due to differences in saturation of dietary fatty acids. They reported a decrease in apparent metabolizable energy when the saturation level in fat was increased.

Feed intake and feed efficiency, unlike growth rate, are highly influenced by the type of fat and fatty acids present in the diet. Vegetable fat based diets result in lower feed intake and higher feed efficiency, whereas, the opposite is normally the case in birds fed diets containing animal based fats. By contrast higher caloric efficiencies (energy gain/energy absorbed) of 0.31, 0.27, 0.29 and 0.20 was obtained for tallow, hydrogenated coconut, soyabean and corn oil based diets respectively (Fuller and Rendon, 1979). As previously stated the major effects of dietary fat sources on lipid utilization arise from their difference in specific fatty acid

component. A study involving palmitic, stearic, oleic and linoleic as major sources of fat, at a dietary inclusion level of 8 percent of the diet, revealed significant differences in feed intake and feed efficiency (Atteh and Leeson, 1983). Higher feed intake accompanied by lower feed efficiency were obtained with enhanced dietary inclusion of both palmitic and stearic acids, whilst improvements in both feed efficiency and feed intake were obtained by dietary enhancement of oleic and linoleic acids (Atteh and Leeson, 1983).

The absolute concentration or proportion of fat in the diet affects many aspects of metabolism of the major nutrients and in turn the overall performance of the bird. Most of the experiments performed to determine optimum levels of fat supplementation have shown that the average daily liveweight gain is improved by dietary fat supplementation regardless of the type fat (Table 2.7). However, Fedde *et al.* (1960) showed that weight gains were not affected by either fat source or fat level in the diet when beef tallow, safflower oil, corn oil and hog grease at levels of dietary inclusion of 10 and 20 percent were used as fat supplements during the first two weeks of life. It was therefore suggested that the observations might have been the result of the inability by the chick to utilize fat efficiently during the early stages, coupled also with a reduced fat requirement. Similar findings have also been reported by Velu and Baker (1974), Fuller and Rendon (1979), Atteh and Leeson (1983) and Bartov (1987) and similarly in other species such as piglets (Li *et al.*, 1990). Bartov (1987) found out that feeding high levels of fat during the early stages of growth was later reflected in the abdominal pad and carcass lipid amounts and their composition. Generally feed intake and feed efficiency are improved by the addition of fat in the diet, a pattern that seems to be only true at low levels of dietary fat (Ketels and De Groote, 1987). Ketels and De Groote (1987) observed an increase in fat utilization of both the basal and added fat in soyabean and tallow oil when the

Table 2.7: Effects of fat and fatty acids supplementation on body weight, feed co nversion, feaces in fat and feed intake in 4 week broiler chicks

diets	weight (gm)	feed:gain	fat in dry faeces (%)
Basal	283	2.1	1.18
+ 5% white grease	311	1.92	3.17
+ 10% white grease	289	1.91	5.80
+ 5% oleic acid	292	1.92	3.68
+ 5% hydrogenated fat	309	2.21	9.27
	no fat	added fat	ratio of non fat to supplement fat
	feed intake (gm)		
age (days)			
0 - 7	380	545	1:1.4
0 - 14	1100	2367	1:2.15
0 - 21	2315	5380	1:2.3
0 - 28	4950	9610	1:1.9

Source: Sunde, (1956)

Table 2.8: Weight at 27 days of broilers chicks fed diets varying in level of dietary protein, with and without 10% supplemental tallow

	tallow	
	(+)	(-)
protein level (%)	weight (gm)	
13	190	195
16	266	268
20	307	334
25	314	380
31	292	343

Source: Waibel, (1955)

level of supplemental fat was 2.5 percent. However, the coefficients of fat utilization remained high at 94 and 92 percent when soyabean oil was respectively, added to the diet at 5 and 12.5 percent; whereas, a decreasing trend in fat utilization was observed when the level of tallow in the diet was increased above 2.5 percent. The variations observed were attributed to differences in the extent of emulsification. Whereas the unsaturates from soyabean oil required little emulsification hence, posing no limit to the chick's absorption capacity, tallow fat was difficult to digest, hence, required extensive emulsification. The process which might have been limited by the level of bile salt production and lipase activity (Ketels and De Groote, 1987). Studies have also shown that incorporation of high levels of fat (more than 10 percent of the diet), in chicken diets normally results in reduced feed intake and feed efficiency (Cunningham and Morrison, 1977; Fuller and Rendon, 1979; Akinwande, 1981). High levels of fat in the diet are also known to be among the major causes of protein and amino acid deficiencies arising from low feed intake. The performance of broiler chicks in terms of growth rate was found to be higher in chicks fed a high protein diet (25 percent) without tallow oil (see Table 2.8) (Waibel, 1955). Notably therefore, it is important to maintain a required energy:protein ratio during feed supplementation. Increased soap formation occurs when high levels of fat particularly those high in saturated fatty acids are added to the diet, thus limiting the utilization of minerals (Atteh and Leeson, 1983).

2.4.2 Age of the chick

Many studies have been undertaken to show that the chick emerges with a selection of incomplete physiological systems; in particular those involved in the processes of digestion. Carew *et al.* (1972) found that young chicks did not possess the full physiological capacity for fat absorption, but it developed with age; the rate

of development differing among the various types of fat. A recent review by Krogdahl (1985) also indicates that the newly hatched chick has significantly reduced levels of bile production, pancreatic secretions and enzymatic activities during the early post-hatch period. Such features severely limit the chick's ability to efficiently utilize a range of dietary nutrients during this period. Akiba *et al.* (1988) concluded that both the nutritional status and the metabolism of the chick during the early stages of growth were not analogous to those found in older growing chicks or the adult chicken. In spite of these inadequacies, the chick is able to exhibit a high daily growth rate, reaching a peak at about day 4-6 post-hatch. Maintenance of such a growth rate has been associated with high energy utilization from the residual yolk and this is reflected by the rapid regression of the yolk sac membrane (Bjornhag, 1979; Nir *et al.*, 1988; Akiba *et al.*, 1988). The chick undergoes rapid changes during the post-hatch period amongst which is the rapid growth of the GIT and also associated with it are increases in bile and pancreatic secretions (Freeman, 1976; Bjornhag, 1979; Krogdahl and Sell, 1989). Sell *et al.* (1991) showed that in relative terms segments of the GIT increased in weight more rapidly in comparison with other parts of the body during the early post-hatch period. These rapid changes were most marked in the proventriculus, small intestine and pancreas and were most prominent in the first week of age. A similar trend was reported in the poults; in which a 10 fold increase in pancreatic weight over the initial 30 days was observed, the weight reaching about 102 times that of initial weight by the day 56 post-hatch. A response to a combination of increased feed intake, enzyme levels and activities was suggested. In support of this view Nir *et al.* (1988) reported that the chick was subject to a high feed consumption of some 20 percent above its body weight requirement during the first few weeks of its life. The activities of major pancreatic enzymes i.e amylase, lipase trypsin and chymotrypsin were initially very low

following hatching and reached maximum levels at about three weeks of age. The development of intestinal lipase ability is closely related to the amount of dietary fat. Low lipase activities were observed in chicks fed low fat diets, remaining low throughout a period of lipid deprivation. By comparison a five fold increase was observed in chicks fed high fat diets although the response was not observed until the chicks were three weeks old (Krogdahl and Sell, 1989). These results showed that chicks were unable to utilise high fat diets during the early growth period and that an early initiation into a high fat dietary regime may considerably alter the development of lipase and other enzymes. Undoubtedly though a certain amount of fat is required in the diet in order to attain maximum development of the intestinal and pancreatic lipase (Escribano *et al.*, 1988; Sell *et al.*, 1991).

The absorption and utilization of fat by the chick improves markedly with age and this effect is more pronounced for saturated fats than the unsaturates (Renner and Hill, 1960; Fedde *et al.*, 1960; Carew *et al.*, 1972; Hakansson, 1974; Polin *et al.*, 1980; Krogdahl and Sell, 1989). Carew *et al.* (1972) found out that the apparent absorbability of fat in a corn oil based diet was at 84 percent between day 2 and 7 post-hatch and 95 percent between day 8 and 15 post-hatch. Corresponding figures for a tallow based diet were 40 and 79 percent, respectively. From these findings it was concluded that fats containing high polyunsaturated fatty acid levels such as corn oil and safflower oil were well absorbed even as early as two weeks, whereas, a tallow based diet high in saturated fatty acids was poorly absorbed during the early post-hatch days but the absorption improved markedly with age. This improvement was thought to be a result of improved enzyme activities and increased bile secretions.

Fat excretion is undoubtedly a major indicator of fat digestibility in the chick (Pattison, 1989). Excess fat in the diet results in sticky faeces which adheres to the foot pads. The amount of fat excreted by chicks fed a low fat diet between the ages of 2-7 days was shown to be about 10 times greater than fat intake, the level declining to twice that of intake between day 8 and 15 post-hatch (Carew *et al.*, 1972). Similar observations were also made by (Fedde *et al.*, 1960). Fat excretion was higher in high fat diets, in particular in diets containing high levels of saturated fatty acids and hydrogenated fats and lower in chicks fed diets containing high levels of unsaturated fatty acids (Table 2.7). Following these observations, high levels of vegetable fat based diets are sometimes recommended for use in starter rations (Pattison, 1989). Fat excretion in tallow based diets was reduced when ox bile was added to the diet. This was a further indication that bile production was among the limiting factors in fat absorption during the early stages of growth (Fedde *et al.*, 1960). This condition, whereby there is large excretion of nutrients is known as flushing and sometimes has adverse effects on the chick, because other nutrients such as fat soluble vitamins are also excreted with the fat, as a result deficiencies and physiological disorders are a normal accompaniment (Carew *et al.*, 1972). It is therefore important to optimise dietary levels of fat particularly during the early stages of growth in order to attain rapid growth and normal physiological development of the bird (Hakansson, 1974; Pattison, 1989).

2.5 THE EFFECTS OF DIETARY FAT SOURCES ON THE LIVER AND BILE LIPID COMPOSITIONS

In lipid terms, the liver is by far the largest and most important organ in the body of the developing bird (McCormick, 1990). Other naturally important functions carried out in the liver include secretion of bile, metabolism of

carbohydrates and proteins, detoxification of harmful substances and storage of vitamins (McCormick, 1990). In the bird the liver is the main site for fatty acid synthesis in contrast to the dominant role of the adipose tissue in other species (Leveille *et al.*, 1975; Annison, 1983; Hill, 1983). In common with other animal species the steps in fatty acid synthesis in the chicken are:

(i) the activation of acetate to acetyl-CoA

(ii) carboxylation of acetyl-CoA to malonyl-CoA and

(iii) the conversion of malonyl-CoA to fatty acids (Abraham, 1970).

A combination of the above with other processes of elongation and desaturation results in the formation a full range of fatty acids (Annison, 1983). Hepatic lipogenesis in the chicken is low during the embryonic stage but develops rapidly after hatching (Hill, 1983; Donaldson, 1990). The ability of the liver to synthesize fatty acids is mainly affected by carbohydrates, fats and a range of minor nutrients such as vitamins and minerals. Hepatic lipogenesis is reduced when high levels of dietary fat and lower levels of carbohydrates are fed (Abraham, 1970; Madapally *et al.*, 1971; Shapira *et al.*, 1978; Rosebrough *et al.*, 1981; Annison, 1983). Although all types of fats fed at high levels limit hepatic lipogenesis, the effect seems to be far greater for fats containing higher levels of unsaturated fatty acids than those with saturated fatty acids (Abraham, 1970). Akiba *et al.* (1983) observed a reduction in hepatic lipogenesis when high levels of fish solubles were added to the diet, whereas, Sklan (1983) found increased activity when vitamin A levels were increased in the diet. From this it can clearly be seen that the capacity of the liver to synthesize fatty acids is open to considerable variations, as a result of increases and reductions in certain dietary nutrients.

The type of fat and fatty acid composition of the liver, like many other tissues in the body is directly influenced by dietary composition (Sim *et al.*, 1973). Shapira *et al.* (1978) showed that the lipid content of the liver and liver weight were both increased to a greater extent by supplementation of glucose as opposed to oil supplementation. The liver weight and fat content were 31.3 g and 17.3 percent; 17.5 g and 6.5 percent for glucose and oil supplementation respectively (Shapira *et al.*, 1978). Glucose supplementation led to a marked increase in oleic acid content and a decrease in linoleic acid, whilst, soyabean oil supplementation increased linoleic acid at the expense of all other fatty acids. However, Abraham (1970) found that the effect of diet was more obvious on fatty acid composition than on the amount of fat present in the liver. This was further shown by Sim *et al.* (1973) who found out that chicks fed low fat diet and tallow diet deposited low linoleic acid levels, whereas, feeding soyabean or sunflower oil increased the linoleic acid in the liver (Table 2.9). The liver of the newly hatched chick contains high levels of esterified cholesterol (Svanberg, 1971). The esterified cholesterol present in the liver of the chick is derived from yolk synthesis during incubation and its accumulation is most prominent during the last 2-3 days of incubation (Noble, 1987). Cholesterol esters are important during incubation time because they aid in the transportation of the yolk lipid constituents into the embryo and subsequently in the young chick (Noble and Moore, 1964). A sharp decline in liver cholesterol esters is normally observed soon after hatching with liver lipid composition comparing well with that of an adult bird at about 2 weeks of age. Significant differences exists in liver lipid composition between immature and laying birds (Table 2.10) (Noble *et al.*, 1988). High levels of triglycerides in laying hens are thought to be a result of large demands for egg production since triglycerides are an important egg constituent of yolk lipid (Gilbert, 1971; Noble *et al.*, 1988).

Table 2.9: Effect of dieatry treatments on fatty acid composition of liver lipids

	fatty acid (percentage)					
	14:0*	16:0	16:1	18:0	18:1	18:2
treatment:						
basal	0.7	28.7	4.2	6.5	51.2	8.7
animal tallow						
1%	2.1	23.0	2.1	19.7	44.4	8.7
2%	0.7	26.1	4.4	11.2	51.2	6.4
4%	0.6	25.9	4.1	9.5	53.6	6.3
8%	0.6	25.6	4.6	10.6	53.6	5.1
soyabean oil						
1%	0.5	20.5	5.0	9.4	50.2	13.0
2%	0.8	28.5	3.4	9.6	39.0	16.8
4%	0.6	23.4	2.2	13.4	35.2	23.4
8%	0.7	21.0	4.6	20.9	24.3	26.5
sunflower oil						
1%	0.5	29.4	2.9	10.7	46.1	10.1
2%	0.3	26.5	2.3	13.5	40.0	17.4
3%	0.5	29.5	2.1	14.7	29.2	24.0
4%	0.9	24.4	0.8	18.0	27.7	28.2

* The common names of the fatty acids as per description in Table 3.1

Source: Sim *et al.* (1973)

Bile salts play an important role in the excretion and turnover of lipids (Cross *et al.*, 1987). Bile salts in the fowl are secreted by the liver via an active transport process. The secreted bile then enters the gall bladder and undergoes considerable concentration. The secretion of bile salts by the liver is influenced among other factors by age and dietary composition (Hill, 1983). The rate of bile synthesis was found to be low in young chicks, but increased gradually with age particularly when dietary components enhancing lipogenic activities such as carbohydrates were fed (Pearce, 1974; Hill, 1983). Sklan (1983) showed that there were increases in the secretion of bile acids and cholesterols when vitamin A was added to the diet. It is estimated that the bile acid pool in the fowl is about 18-23 mg/100g body weight and has a half life of 6-9 days (Serafin and Neishem, 1970). The same authors observed high concentration of bile in the proximal part of the jejunum in correspondence with entry of the bile at the duodenal-jejunal junction and that over 90 percent of the bile secreted into the duodenum was absorbed in the jejunum and ileum and only a small amount was found in large intestine. Similar observations were also made by Sklan *et al.* (1973). The amount of bile excreted in the faeces to some extent depends on the nature of the dietary fat whereby, it is increased by high levels of unsaturated fats and decreased by high levels of saturated components in the diet (Hill, 1983; Lindsay *et al.*, 1970). However, there was a reduction in the absolute amount of excreted bile salts when dietary oils were added to the diet. This was attributed to the reduced feed intake resulting from increased caloric density (Lindsay *et al.*, 1970). The lipid and fatty acid composition of the bile in the chick embryo and adult birds differs markedly from that of other animal species (Noble and Connor, 1984). In other monogastric species phospholipids is the major lipid group whereas, triglycerides and cholesterol esters are the major lipid fractions in the chicken bile (Noble *et al.*, 1988). Oleic acid is the major fatty acid

Table 2.10: Lipid compositions (major fractions, weight percentage of total lipid present) of the liver and bile of the broiler breeder at 7, 20 and 42 weeks of age

	age (weeks)		
	7	20	42
lipid:			
liver			
cholesterol ester	5.29 ± 0.97	5.92 ± 0.37	3.82 ± 0.61
triglyceride	16.5 ± 2.23	16.5 ± 2.04	74.7 ± 3.25
free fatty acid	2.61 ± 0.21	1.15 ± 0.12	1.47 ± 0.30
phospholipid	64.4 ± 1.68	63.3 ± 1.53	17.7 ± 2.66
bile			
cholesterol ester	trace	trace	6.14 ± 1.14
triglyceride	31.6 ± 2.86	1.20 ± 0.08	3.71 ± 0.84
free fatty acid	5.16 ± 1.03	1.09 ± 0.14	2.35 ± 0.17
phospholipid	56.5 ± 2.37	88.4 ± 0.90	82.0 ± 1.60
free cholesterol	6.75 ± 0.98	9.32 ± 0.73	5.76 ± 1.40

Source: Noble *et al.*, (1988)

found in most of the bile lipid fractions (Noble and Connor, 1984; Noble *et al.*, 1988). Substantial changes in bile lipid composition with age have been reported in the chicken (see Table 2.10) (Noble *et al.*, 1988). Lindsay *et al.* (1970) noted that the percentage of the triglyceride fraction in bile lipids was related to age being lower in immature birds. As in the liver the onset of laying in the chicken alters the bile lipid composition whereby, high levels of triglycerides are observed. In general lipid compositional changes in the chicken bile are a reflection of changes in the liver.

2.6 EFFECTS OF DIETARY FAT SOURCES ON THE ESTABLISHMENT AND GROWTH OF INTESTINAL MICROFLORA

The intestinal tract of animals is a host to many kinds of micro-organisms with varying contributions; which may be both beneficial and harmful to the animal (Salanitro *et al.*, 1978). Studies to elucidate both the interrelationships that may exist between micro-organisms and the host (Shapiro and Sarles, 1949) and the mechanism by which antibiotics stimulate growth in young animals (Lev *et al.*, 1957) have been reported. The intestinal flora affects growth and development of the animal through a range of factors. These include modification of both exogenous and endogenous substances presented to the GIT, alteration of the morphogenesis of the GIT which in turn may affect nutrient absorption, prevention of the establishment within the GIT of foreign organisms and supplementation or competition with the host animal for a range of nutrients (Schaedler, 1973).

Nevertheless, the dietary and other roles of the intestinal flora in the chicken are not as distinct as it has been demonstrated in other monogastric species probably due to the short transit retention time with which nutrients stay within the GIT (Schaedler, 1973; Shapiro and Sarles, 1949; Salanitro *et al.*, 1978). Few studies have indicated that there may be limited vitamin synthesis and fermentation in the caeca

yielding volatile fatty acids similar to those found in other animals, although the nutritional benefits of the processes is yet to be established (Hegde *et al.*, 1982). The necessity of maintaining a balanced GIT flora in the chicken has been advocated by several workers (Boyd and Edwards, 1967; Eyssen and De Somer, 1967; Furuse and Yokota, 1984). In spite of the vagueness about the role played by micro-organisms present in the chicken GIT, it is assumed that an alteration in the microflora balance might result in increased susceptibility to diseases and poor performance (Ferket, 1991).

The newly hatched chick harbours few micro-organisms in the gut, but a rapid establishment appears to begin soon after feeding (Shapiro and Sarles, 1949; Salanitro *et al.*, 1978; Lev and Briggs, 1956; Lev *et al.*, 1957; Furuse *et al.*, 1991). The rapid microbial colonization of the GIT which takes place soon after feeding is completed when the chick is 9-13 days old, as shown in Figure 2.4. As a result it has been sometimes suggested that the chick might not have an obligate flora and that most of the micro-organisms within the GIT of the young chick are a mere reflection of the diet and the environment. Similar conclusions have also been made for the pig (Shapiro and Sarles, 1949; Willingale and Briggs, 1955). However, other studies have shown that there are species within the GIT of the chick which may be regarded as obligate flora; these include *Bacterium coli commune, Baccilus vegetus* and *Pseudomonas granulata* (see Table 2.11) (Barnes *et al.*, 1978).

The establishment of the various types of micro-organisms found in the chick GIT is affected by age, diet and environment (Lev and Briggs, 1956; Barnes *et al.*, 1978; Salanitro *et al.*, 1978; Barnes *et al.*, 1980). Studies carried out show that the establishment of the GIT flora is open to considerable variation (see Figure 2.4), estimates ranging from 6 hours after feeding up to 4-6 days of age (Shapiro and Sarles, 1949; Lev and Briggs, 1956; Lev *et al.*, 1957; Barnes *et al.*, 1980). By week

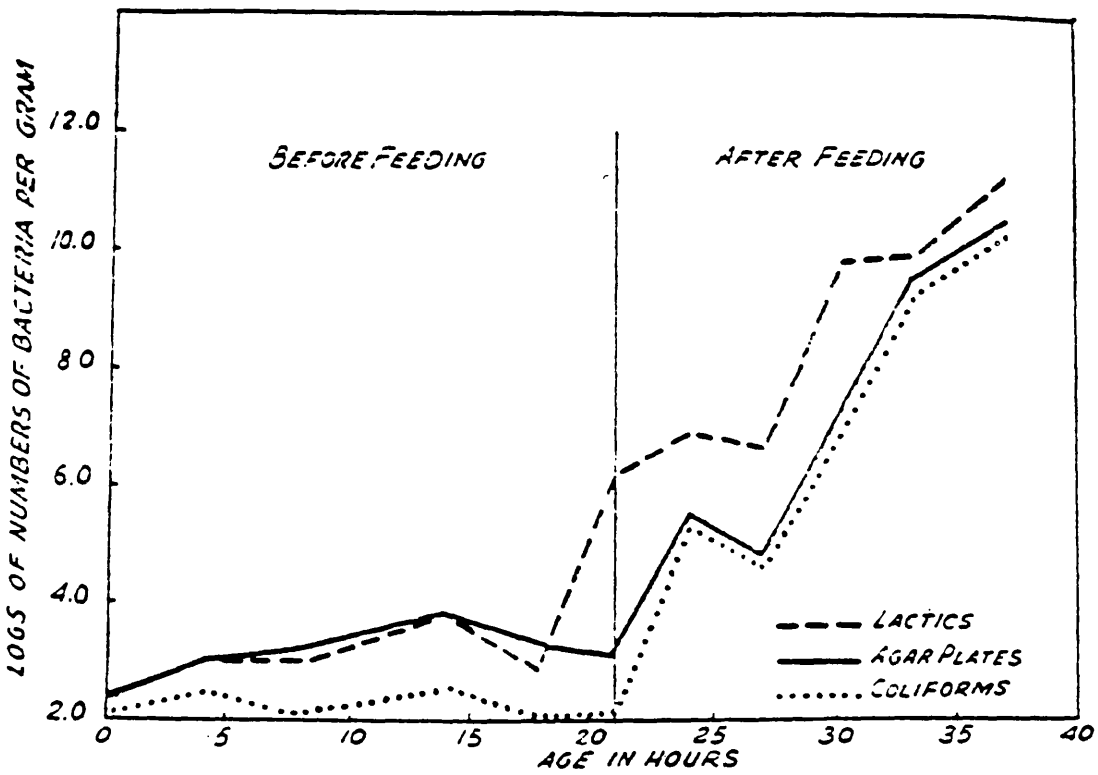
Table 2.11: The flora of crop, jejunal and caecal contents of conventional chicks aged 5 and 14 days fed on a diet containing lactose or a control diet

	$\log_{10}$ CFU/ gram fresh weight					
	crop		jejunum		caecum	
age (days):	5	14	5	14	5	14
organism:						
E. coli						
lactose	4.5	5.8	5.1	5.7	9.6	9.4
control	4.8	5.3	5.3	5.1	9.1	9.1
Streptococci						
lactose	4.7	4.2	5.4	4.9	9.0	7.4
control	4.8	4.1	5.1	5.0	8.5	7.8
L. acidophillus						
lactose	6.6	6.7	6.2	6.1	8.0	4.4
control	6.6	6.7	6.6	6.6	6.9	7.2
L. salivarius						
lactose	6.8	7.5	6.7	7.1	8.5	5.2
control	7.5	7.1	7.3	7.1	7.3	7.5
Bacteriodaceae						
lactose	0.0	2.8	2.9	3.7	8.5	4.3
control	2.6	4.1	3.9	4.1	9.3	9.9
Clostridia						
lactose	0.0	0.0	0.0	0.0	4.1	5.9
control	0.0	2.6	0.0	3.4	8.3	9.0

Source: Morishita *et al.* (1982)

Figure 2.4: The cecal microflora of young chicks

Source: Salanitro *et al.* (1978)



The cecal microflora of young chicks at various ages.

post-hatch, distribution of the flora in the chick is similar to that of adult birds (Salanitro *et al.*, 1978). The most common micro-organisms present in the GIT of the young chick and the chicken include Streptococci, Clostridia, Enterobacteria, Lactobacilli and a large number of other groups which are identifiable and unidentifiable (Salanitro *et al.*, 1978). Differences in the rate of establishment and distribution of micro-organisms among the various parts of the chick GIT have been observed (Shapiro and Sarles, 1949; Salanitro *et al.*, 1974; Barnes *et al.*, 1980). Gram positive aerobic organisms such as Lactobacilli, Streptococcus, Staphylococcus and *Escherichia coli* are predominantly found in the crop and the small intestine, while Clostridia, Bacteriodes and other gram negative anaerobic organisms predominate the caecum and the colon.

The duodenum contains the least number of micro-organisms when compared to the other parts of the GIT and in most cases the numbers are less than 10^8 per g fresh wt (Shapiro and Sarles, 1949; Barnes *et al.*, 1978; Salanitro *et al.*, 1978). Soon after hatching the duodenum becomes dominated by Clostridia and Streptococci with very few Coliforms and Lactobacilli. However, by the second week of age the GIT microflora is dominated by Lactobacilli (Barnes *et al.*, 1972). Various reports show that Lactobacilli remain dominant throughout the broiler's life (Kimura *et al.*, 1986). The ileum also contains relatively few micro-organisms although their numbers are slightly higher than those found in the duodenum (Shapiro and Sarles, 1949). During the first few days of life the ileal flora is composed mainly of Lactobacilli, Streptococci and other species, but after 1-2 weeks of age the entire population is made up of Lactobacilli with occasional appearances of Streptococci and Enterobacteria (Shapiro and Sarles, 1949; Willingale and Briggs, 1955; Barnes *et al.*, 1972; Salanitro *et al.*, 1978; Kimura *et al.*, 1986). Both heterofermentative and homofermentative Lactobacilli have been

isolated from the chick's ileum (Barnes *et al.*, 1980). Shapiro and Sarles (1949) speculated that Lactobacilli could play an important role in the vitamin requirements of the chick since they were found in large numbers and they also had high vitamin requirements. The caecum contains the highest numbers of micro-organisms in comparison to other parts of the GIT (Table 2.12). The initial flora in the caeca is thought mainly to be composed of *Streptococcus faecalis, Coli aerogenes* and few Lactobacilli which are established slowly (Barnes *et al.*, 1978; Baba *et al.*, 1991). Lev and Briggs (1956) suggested that the bacterial flora observed in the caeca soon after hatching is the most representative of the indigenous population, a view not entirely agreed with by other workers (Drasar *et al.*, 1973; Salanitro *et al.*, 1974).

The chemical nature of the diet has long been considered to exert a limited influence on numbers and type of micro-organisms present in the GIT of the chicken. Investigations to determine the effect of different dietary nutrients on the GIT flora have been performed (Drasar *et al.*, 1973). Nevertheless interpretation of most of the results is difficult because of the heterogeneity of the microbes present in the GIT. An increase in Bifidobacteria (Drasar *et al.*, 1973), aciduric flora (Nath *et al.*, 1948) and increases in Lactobacillus production (Barnes *et al.*, 1972) and yeast production (Jayne-Williams and Fuller, 1966) were observed, when dietary carbohydrates were fed to chickens. The ratio between aerobes and anaerobes was highly influenced by differences in dietary carbohydrates. Aerobic:anaerobic ratio of 1:1, less than 1:2 and less than 1:4 were observed respectively, when dextrin, sucrose and lactose were added to the diet. Additionally it has been found that lactose fed chickens had higher caecal contents (Nath *et al.*, 1948). Morishita *et al.* (1982) found decreases and increases in Lactobacilli and Clostridia species in the caeca and small intestine respectively, when lactose was added to the diet (Table 2.12). In contrast to carbohydrates, the influence of protein and fats on the GIT flora

Table 2.12: The intestinal flora at 2, 3 and 4.5 weeks in chickens fed diet A (9% fishmeal) and diet C (25% fishmeal)

			1[a]	2	3	4
age (weeks):	gut section:	diet:				
2	duodenum	A	2.9	8.4	N.F	N.F
		C	3.2	6.3	N.F	N.F
	small intestine	A	N.F	6.8	3.4	N.F
		C	3.4	6.8	4.9	N.F
	caecum	A	5.3	9.2	6.2	8.7
		C	7.2	8.9	4.3	8.1
3	duodenum	A	4.8	N.F	3.9	N.F
		C	2.9	N.F	N.F	N.F
	small intestine	A	3.5	4.4	4.4	N.F
		C	N.F	N.F	N.F	N.F
	caecum	A	7.7	6.5	7.0	8.2
		C	8.3	4.5	6.1	8.2
4.5	small intestine	A	3.6	5.2	N.F	N.F
		C	3.3	5.5	6.0	N.F

Source: Barnes *et al.* (1972)

N.F = Clostridia are below 10^2/gram; others are below 10^3/gram

[a] = 1 Total clostridia, 2 Lactobacilli, 3 *Faecal streptococci* and 4 *Coli aerogenes*

are far more limited. High protein favours the growth of proteolytic flora, an increase of *Clostridium welchii* (Drasar *et al.*, 1973) and a reduction in the number Lactobacilli (Jayne-Williams and Fuller, 1966). However, Harrison and Coates (1972) showed that the addition of fish solubles was without effect on most of the GIT flora, particularly *Streptococcus faecalis*. The ability of most of the chicken GIT flora to degrade fat is low, hence addition of high levels of fat tend to decrease some of the flora already present (Nath *et al.*, 1948). Coliforms and Enterobacteria were reduced when dietary fat was increased while Lactobacilli were unaffected (Drasar *et al.*, 1973). In a review by Ferket (1991) it was pointed out that the practice of modern broiler feeding whereby high levels of soyabean meal and fats are added to the diets of the newly hatched chicks would most likely cause destabilization of the gut flora. This is because the oligosaccharides and sucrose in soyabean meal are not well digested by the chick and at the same time sucrase activity is reduced by high fat levels. Young chicks are more susceptible to enteric disorders because of their low microbial diversity and the proportionately greater influence of the environment and diet (Ferket, 1991).

The combination of large and differing populations of micro-organisms within the GIT of the chicken, a short transit time of the feed and large variations between and within birds arising from difficulties in culturing and identification makes it very difficult to study the effect of intestinal flora on chicken nutrition (Coates and Jayne-Williams, 1966). As a result, the nutritional contribution to the chicken by the intestinal flora is vaguely understood. Nevertheless, significant differences in growth performance and nutrient utilization between germ free and conventional birds, attributable to differences in microbial populations have been observed. Body weight and feed intake in most cases has been found to be higher in germ free than in conventional chicks (Eyssen, 1973) and (Furuse and Yokota,

1984b), although the addition of fish solubles to the diet led to better weight gain in conventional chicks (Harrison and Coates, 1972). The improved performance by germ free chicks is thought to be a result of various factors which include morphological changes to the GIT (Harrison and Coates, 1972; Furuse and Yokota, 1984b; Furuse *et al.*, 1991). Thus, Furuse and Yokota (1984b) showed that the size and weight of the proventriculus, gizzard, duodenum, jejunum, ileum, caeca, colon, liver and pancreas were smaller and lighter in germ free chicks compared to conventional chicks. The thin intestinal wall favoured the absorption of nutrients and thereafter led to improved performance by the germ free chicks. Variations in the utilization of individual nutrients by germ free and conventional birds have also been observed (Boyd and Edwards, 1967; Eyssen and De Somer, 1967; Furuse and Yokota, 1984a). Higher metabolizable energy (Furuse *et al.*, 1991) and fat excretion in faeces (Cole *et al.*, 1981) have been observed in conventional birds (Table 2.13). Fat excretion was particularly high during the early stages of growth. Intestinal microflora play an important role in the metabolism of bile acids and thereby indirectly affect lipid metabolism (Schaedler, 1973). Clear differences in bile acid secretions and excretions between germ-free and conventional animals have been reported (Eyssen, 1973). Germ free animals excrete only conjugated primary bile acids, both in the bile and faeces, whilst in conventional animals the same bile acids are subjected to deconjugation and dehydroxylation by microbial enzymes in the intestine to yield secondary bile acids. The secondary bile acids may be partly absorbed and transformed again in the liver but in some cases the level of free bile acids is increased thereby reducing the efficiency of micelle formation with consequential effects on fat absorption. Eyssen (1973) reported that the amount of bile acids excreted through the faeces in germ free chicks and rats was 30-40 percent less than conventional counterparts. As a result the small intestine of the germ free

Table 2.13: Three and 14 day lipid analyses (Folch method)

age (days):	3		14	
	*GF	CV	GF	CV
parameters:				
faeces weight (g)	17.3	22.5	52.1	52.1
lipid/g faeces	0.034	0.088	0.045	0.089
total faecal lipid	0.57	1.87	2.24	4.44
food eaten (g)	48.2	52.4	120.0	123.3
total lipid retained (g)	6.41	5.79	15.12	13.4
lipid retained/ g food eaten	0.133	0.11	0.125	0.10
lipid retained/ g lipid eaten	0.92	0.76	0.87	0.75
weight gained (g)	37.3	33.4	57.3	55.6
lipid retained/ g weight gained	0.181	0.175	0.269	0.284

Source: Cole *et al.* (1981)

* GF = germ free; CV = conventional

chick contains at least twice the level of bile acids than those present in the small intestine of conventional chicks. A consequential enhancement of neutral sterols and fatty acid absorption occurs as a result. Although in most instances the germ free chick displays an enhanced performance when compared to a conventional chick, the underlying importance of a balanced GIT flora cannot overlooked.

The effect of different dietary fat sources on the viability and performance of the chick during the early post-hatch days have been extensively reviewed. From the present review it was evident that using high fat diets regardless of their lipid and fatty acid compositions was not entirely beneficial to the chick and it might cases it lead to poor performance. However, varied responses were noted when chicks were fed diets containing fats differing in fatty acid compositions. Nevertheless, the benefits arising from incorporating high levels of fats during the early post-hatch period are vaguely understood and sometimes they are exceeded by the physiological impacts to the chick which are observed during this period. Following this the present study was therefore undertaken so as to evaluate the effects of incorporating different fat sources (varying in their lipid and fatty acid compositions) in chick starter diets on the performance of chicks during the early post-hatch period.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The main objective of the present study was to investigate the effects of fat sources, differing considerably in their fatty acid composition on the performance of broiler chicks during the early stages of growth. The dietary fat sources were based on; tallow oil (containing high levels of saturated fatty acids), soyabean oil (containing high levels of polyunsaturated fatty acids) and a proprietary dietary fat source (also containing high levels of polyunsaturated fatty acids). For comparative purposes a broiler starter diet (from Dalgety Agriculture Ltd: (TU), Bristol, England) was additionally used. The study was divided into four distinctive parts (experiments 1 - 4), the outlines of which are detailed individually in the relevant sections.

3.2 LIPID EXTRACTION

The basic lipid extraction methods were similar for the diets, their fat sources and the tissues sampled from the chicks over the experimental period. In all cases an appropriate amount of sample was exhaustively homogenized in appropriate volumes of chloroform:methanol (2:1, v/v) according to standard procedures (Folch *et al.*, 1957; Christie, 1982). The homogenates were filtered using slow flow Whatman filter papers (Whatman Labscales Ltd, Kent, U.K.) into a measuring cylinder. The residue on the filter paper was retained and dried in a laboratory oven to aid in subsequent calculations of dry matter content. Where appropriate, extraction of lipid from the feed and tissue samples was promoted by heating in a water bath at 60^{o}C or in certain instances by refluxing for 20 minutes.

Lipid extraction using chloroform:methanol is known to dissolve a limited amount of sugars, amino acids, salts and other impurities (Christie, 1982). In order to remove these contaminants the chloroform:methanol extract was vigorously

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Lipid extraction using chloroform:methanol is known to dissolve a limited amount of sugars, amino acids, salts and other impurities (Christie, 1982). In order to remove these contaminants the chloroform:methanol extract was vigorously

shaken with 0.88 percent (w/v) potassium chloride solution (5:1 v/v) (Folch *et al.*, 1957). The mixture was then allowed to partition into a biphasic separation by leaving it to stand for a minimum of 12 hours. After separation the upper layer of the mixture was removed and then discarded. The lower phase containing the extracted lipids was transferred to a suitable sized round bottom flask and dried on a rotary film evaporator at 65°C. Several additions of chloroform:methanol (2:1 v/v) were made in order to ensure complete removal of water from the lipid sample. After drying, chloroform was added and the lipid sample was transferred to a glass vial. The samples in the vials were then placed in a heating block at 55°C and dried using a stream of nitrogen gas. To the dried sample a suitable volume (5-10 ml) of chloroform was added and then stored in a refrigerator to await subsequent analyses.

3.3 LIPID AND FATTY ACID DETERMINATIONS

Prior to analyses, lipid samples in the vials were again dried under a stream of nitrogen gas after which a known volume of chloroform depending on the concentration of the sample was added. Part of the sample was analysed for total fatty acids and the remaining portion was used for lipid separations, quantification and determination of fatty acids according to procedures described in the following sections.

3.3.1 Lipid separation

The major lipid fractions were separated according to their polarity using established procedures of Liquid Chromatography (Christie, 1982). Dry glass plates of a suitable size (20 x 20 cm or 20 x 10 cm) were aligned on a commercial plate holder and wiped with cotton wool dampened with hexane to remove any contamination. A suspension containing 22.5 g Silica gel G (Merck, ATG,

shaken with 0.88 percent (w/v) potassium chloride solution (5:1 v/v) (Folch *et al.*, 1957). The mixture was then allowed to partition into a biphasic separation by leaving it to stand for a minimum of 12 hours. After separation the upper layer of the mixture was removed by pippeting and then discarded. The lower phase containing the extracted lipids was transferred to a suitable sized round bottom flask and dried on a rotary film evaporator at 65°C. Several additions of chloroform:methanol (2:1 v/v) were made in order to ensure complete removal of water from the lipid sample. After drying, chloroform was added and the lipid sample was transferred to a glass vial. The samples in the vials were then placed in a heating block at 55°C and dried using a stream of nitrogen gas. To the dried sample suitable volume (5-10 ml) of chloroform was added and then stored in a refrigerator to await subsequent analyses.

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Darmstadt, Germany) in 50 ml glass distilled water was prepared by shaking the mixture vigorously. Using an aluminium spreader, the suspension was evenly applied to the surface of the glass plates to form a thin layer of 0.25 mm thick. The plates were left to dry at room temperature for about 30 minutes. After drying the coated plates were transferred to an oven and then activated by heating at 110^{o}C for a minimum of 2 hours. The plates were then stored in a heated cabinet until just before use. The coated plates were allowed to cool at room temperature before samples to be separated were applied. Suitable aliquots of the lipid extracts in chloroform were carefully applied to the plates as discrete narrow bands at about 1.0 cm from the base of the thin layer plate using a fine 100 µl syringe. The chloroform was allowed to evaporate before lipid separation was undertaken.

Separation of simple and complex lipid components was performed using a freshly prepared eluting mixture of hexane:diethyl ether:formic acid (80:20:1, v/v/v). In order to promote lipid separation the chromatographic tank was lined with a filter paper saturated in the above solvent mixture. The chromato plate was then placed carefully in the chromatographic tank containing the solvent with minimum disturbance and the tank was sealed with a glass cover. Development time for each plate was about 30 minutes, by which time the solvent front had reached the top of the plate (approximately 1 cm from the top). The major lipid classes separated according to their relative polarities, i.e less polar moieties migrating faster than the more polar moieties. The plate was then carefully removed from the tank and excess solvent was allowed to evaporate by brief exposure to air for drying. The dry plates were then sprayed with an ultraviolet fluorescent dye, (0.1 percent, w/v) of 2,7,-dichloro-fluorescein in methanol in order to facilitate the identification of each separated lipid moiety. This method allows lipids to resolve into separate bands which in each case were identified as cholesterol esters, triglycerides, free fatty

acids, free cholesterol and phospholipids by their retention times relative to standards. The lipid moieties were visualized under the ultra violet light in a specialist viewing chamber and each individual lipid band was removed from the plate and transferred into 10 ml test tubes. The lipids were recovered from the Silica gel G by consecutive elutions with 5 ml of diethylether for cholesterol esters, triglycerides, free fatty acids and free cholesterol and 5 ml of methanol for phospholipids. In each case the tubes were shaken vigorously following the addition of appropriate solvent to the Silica gel G/sample. The Silica gel G was separated from the lipids by centrifuging at 2000 rev/min for 10 minutes. The solvent layer containing the lipid was transferred to a suitably sized round bottom flask. The elution process was repeated three times.

3.3.2 Fatty acid determinations

The method of Christie (1982) was adapted for fatty acid determinations. To 10-15 ml aliquot of the recovered esterified lipids or total lipid 1 ml of a pentadecaenoic acid in methanol (C15) as a an internal standard was added and the total volume was dried down on a rotary film evaporator under nitrogen gas. After drying 4 ml of a transmethylating agent were added. The mixture was then refluxed at 66^{o}C for a minimum of 30 minutes. After cooling 10 ml of hexane and glass distilled water, respectively were added and the mixture shaken vigorously. The mixture was immediately transferred to a 60 ml glass tube and allowed to partition into two phases. The top hexane layer containing the methyl esters was carefully pippeted into 30 ml test tube containing a drying agent (Na_2SO_4: $NaHCO_3$ 4:1, w/w) to remove any contaminating water, capped and was left to stand for a minimum of 30 minutes. The lipid sample in hexane layer was then transferred to a 15 ml test tube and dried under a stream of nitrogen gas. After drying the mixture

containing methyl esters were redissolved in about 100 µl of hexane. At this point the sample was ready for injection into a Gas Liquid Chromatography. The above procedure were done for each individual lipid class.

3.3.3 Determination of Fatty Acids by Gas Liquid Chromatography

The gas liquid chromatograph used for fatty acid analyses was PYE (Philips Model No. 4500). The chromatograph was fitted with dual parked columns. Detection was by flame ionisation, whereby hydrogen and air were supplied at 100 kN/m^2 and 145 kN/m^2, respectively. Column parking was on a Gaschrom P solid support containing 15 percent silicone treated ethylene glycol succinate (EGSSX). The carrier nitrogen gas was supplied at a pressure of 340 kN/m^2, with an inlet pressure of 185 kN/m^2 and to deliver at a flow rate of 40 ml per minute. The column temperature was 190°C and the injection block temperature was 210°C. About 1-2 µl (depending upon sample concentration) of the fatty acid methyl esters in hexane was injected onto the analyser column each time. Analysis time for the methyl esters of all the fractions was such that all fatty acids up to and including docosahexaenoic acid were determined. The common names of the fatty acids and their shorthand form which were determined are shown in Table 3.1. Identification of the fatty acids was from a series of parameters that included comparative retention and relative retention times with known standards and carbon numbers versus semi logarithmic plots. The relative proportions of fatty acids were quantified by integration of the amplifier signal using an electronic intergrator (Spectro-physics Model No. 4270). The resultant peaks not only provided the relative proportions of the major fatty acids present, but also their absolute amount through comparison with an added pentadecaenoic acid (Christie *et al.*, 1970). The proportion of each individual fatty acid in a methyl ester sample plus the unnormalized total of the

Table 3.1: The common names and the description of the major long chain fatty acids

Common name	shorthand notation	common name	shorthand notation
palmitic	16:0	eicosatrienoic	20:3
palmitoleic	16:1	arachidonic	20:4
stearic	18:0	eicosapentaenoic	20:5
oleic	18:1	docosapentaenoic	22:5
linoleic	18:2	docosahexaenoic	22:6
linolenic	18:3		

Source: Gurr (1984)

The shorthand designation used e.g 16:0, 16:1, 18:0 etc. is composed of n which is the length of the carbon chain followed by a colon; the number after the colon denotes the number of double bonds present in the fatty acid. Thus 18:2 equal is a C18 chain length fatty acid with 2 double bonds. In the present study the shorthand notation will be used in the tables, whereas common names will be used in the text.

Table 3.2: The correction factors used to convert the total amount of fatty acid in a lipid class (determined by gas liquid chromatography analysis) to weight of lipid

Lipid class	factor
cholesterol esters	2.46
triglycerides	0.995
free fatty acids	0.991
phospholipids*	1.371

Source: Christie *et al.* (1970)

* As an approximation, it is assumed that the phospholipids consist of phosphatidylcholine only

Experiment.....
Date.....
Sample Number.....

	Cholesterol ester	Triglyceride	Free fatty acid	Phospholipid	Partial glyceride
Fatty acid 16:0*					
16:1					
18:0					
18:1					
18:2					
18:3					
20:3					
20:4					
20:5					
22:5					
22:6					
FC					
UNT					
NT					

*The fatty acids as per description in Table 3.1

particular lipid class were recorded at the end of each run, (see data sheet). From the amount of fatty acid thus determined, concentrations of individual lipid classes could be calculated using factors based on relative proportions of fatty acids associated with individual lipid classes. These correction factors are given in Table 3.2. The concentrations of the fatty acids in a lipid plus the value for free cholesterol (see below) enabled the total amount of lipid in a sample to be determined and also the proportion of each lipid class within the total lipid.

$$\frac{\text{normalised total of lipid class}}{\text{total amount of lipid in the sample}}$$

3.4 QUANTIFICATION OF FREE CHOLESTEROL

This was determined using a modified charring procedure based on the use of a liquid scintillation counter as described by Shand and Noble (1980). The stored samples containing free cholesterol following elution from the thin layer chromatoplate were dried under a stream of nitrogen gas. After drying the free cholesterol were redissolved in 300 µl of hexane. Aliquot 150 µl of the reconstituted sample was applied as a discrete band onto a Silica gel G plate and the plate was then sprayed with a charring agent; a mixture of 3 percent (w/v) cupric acetate in 8 percent (w/v) orthophosphoric acid (Fewster *et al.*, 1969). After heating at 180°C for 15 minutes in a forced draught oven, the plates were cooled and thereafter the charred individual discrete bands were scrapped into scintillation vials. Following resuspension in 2 ml of distilled water and addition of 10 ml of an emulsifier (a commercial scintillation gelling cocktail, BDH Chemicals LTD, Poole, England), the samples were warmed in a water bath and the vials were then shaken vigorously to trap the charred free cholesterol in a resultant firm gel. The amount of quenching due to the presence of the charred material was then measured by counting each

sample for 1 minute in the external standard ratio mode (radium^{226}) of a liquid scintillation counter (Packard Instruments, Carversham, England, Model 2425). The interpolation of the external standard channels ratio obtained from a previously derived standard curve of cholesterol concentration versus external standard channels ratio of the sample enabled the amount of cholesterol to be determined directly (see Figure 3.1). A standard calibration curve was prepared by applying various amounts 1 mg/ml to 20 mg/ml of cholesterol as discrete bands to thin chromatoplates and treated as described above.

3.5 PREPARATION OF REAGENTS

(i) *2,7,-dichloro-fluorescein*

0.1 g of 2,7,-dichloro-fluorescein was dissolved in 96 ml of methanol and 4 ml of distilled water.

(ii) *Methylating agent* (Methanol: toluene: sulphuric acid(20:10:1, v/v/v))

To 500 ml of super dry methanol in a large round bottom flask was added 250 ml of toluene. 25 ml of concentrated sulphuric acid was added to the mixture, proper carefulness being taken to avoid overheating.

(iii) *Internal standard the pentadecaenoic acid (C15)*

20 ml of purest grade stock of pentadecaenoic acid was dissolved in 230 ml of methanol. The resultant delivered 0.322 mg/ml of pentadecaenoic acid.

NOTE: All the reagent preparations and most of the work were done in a ventilated fume cupboard.

Most of the equipments, reagents and solvents used for lipid and fatty acid determinations unless otherwise stated were obtained from the following companies:

(i) BDH/ Merck LTD. Poole, England.

(ii) Rhone Poulene LTD. Manchester, England.

(iii) Fisons Scientific Equipments, Leicestershire, England.

(iv) Packard Instruments, Carversham, England.

Figure 3.1: The standard curve for the determination of free cholesterol

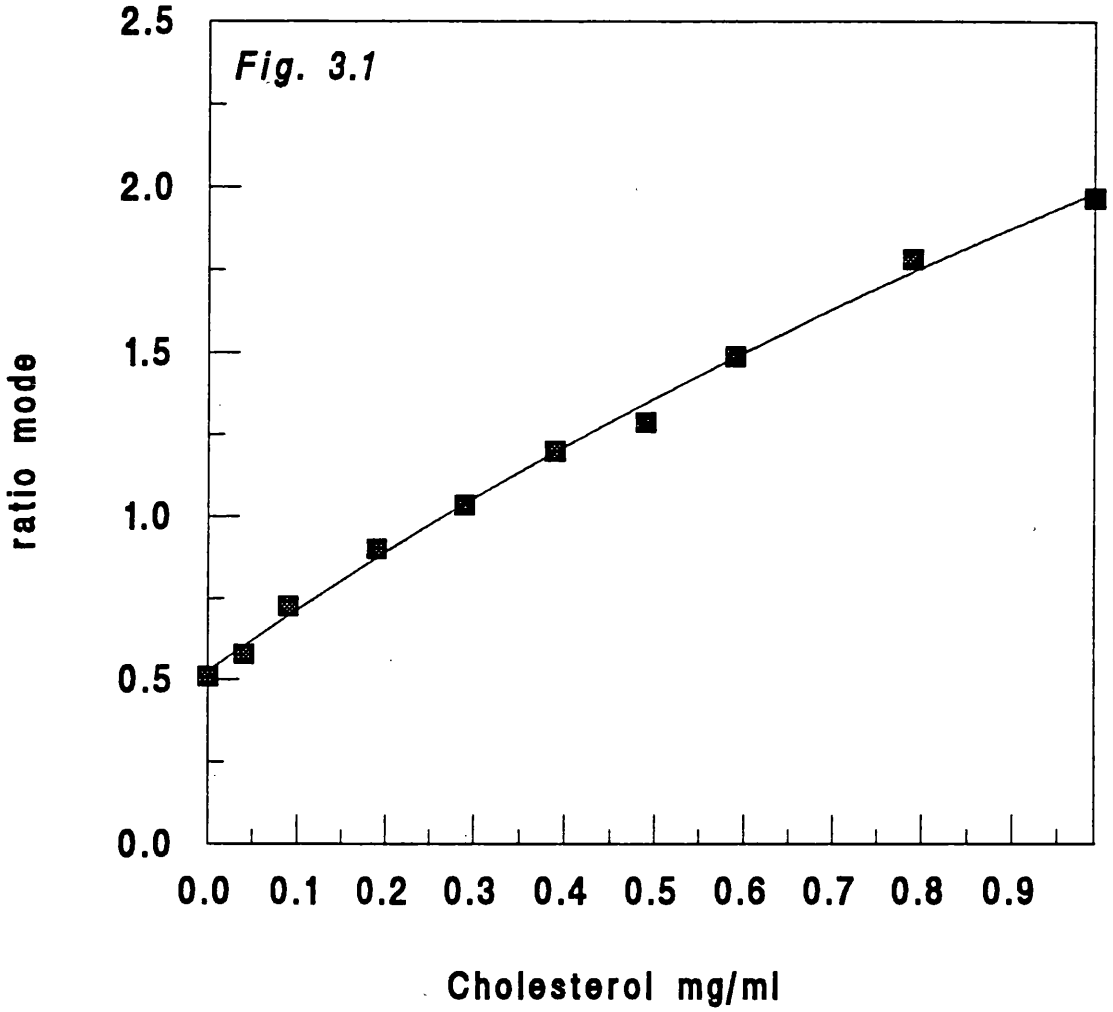
Fig. 3.1
2.5
2.0
1.5
1.0
0.5
0.0
ratio mode
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
Cholesterol mg/ml

CHAPTER 4

THE EFFECTS OF DIETARY SATURATED AND UNSATURATED ACIDS ON LIPID COMPOSITIONAL CHANGES OF THE RESIDUAL YOLK SAC MEMBRANE, LIVER AND GALL BLADDER BILE IN GROWING BROILER CHICKS

4.1 INTRODUCTION

Emergence of the chick from the egg is associated with very high concentrations of lipids in the liver, yolk sac and blood (Entenman *et al.*, 1940). The yolk material complements nutrient intake and fortifies efficient utilization of dietary energy and protein by the newly hatched chick (Bjornhag, 1979; Calder, 1979; Murakami *et al.*, 1988; Nir *et al.*, 1988; Skewes *et al.*, 1988). The liver of the newly hatched chick displays particularly high concentration of lipid which accounts for 20 percent of total tissue weight. Most of this lipid is in the form of esterified cholesterol (Entenman *et al.*, 1940; Noble and Ogunyemi, 1989). The high concentration of lipid found in the chick's body tissues at hatching differs markedly both in distribution and fatty acid composition from that of the adult birds (Akiba *et al.*, 1988). As a result, the chick undergoes dramatic physiological and biochemical changes during the early post-hatch period in order to adapt to the new environmental conditions and feeding regimes in which it finds itself. The regression of the yolk sac and the lipid compositional changes of the liver are amongst the many notable important changes associated with the early post-hatch period (Entenman *et al.*, 1940; Romanoff, 1960; Noble and Ogunyemi, 1989; Daly and Peterson, 1990).

The normal development of the chick and the associated metabolic features are highly influenced by early feeding regimes. The type and range of fatty acids available from the diet are known to play an important role in the development of the neonatal chicken (Ewing, 1963). Thus substantive evidence exists to suggest the importance of polyunsaturated fatty acids in normal neonatal development. Under normal circumstances the standard chick starter diet is designed in such a way as to contain very high levels of unsaturated fatty acids in particular linoleic (Watkins, 1987). However, considerable doubt has been cast upon whether exposure of the

chick to such high levels of polyunsaturated unsaturated fatty acids is beneficial during the critical period immediately after hatching (Fedde et al., 1960); Renner and Hill, 1960; Noble and Ogunyemi, 1989) and if so whether benefit may be derived by specific dietary lipid composition. In addition the presence of free fatty acids mainly from the diet pose problems to the chick's development (Renner and Hill, 1961; Krogdahl, 1985). The aim of present experiment was therefore, to investigate the possible effects of three diets (commercial and 2 artificial), differing substantially in their fatty acids compositions with respect to saturation and degree of esterification, on a range of major metabolic developmental lipid features in the neonatal chicken. During this study the following parameters were measured:

(i) the regression of the residual yolk sac, its changes in lipid and fatty acid composition over the 12 day post-hatch period

(ii) the changes in lipid and fatty acid composition of the liver over the 12 day post-hatch period

(iii) the changes in lipid and fatty acid compositions of the gall bladder bile over the 12 day post-hatch period

(iv) associated weight gain changes of broiler chicks during this period

4.2 MATERIALS AND METHODS

4.2.1 Treatments

Three dietary treatments differing considerably in dietary fat sources and therefore fatty acid compositions were used. The basic descriptions and the estimated proximate composition of the experimental diets are shown in Table 4.1. experimental diets were designated as Diet 1 (Dalgety chick starter, Bristol, England), Diets 2 and 3 in which the tallow oil and soybean oil provided the major

Table 4.1: Basic ingredients and estimated proximate composition of the experimental diets

	Diet 1	Diet 2	Diet 3
Ingredient, g/kg:			
barley		100	100
maize		250	250
wheat		245	245
herring meal		50	50
soyabean meal		220	220
grass meal		50	50
limestone		5.3	5.3
dicalcium phosphate		21.7	21.7
vitamin mix		2.5	2.5
mineral mix		2.5	2.5
salt		2.5	2.5
amprol mix		0.5	0.5
tallow oil		50	2.5
soyabean oil		-	47.5
calculated analysis			
ME (MJ/kg)	12.6	12.8	12.8
crude protein(%)	23.0	20.0	20.0
oil (%)	5.5	5.75	5.75

Diet 1= Commercial chick starter diet; Diet 2= Diet containing tallow oil as a major fat source and Diet 3= Diet containing soyabean oil as a major fat source

dietary fat source, respectively.

4.2.2 Lipid and fatty acid composition of tallow and soyabean oils and the compounded diets

The lipid composition of the fat sources and the experimental diets

The distributions of the major lipid groups of the tallow and soyabean oils and in the respective compounded diets used in experiment 1 are shown in Table 4.2. Triglyceride was by far the largest lipid group in both tallow and soyabean oils, accounting for more than 94 percent in each case. The other lipid groups were negligible or absent.

The distribution of the lipid groups in the compounded diets differed from those present in the tallow and soyabean oils although the level of triglycerides was still high. Triglycerides accounted for over 60 percent of total lipid in the case of both the tallow and soyabean oil based diets and for about 40 percent in the commercial diet. These differences in triglyceride levels between the dietary fat sources and their respective compounded diets were balanced by increased levels of free fatty acid in the latter. Apart from the mentioned differences the overall lipid composition of the diets were similar.

Fatty acid distribution

The fatty acid composition, weight percent of total fatty acids present in total lipid and the two major lipid components (triglycerides and free fatty acids) of the tallow and soyabean oils and the compounded diets are shown in Tables 4.3, 4.4 and 4.5, respectively. From Tables 4.3 it can be seen that there was a close similarity in the total fatty acid composition of the tallow and the soyabean oils and the subsequent diets based on their incorporation. The levels of unsaturated fatty acids

Table 4.2: Lipid compositions (major lipid fractions, weight percentage of total lipid present) of the dietary fat sources and compounded diets

	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
lipid group:					
cholesterol ester	0.3	2.1	2.5	2.0	1.4
triglyceride	94.3	97.2	48.5	67.6	66.0
free fatty acid	4.7	<0.1	38.1	18.4	22.0
partial glycerides	<0.1	<0.1	2.0	5.5	4.7
phospholipid	<0.7	<0.8	7.9	6.5	5.9

Table 4.3: Fatty acid compositions (major long chain fatty acids, weight percentage of total present) in the total lipids of the dietary fat sources and the compounded diets

	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet3
fatty acid:					
16:0*	24.8	10.2	13.5	21.8	14.3
16:1	5.6	<0.1	1.3	3.9	1.0
18:0	21.5	3.1	5.5	15.0	4.8
18:1	43.8	28.5	32.7	33.4	25.1
18:2	3.9	50.2	38.8	19.6	46.4
18:3	0.1	7.6	7.1	3.5	6.0
20:3	<0.1	0.3	0.3	0.9	0.7
20:4	0.1	<0.1	0.2	<0.1	<0.1
20:5	<0.1	<0.1	0.4	0.8	1.0
22:5	<0.1	<0.1	<0.1	0.2	<0.1
22:6	<0.1	<0.1	0.3	0.7	0.8
S:U	1:1.16	1:6.5	1:4.4	1:1.72	1:1.43

* The common names of the fatty acids as per description in Table 3.1

S:U = The ratio between total saturated and total unsaturated fatty acids

Table 4.4: Fatty acid compositions (major long chain fatty acids, weight percentage of total present) of triglyceride lipid fraction in dietary fat sources and compounded diets

	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
fatty acid					
16:0*	24.9	10.2	12.7	23.9	13.8
16:1	5.4	0.2	1.2	4.4	1.1
18:0	21.5	3.4	3.2	18.9	5.5
18:1	42.6	29.3	23.5	35.6	27.7
18:2	3.9	49.3	50.4	15.0	45.5
18:3	1.6	6.9	5.3	1.1	5.1
20:3	<0.1	0.7	0.2	0.5	0.6
20:4	0.1	<0.1	1.6	<0.1	<0.1
22:5	<0.1	<0.1	<0.1	0.1	<0.1
22:6	<0.1	<0.1	1.0	0.3	0.4
S:U	1:1.16	1:6.38	1:5.31	1:1.34	1:4.20

Table 4.5: Fatty acid compositions (major long chain fatty acids, weight percentage of total present) of free fatty acid lipid fraction in the dietary fat sources and compounded diets

	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
fatty acid:					
16:0*	25.3	N.D	16.3	26.1	19.4
16:1	4.9		1.4	3.5	1.2
18:0	20.7		3.4	12.0	5.6
18:1	43.0		20.4	29.9	24.3
18:2	3.8		48.1	20.9	42.5
18:3	1.8		5.6	3.6	5.8
20:3	<0.1		2.2	2.7	<0.1
20:4	0.2		<0.1	0.7	<0.1
20:5	<0.1		1.0	0.8	<0.1
22:6	<0.1		0.8	0.9	0.7
S:U					

* The common names of the fatty acids as per description in Table 3.1

S:U = The ratio between total saturated and total unsaturated fatty acids. N.D = The level of free fatty acid in soyabean oil was insufficient for determination

were higher in soyabean oil and the corresponding compounded diet, whilst tallow oil showed much higher levels of saturated fatty acids. The C20 and C22 polyunsaturated fatty acids were extremely low throughout in all the tallow and soyabean oils and the compounded diets. Within the tallow and the soyabean oils there was a close similarity between the fatty acid composition of the triglyceride and the free fatty acid lipid fractions (see Tables 4.4 and 4.5). Likewise the fatty acid compositions within the total lipid of tallow and soyabean oils and their compounded diets showed a close similarity. In overall terms therefore the diets fed to the chicks provided three different lipid/fatty acid spectrum:

(i) diet in which there were high levels of free fatty acids and high levels of C18 polyunsaturated fatty acid throughout (Dalgety chick starter).

(ii) a diet which contained significantly lower levels of free fatty acids and high levels of triglycerides but in which levels of C18 polyunsaturated fatty acids were low throughout (tallow oil based diet)

(iii) a diet which levels of triglycerides and free fatty acids fractions were similar to (ii) but contained high levels of C18 polyunsaturated fatty acids throughout (soyabean oil based diet)

4.2.3 Rearing of chicks

One hundred and ninety five day old Ross 1 male broiler chicks were obtained from Ross Poultry Limited in Inverurie, Aberdeenshire. The chicks were weighed and then randomly allocated to the three dietary treatments. The experiment was conducted under controlled environmental conditions for both temperature and humidity. Access to the house was limited to the persons involved in the experiment in order to minimize disease transmission. The chicks were reared on the floor covered with wood shavings to a depth of about 2.5 cm thick. All the

chicks in each dietary treatment were kept as a single large flock in a pen with a stocking density of 13 chicks/m^2. Since the experimental period was only 12 days, a continuous uninterrupted lighting regime was employed using about 20 lux fluorescent light source. Brooding temperature was $35^oC \pm 0.5$ during the first week and was reduced to about $32^oC \pm 0.5$ on the second week. The chicks were fed the diets *ad libitum.* Clean water was also available at all times. As far as possible the chicks were reared according to good husbandry management.

4.2.4 Sampling

Tissue samples of the remnant yolk sac, liver and the gall bladder bile were obtained on day 1, 3, 6, 9 and 12 post-hatch. Twelve chicks from each dietary treatment were randomly selected for sampling after weighing. Each chick was killed by neck dislocation, followed by careful laparotomy to reveal the yolk sac membrane, liver lobes and the gall bladder. Using tweezers the yolk sac was carefully teased out, weighed and temporarily stored in a suitable sample bottle and maintained at approximately 5^oC in an insulated container filled with ice. A similar procedure was adopted for the liver, taking both lobes and the gall bladder. Samples from three chicks were pooled to form one sample and each dietary treatment had four replications. After weighing all the samples were stored in suitable containers at -20^oC to await analyses.

4.2.5 Lipid analyses

These were conducted as per procedures described in detail in Chapter 3.0 Materials and Methods: sections 3.1-3.3

4.2.6 Statistical analyses

All the data obtained were subjected to a one way analysis of variance using the Minitab Release 7.1 Version of 1989 and the results are presented in the Appendices. Results of $P<0.05$ and above were regarded as significant.

4.3 RESULTS

4.3.1 Body weight changes

The mean body weights of chicks during the experimental period are shown in Table 4.6. Allowing for differences in the initial hatching weights of the chicks, it can be seen that dietary treatment, had no significant effect on weight gains. However, in overall terms some differences in the pattern of weight gains were observed. Thus, during the first 3 days post-hatch weight gains in chicks receiving the Dalgety chick starter and soyabean oil based diets averaged 14 g per day compared to those receiving the tallow oil based diet which averaged 12 g per day. Subsequently, however, up to day 12 post-hatch weight gains in chicks receiving the tallow oil based diet were highest. The highest relative weight gains in chicks were observed during days 1-3 post-hatch in all the dietary treatments.

4.3.2 Yolk sac changes in weights, lipid and fatty acid compositions

Overall weight changes

The rate of regression of the yolk sac measured by weight changes are shown in Table 4.6. As can be seen the regression of the yolk sac was significantly affected by post-hatch age. A decrease in the weight of the yolk sac of about 50-75 percent of its initial weight occurred during the first 3 days post-hatch. The largest decrease was found in chicks receiving the tallow oil based diet. Between days 3 and 6 post-hatch a further sharp decline in the weight of yolk sac by more than 75 percent

Table 4.6: The effect of age and dietary fat sources on the body weight changes, residual yolk sac materials and liver weight changes

		age (days)				
		1	3	6	9	12
Parameters:	Diets					
Body weight						
(g)	1	38.6 ± 1.12	53.0 ± 1.48	91.8 ± 1.28	156.4 ± 2.81	253.3 ± 8.22
	2	48.0 ± 1.20	60.3 ± 1.18	99.8 ± 1.52	169.8 ± 2.96	277.1 ± 10.9
	3	46.7 ± 1.40	61.1 ± 2.01	102.9 ± 2.11	162.3 ± 3.66	253.0 ± 8.56
YSM remnants						
(g)	1	7.8 ± 0.90	3.9 ± 0.22	1.0 ± 0.43	0.1	0.22
	2	15.7 ± 0.23	3.7 ± 0.66	0.8 ± 0.27	0.3 ± 0.08	0.1 ± 0.03
	3	15.4 ± 1.61	5.0 ± 0.44	0.3 ± 0.14	0.1 ± 0.06	0.1 ± 0.06
Liver weight						
(g)	1	2.6 ± 0.08	6.4 ± 0.29	11.3 ± 0.60	20.6 ± 0.60	27.4 ± 0.60
	2	2.6 ± 0.31	6.2 ± 0.32	9.3 ± 1.09	16.7 ± 0.28	21.2 ± 0.84
	3	2.5 ± 0.45	6.2 ± 0.22	12.9 ± 0.47	17.0 ± 0.58	22.0 ± 0.97

of that remaining was observed in chicks receiving both the tallow oil based diet and the Dalgety chick starter diets; this compared with less than 20 percent reduction in the chicks receiving the soyabean oil based diet. Nevertheless, by day 9 post-hatch the weights of the yolk sac were similar in all dietary treatments. A similar pattern was observed when the weights of the yolk sac were expressed as percentage of total body weight (see Figure 4.1). The yolk sac constituted between 6-11 percent of the total body weight in the newly hatched chick on day 1 post-hatch but had declined to less than 1 percent on day 12 post-hatch. Like body weight changes, in overall terms the rate of disappearance of the yolk sac was not significantly affected by diet.

Lipid changes

The distribution of the major lipid classes in the yolk sac following hatching are shown in Figures 4.2(i)-(iv). Triglyceride accounted for more than 60 percent of the total lipid on day 1 post-hatch, followed by phospholipid at about 20 percent, whilst cholesterol ester, free cholesterol and free fatty acid together accounted for less than 15 percent. Irrespective of the diet the yolk sac underwent major lipid compositional changes following hatching. These were characterised by decreasing proportions of both triglyceride and phospholipid fractions and increases in both cholesterol ester and free cholesterol fractions. Significant effects of diet on lipid composition of the yolk sac were observed on days 3 and 6 post-hatch. Proportions of triglyceride and phospholipid in the residual yolk sac materials of chicks receiving the soyabean oil based diet were higher than those of chicks receiving the Dalgety chick starter and tallow oil based diets. Concomitantly there were increases in the proportions of both cholesterol ester and free fatty acid. Analyses of data of the residual yolk sac material were not possible after 6 day post-hatch due to insufficient material. The absolute amount of lipid in the yolk sac was reduced very rapidly over the first 6 days following hatching.

Figure 4.1: The effect of age and dietary fat sources on the proportion of the residual yolk sac materials to the chick body weight

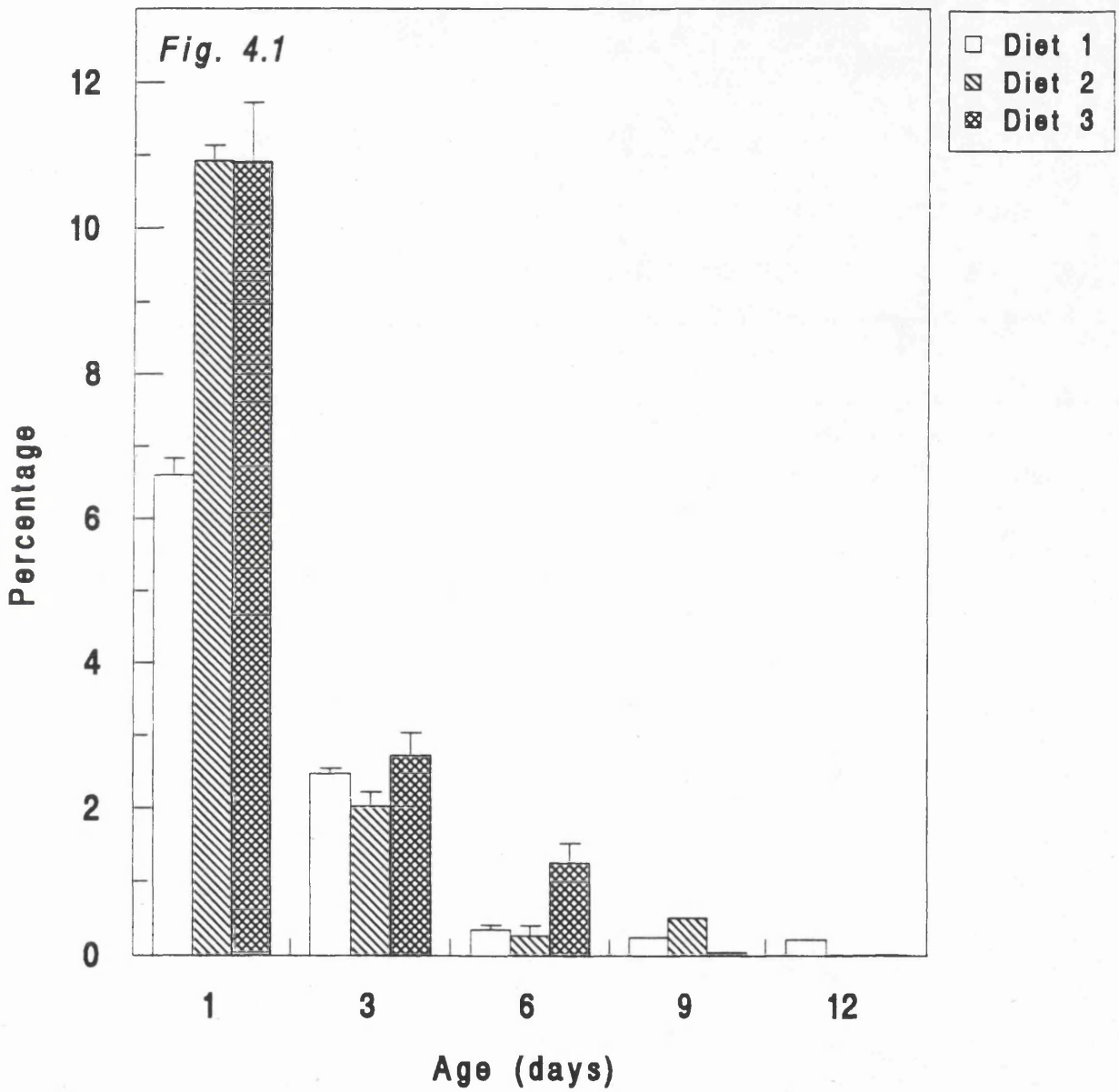
Fig. 4.1
Diet 1
Diet 2
Diet 3
Percentage
0
2
4
6
8
10
12
1
3
6
9
12
Age (days)

Figure 4.2(i-iv): The effect of age and dietary sources on the distribution of the major lipid fractions in the residual yolk sac materials

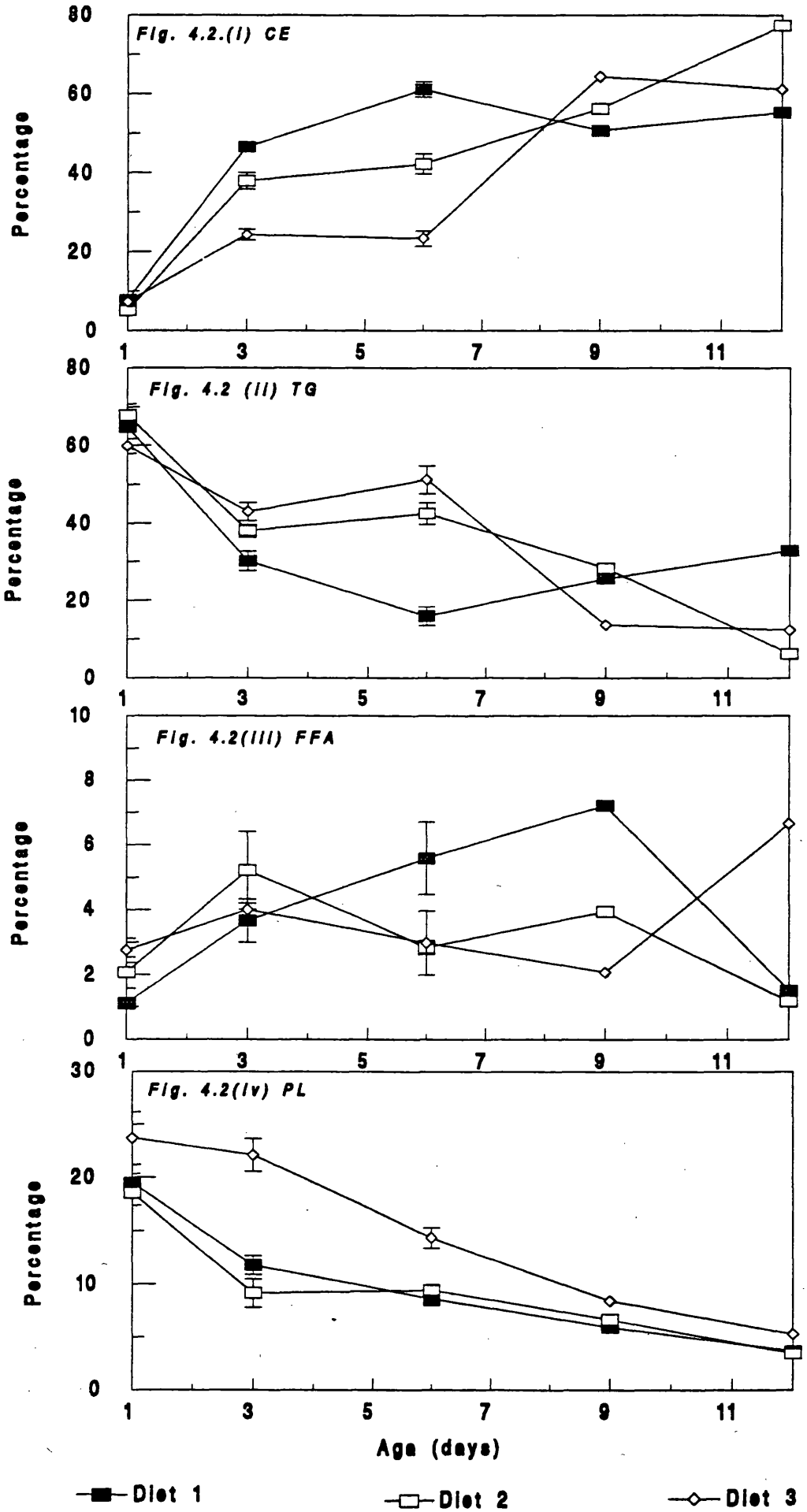
Fig. 4.2.(I) CE
Fig. 4.2 (II) TG
Fig. 4.2(III) FFA
Fig. 4.2(IV) PL
Percentage
Age (days)
Diet 1
Diet 2
Diet 3

Fatty acid changes

The major fatty acids present in cholesterol esters and triglycerides of the residual yolk sac materials following hatching are shown in Tables 4.7 and 4.8, respectively. Oleic acid accounted for more than 75 percent of the total fatty acids within the cholesterol ester on day 1 post-hatch. Although the proportion of oleic in the cholesterol ester remained high in all dietary treatments, both the tallow and soyabean oils based diets were associated with slight increases following hatching. Linoleic acid accounted for 10 percent of total fatty acids on day 1 day post-hatch. The combined levels of palmitic and stearic acids were only 10 percent of total fatty acids in cholesterol ester. There were no apparent changes in the proportions of these acids following hatching.

As in the case of cholesterol ester, oleic acid was the major fatty acid present in the triglyceride lipid fraction accounting for about 45 percent of total fatty acids present on day 1 post-hatch, followed by palmitic, linoleic and stearic acids. The fatty acid composition remained fairly constant following hatching and there were no apparent changes due to dietary treatments.

The distribution of fatty acids in the phospholipid and free fatty acid fractions are shown in Table 4.9 and 4.10, respectively. As can be seen their composition differed markedly from those of cholesterol ester and triglyceride fractions. Although oleic was still the major fatty acid, both fractions also contained considerable proportions of palmitic, stearic and linoleic compared to levels present in the triglyceride and cholesterol ester fractions. The levels of palmitic, stearic and linoleic acids were 27, 18 and 16 in the phospholipid fraction and 14, 7 and 23 percent in the free fatty acid fraction on day 1 post-hatch. Both fractions also contained about 5 percent of arachidonic acid. A slight decrease of oleic acid and increases in stearic and arachidonic acids with post-hatch age were observed in both

Table 4.7: The effect of age and dietary fat sources on fatty acid composition (major long chain fatty acids, weight percentage of total present) of cholesterol ester lipid fraction in the yolk sac materials

		age (days)				
		1	3	6	9	12
fatty acid:	Diet					
16:0*	1	4.9 ± 0.25	3.6 ± 0.15	4.7 ± 0.56	3.9a	4.5
	2	5.6 ± 0.37	3.0 ± 0.24	3.5 ± 0.26	4.1	1.2
	3	5.7 ± 0.55	3.2 ± 0.34	3.3 ± 0.78	1.6	1.1
16:1	1	1.4 ± 0.12	1.5 ± 0.32	1.4 ± 0.24	1.4	1.3
	2	1.3 ± 0.13	0.8 ± 0.04	1.1 ± 0.17	0.7	0.9
	3	1.8 ± 0.42	1.4 ± 0.28	1.6 ± 0.61	0.7	0.8
18:0	1	4.5 ± 0.19	4.7 ± 0.27	5.1 ± 0.34	4.8	5.1
	2	4.5 ± 0.94	3.4 ± 0.27	3.5 ± 0.39	3.0	2.9
	3	4.0 ± 0.42	3.3 ± 0.16	4.5 ± 0.75	3.9	3.5
18:1	1	75.2 ± 0.64	76.6 ± 0.20	78.4 ± 0.59	76.4	75.1
	2	73.7 ± 0.97	79.5 ± 1.07	80.0 ± 0.94	72.2	82.0
	3	74.7 ± 0.17	78.9 ± 0.60	77.1 ± 0.70	80.2	81.3
18:2	1	12.0 ± 0.90	10.3 ± 0.19	8.5 ± 0.14	10.7	10.9
	2	13.0 ± 0.88	11.7 ± 0.85	10.4 ± 0.66	11.6	10.2
	3	12.0 ± 0.18	11.5 ± 0.71	12.3 ± 0.49	11.7	11.5

* The common names of the fatty acids as per description in Table 3.1
a = only single samples were available for analyses

Table 4.8: The effect of age and dietary fat sources on fatty acid composition (major long chain fatty acids, weight percentage of total present) of triglyceride fraction of the residual yolk sac materials

		age (days)				
		1	3	6	9	12
fatty acid:	Diet					
16:0	1	24.7 ± 0.55	23.6 ± 1.14	24.3 ± 1.67	23.4	22.8
	2	26.2 ± 1.37	27.7 ± 0.78	25.5 ± 0.78	28.9	15.6
	3	25.3 ± 1.76	24.8 ± 1.43	25.3 ± 0.71	23.3	20.8
16:1	1	3.3 ± 0.60	2.2 ± 0.59	2.7 ± 0.32	2.7	2.5
	2	3.6 ± 0.40	2.8 ± 0.22	3.0 ± 0.41	3.1	3.2
	3	2.7 ± 0.79	2.3 ± 0.40	2.6 ± 0.66	0.8	0.8
18:0	1	7.0 ± 1.03	8.7 ± 1.16	11.1 ± 0.75	10.1	9.7
	2	4.9 ± 0.79	6.5 ± 0.62	7.0 ± 0.21	6.5	12.4
	3	5.2 ± 0.99	6.8 ± 0.13	6.4 ± 1.08	10.4	10.7
18:1	1	45.9 ± 1.24	46.1 ± 0.56	47.4 ± 1.61	44.1	44.6
	2	47.7 ± 1.03	44.6 ± 1.99	45.6 ± 0.73	42.7	53.3
	3	48.3 ± 1.36	46.0 ± 1.39	46.8 ± 0.28	45.5	48.8
18:2	1	16.9 ± 1.91	14.7 ± 0.46	14.0 ± 1.73	16.3	16.3
	2	16.4 ± 1.65	17.0 ± 0.48	17.1 ± 0.17	16.1	18.8
	3	16.5 ± 0.72	17.8 ± 1.17	16.5 ± 0.32	18.4	19.1

* The common names of the fatty acids as per description in Table 3.1
a = only single samples were available for analyses

Table 4.9: The effect of age and dietary fat sources on fatty acid composition (major long chain fatty acids, weight percentage of total present) of the free fatty acid fraction in the residual yolk sac materials

		age (days)		
		1	3	6
fatty acid:	diet			
16:0*	1	23.7 ± 0.60	22.8 ± 0.79	21.6 ± 2.04
	2	13.8 ± 0.57	15.2 ± 0.79	14.4 ± 0.99
	3	14.6 ± 0.87	15.5 ± 0.40	16.9 ± 0.59
18:0	1	6.6 ± 0.50	15.3 ± 0.43	18.0 ± 0.33
	2	4.8 ± 0.35	10.6 ± 0.74	16.0 ± 0.59
	3	7.0 ± 0.75	9.9 ± 0.18	12.6 ± 1.00
18:1	1	42.9 ± 1.52	37.1 ± 0.69	36.6 ± 1.11
	2	43.5 ± 1.38	38.0 ± 1.30	35.1 ± 1.89
	3	44.8 ± 1.66	37.4 ± 1.29	39.6 ± 1.87
18:2	1	23.0 ± 0.37	20.2 ± 0.58	11.7 ± 0.27
	2	24.7 ± 0.77	25.4 ± 0.35	19.7 ± 0.65
	3	23.4 ± 0.48	22.7 ± 0.65	21.7 ± 1.52

* The common names of the fatty acids as per description in Table 3.1
Diet names as per description in Table 4.1

Table 4.10: The effect of age and dietary fat sources on fatty acid composition (major long chain fatty acids, weight percentage of total present) of the phospholipid fraction in the residual yolk sac materials

		age (days)		
		1	3	6
fatty acid:	diet			
16:0*	1	27.2 ± 1.43	21.4 ± 0.28	25.5 ± 0.54
	2	29.0 ± 1.46	31.7 ± 1.81	31.0 ± 0.75
	3	27.5 ± 1.21	25.1 ± 0.13	25.1 ± 1.53
18:0	1	19.6 ± 0.78	25.3 ± 0.64	20.7 ± 1.12
	2	19.0 ± 0.51	20.2 ± 0.79	19.2 ± 2.69
	3	18.9 ± 1.07	21.2 ± 0.33	22.3 ± 0.83
18:1	1	26.9 ± 0.93	24.4 ± 1.30	29.0 ± 0.86
	2	28.8 ± 1.05	25.5 ± 1.14	25.0 ± 1.80
	3	28.8 ± 1.18	26.7 ± 0.67	27.5 ± 0.59
18:2	1	16.3 ± 0.46	21.2 ± 1.40	13.4 ± 1.06
	2	16.2 ± 0.49	17.0 ± 0.50	14.0 ± 0.82
	3	16.9 ± 0.41	16.1 ± 0.22	16.9 ± 0.54

* The common names of the fatty acids as per description in Table 3.1
Diet names as per description in Table 4.1

fractions regardless of diet, whereas, the other fatty acids remained unchanged throughout the experimental period.

4.3.3 Liver changes in weights, lipid and fatty acid compositions

Overall weight changes

Table 4.6 shows liver weights from chicks receiving the different dietary treatments over the 12 day post-hatch period. An overall increase in liver weight with age was observed in all dietary treatments. However, the rates of increase in liver weight varied with post-hatching age. Thus a maximum increase in liver weight was observed during the first 3 days post-hatch. Figure 4.3 shows the proportion of the liver weight to the chick's body weight. The liver formed less than 3 percent of the chick's body weight on day 1 post-hatch; reached the maximum value of more than 5 percent on day 6 in chicks receiving the Dalgety chick starter and the soyabean oil based diets but the proportion was slightly lower in chicks receiving the tallow oil based diet.

Lipid changes

Figure 4.4 shows the effect of age and dietary fats on the absolute amount of liver lipid in the chick. A gradual decrease in the level of liver lipid with age was observed in all the dietary treatments, although it was more drastic in chicks receiving both the Dalgety chick starter and soyabean oil based diets.

The distributions of the major lipid fractions in the liver are presented in Figures 4.5(i-vi). Significant effects of age and to a small extent dietary effects on liver lipid composition were observed during the experimental period.

Cholesterol ester was the major lipid component on day 1 post-hatch. Sharp decreases in the proportion of cholesterol ester with age were observed in all the dietary treatments from about over 75 percent on day 1 post-hatch to about 2 percent on day 12 post-hatch. There were no apparent dietary treatment effects.

The level of liver triglyceride on day 1 post-hatch was less than 4 percent of the total lipid. Unlike cholesterol ester increases in the proportion of triglyceride fraction with age were noted in all dietary treatments, but the rate of increase was different between the diets. By day 12 post-hatch triglyceride accounted for 40, 57 and 27 percent of the total liver lipid of chicks receiving the Dalgety chick starter diet, tallow and soyabean oils based diets, respectively.

The proportion of free fatty acid in the liver was less than 6 percent on day 1 post-hatch. Increases in free fatty acid levels were observed in chicks receiving both the Dalgety chick starter and soyabean oil based diets but no apparent changes were noted in chicks receiving the tallow oil based diet.

The phospholipid fraction accounted for about 9 percent of the total liver lipid on day 1 post-hatch. Increases in the proportions of phospholipid were observed in all the dietary treatments; levels obtained on day 12 post-hatch were 31, 28 and 44 percent in chicks receiving the Dalgety chick starter diet, tallow oil and soyabean oil based diets, respectively. Substantial amounts of free cholesterol were present on day 1 post-hatch (about 7 percent) of total lipid present and was unaffected by either age or diet. The liver lipids also contained less than 1 percent of partial glycerides on day 1 increasing to about 3 percent on day 12 post-hatch.

Figure 4.3: The effect of age and dietary fat sources on the proportion of liver to chick body weight

Figure 4.4: Total liver lipid content as influenced by post-hatching age and dietary fat sources

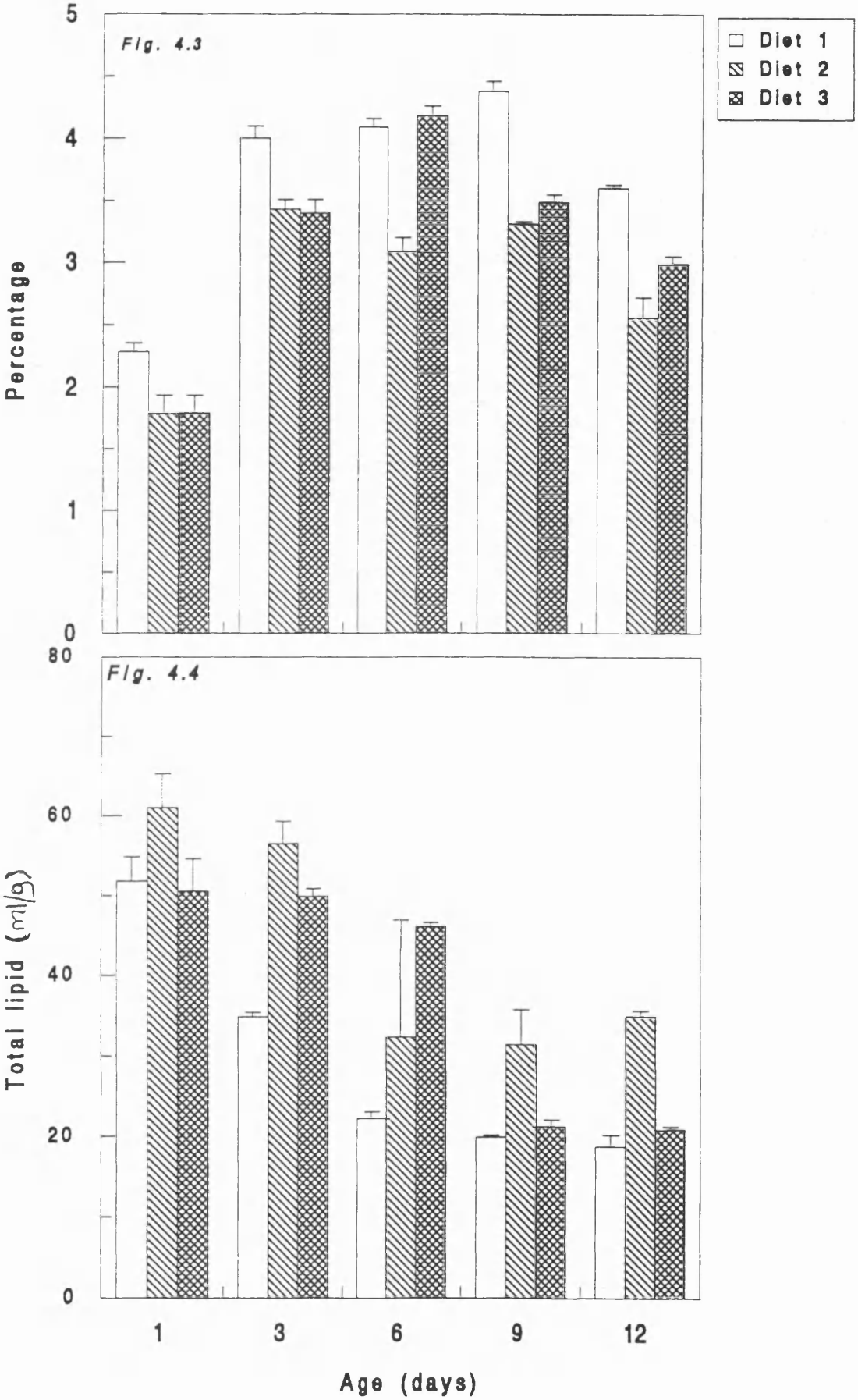
Fig. 4.3
Diet 1
Diet 2
Diet 3
Percentage
0
1
2
3
4
5
Fig. 4.4
Total lipid (mg/g)
0
20
40
60
80
1
3
6
9
12
Age (days)

Figure 4.5(i-vi): The effect of age and dietary fat sources on the distribution of the lipid fractions in the liver

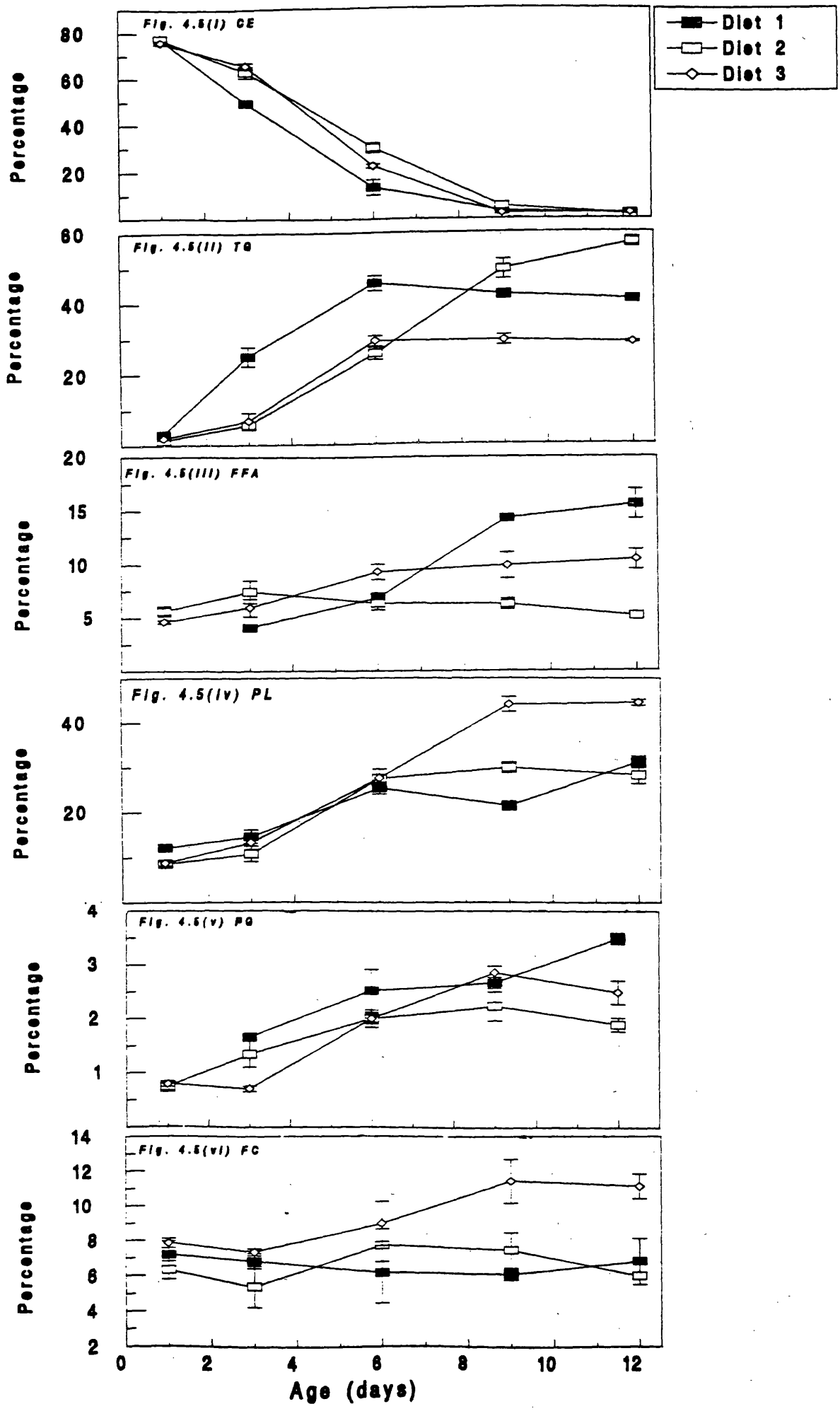
Fig. 4.5(I) CE
Fig. 4.5(II) TG
Fig. 4.5(III) FFA
Fig. 4.5(Iv) PL
Fig. 4.5(v) PG
Fig. 4.5(vI) FC
Diet 1
Diet 2
Diet 3
Percentage
Age (days)

Fatty acid changes

The major fatty acids of the liver cholesterol ester are presented in Table 4.11. Oleic acid formed more than 75 percent of the total fatty acids present in liver cholesterol ester on day 1 post-hatch, followed by linoleic acid which accounted for about 12 percent. An increase in the levels oleic acid was observed during the first 6 days post-hatch, reaching about 85 percent in chicks receiving the Dalgety chick starter diet and 90 percent in chicks receiving either the tallow or soyabean oil based diets. Levels of linoleic acid declined with post-hatching age in all dietary treatments. The levels of palmitic, palmitoleic, stearic, arachidonic and docosahexaenoic were low on day 1 post-hatch. Decreases in the levels of these acids were observed up to day 6 post-hatch. After day 6 post-hatch there were notable increases in palmitic, palmitoleic, stearic and linoleic acid levels at the expense of oleic acid levels in all dietary treatments.

The major fatty acids of the liver triglyceride lipid fraction are shown in Table 4.12. Oleic acid was the major fatty acid on day 1 post-hatch, but there were also substantial proportions of palmitic, stearic and linoleic acids. Slight fatty acid compositional changes characterised by increases in the proportion of palmitic acid and decreases in stearic, oleic and linoleic acids with post-hatch age were observed in all dietary treatments. Most of these changes occurred during the first 3 days post-hatch. The main features of the dietary effects following hatching were high levels of oleic and low levels linoleic acids in chicks receiving the tallow oil based diet and the low oleic and high linoleic acid levels in chicks receiving the Dalgety chick starter and soyabean oil based diets.

The distributions of the major fatty acids in the liver phospholipid fraction are presented in Tables 4.13 and 4.14. In comparison with the cholesterol ester and triglyceride lipid fractions, the main characteristic feature of the phospholipid fatty

Table 4.11: The effect of age and dietary fat sources on the fatty acid composition (major long chain fatty, weight percentage of total present) of liver cholesterol ester

		age (days)				
		1	3	6	9	12
Fatty acid:	Diet					
16:0*	1	2.5 ± 0.15	1.5 ± 0.04	2.4 ± 0.53	14.4 ± 1.23	19.8 ± 1.21
	2	2.3 ± 0.25	1.4 ± 0.04	1.3 ± 0.49	<0.1	12.8 ± 1.69
	3	2.1 ± 0.27	0.9 ± 0.16	1.1 ± 0.47	6.3 ± 0.18	8.9 ± 1.95
16:1	1	1.7 ± 0.07	1.8 ± 0.26	1.7 ± 0.35	3.7 ± 0.08	4.3 ± 0.77
	2	1.3 ± 0.13	1.4 ± 0.11	1.3 ± 0.25	<0.1	<0.1
	3	1.2 ± 0.17	1.2 ± 0.12	0.5 ± 0.02	<0.1	<0.1
18:0	1	3.3 ± 0.09	2.5 ± 0.14	1.8 ± 0.16	8.0 ± 1.46	12.1 ± 0.56
	2	2.8 ± 0.19	1.8 ± 0.11	1.0 ± 0.08	1.4 ± 0.49	8.9 ± 1.26
	3	3.1 ± 0.03	1.9 ± 0.17	0.8 ± 0.14	<0.1	3.6 ± 0.24
18:1	1	78.0 ± 1.10	83.2 ± 0.23	86.0 ± 0.46	62.7 ± 1.35	45.6 ± 1.48
	2	78.3 ± 0.69	83.1 ± 0.04	91.0 ± 0.50	93.5 ± 1.85	68.6 ± 1.08
	2	77.0 ± 0.37	84.3 ± 1.33	92.6 ± 0.47	78.8 ± 0.19	63.0 ± 1.06
18:2	1	11.4 ± 0.38	7.7 ± 0.34	2.8 ± 0.17	8.2 ± 0.78	14.7 ± 1.63
	2	11.6 ± 0.49	9.5 ± 0.20	3.8 ± 0.59	4.7 ± 0.03	10.5 ± 1.79
	3	12.8 ± 0.08	9.6 ± 0.59	4.5 ± 0.17	14.9 ± 1.11	24.2 ± 1.39

* The common names of the fatty acids as per description in Table 3.1

Table 4.12: The effect of age and dietary fat sources on the fatty acid composition (major long cahin fatty acid, weight percentage of total present) of liver triglycerides

		age (days)				
		1	3	6	9	12
fatty acid:	diet					
16:0*	1	21.6 ± 0.86	38.3 ± 0.78	33.8 ± 1.67	36.5 ± 1.21	37.4 ± 1.26
	2	28.1 ± 4.97	41.1 ± 0.55	34.0 ± 0.44	34.9 ± 1.12	32.0 ± 0.39
	3	22.2 ± 1.89	36.6 ± 1.39	34.4 ± 0.66	33.6 ± 1.65	31.7 ± 1.30
16:1	1	1.9 ± 0.09	8.6 ± 0.44	6.7 ± 0.70	4.5 ± 0.10	5.3 ± 0.91
	2	-	4.6 ± 1.02	4.9 ± 0.20	3.4 ± 0.13	3.5 ± 0.62
	3	0.9 ± 0.02	3.0 ± 0.50	4.6 ± 1.07	4.4 ± 0.19	4.2 ± 0.15
18:0	1	12.3 ± 0.16	8.5 ± 0.62	12.4 ± 0.67	16.9 ± 0.32	14.6 ± 1.75
	2	14.8 ± 1.02	12.2 ± 1.42	13.5 ± 0.19	13.5 ± 0.08	14.1 ± 1.02
	3	12.1 ± 1.21	9.4 ± 1.53	11.1 ± 0.45	11.3 ± 0.46	10.9 ± 1.08
18:1	1	44.6 ± 1.77	37.1 ± 0.48	38.2 ± 0.97	36.5 ± 0.85	37.1 ± 1.16
	2	43.4 ± 1.83	34.0 ± 0.45	41.5 ± 1.28	45.9 ± 1.57	47.1 ± 1.20
	3	47.6 ± 2.69	39.0 ± 1.48	41.1 ± 0.63	41.2 ± 0.84	43.8 ± 1.15
18:2	1	12.4 ± 0.61	6.3 ± 0.29	4.9 ± 0.12	3.7 ± 0.48	4.9 ± 0.47
	2	11.6 ± 1.10	7.0 ± 0.36	4.4 ± 0.36	3.4 ± 0.66	2.4 ± 0.26
	3	14.9 ± 0.20	11.2 ± 1.49	7.9 ± 0.37	8.3 ± 1.20	8.4 ± 0.81

* The common names of the fatty acids as per description in Table 3.1

Table 4.13: The effect of age and dietary fat sources on the fatty acid composition (major long chain fatty acids, weight percentage of total present) on liver phospholipids.

		Age (days)				
		1	3	6	9	12
16:0*	1	19.1 ± 0.34	23.4 ± 0.78	23.7 ± 1.20	23.0 ± 0.81	24.1 ± 0.84
	2	20.8 ± 2.24	21.2 ± 0.31	22.4 ± 0.82	21.2 ± 1.25	21.6 ± 0.62
	3	20.1 ± 0.41	19.7 ± 1.15	20.4 ± 0.35	18.6 ± 0.37	18.1 ± 1.00
16:1	1	0.1 ± 0.02	2.5 ± 0.26	2.8 ± 0.59	2.5 ± 0.21	2.4 ± 0.26
	2	-	1.0 ± 0.12	2.1 ± 0.21	1.4 ± 0.06	1.9 ± 0.25
	3	0.5 ± 0.01	0.7 ± 0.20	0.5 ± 0.13	0.4 ± 0.03	0.4 ± 0.03
18.0	1	25.5 ± 0.14	25.5 ± 0.72	25.8 ± 0.94	25.2 ± 0.80	26.6 ± 1.17
	2	31.1 ± 0.75	27.9 ± 0.76	24.8 ± 0.44	24.1 ± 1.02	25.0 ± 0.36
	3	30.2 ± 0.88	29.2 ± 0.60	25.5 ± 1.29	26.6 ± 0.99	27.7 ± 0.84
18:1	1	8.8 ± 0.61	14.7 ± 0.76	17.5 ± 0.60	18.0 ± 0.75	17.8 ± 1.00
	2	9.1 ± 0.16	15.2 ± 1.22	19.7 ± 1.55	22.9 ± 0.83	21.4 ± 1.62
	3	9.8 ± 1.72	10.1 ± 2.08	15.4 ± 0.52	15.8 ± 0.36	15.1 ± 0.30
18:2	1	13.8 ± 0.61	16.8 ± 0.93	19.1 ± 0.73	15.0 ± 1.89	17.9 ± 0.59
	2	14.7 ± 0.68	17.8 ± 0.75	16.6 ± 0.45	16.4 ± 0.15	15.9 ± 0.47
	3	14.3 ± 0.93	21.8 ± 1.07	21.7 ± 1.36	22.2 ± 0.62	22.4 ± 0.27

*The common names of fatty acids as per description in Table 3.1

Table 4.14: The effect of age and dietary fat sources on the percentages of arachidonic and decosahexaeonic acid in the liver free fatty acids and phospholipids

		Age (days)				
		1	3	6	9	12
	Diets					
Free fatty acids	1	<0.1	6.8 ± 1.00	3.2 ± 0.98	3.2 ± 0.62	3.0 ± 1.69
20:4*	2	16.8 ± 0.07	11.0 ± 0.46	8.2 ± 0.55	2.5 ± 0.24	2.3 ± 0.42
	3	17.2 ± 2.80	13.1 ± 0.72	4.3 ± 0.82	4.6 ± 0.91	3.5 ± 0.52
	1	<0.1	3.0 ± 2.21	2.4 ± 0.15	2.7 ± 0.44	2.8 ± 0.82
22:6	2	15.7 ± 0.97	7.1 ± 3.42	5.1 ± 1.15	2.9 ± 0.27	3.4 ± 0.65
	3	12.4 ± 1.09	4.9 ± 2.49	3.2 ± 0.82	2.8 ± 1.03	2.1 ± 0.08
Phospholipids						
	1	19.7 ± 0.51	9.3 ± 0.16	7.0 ± 0.46	7.7 ± 2.28	4.3 ± 0.37
20:4	2	16.2 ± 0.53	11.3 ± 0.60	6.7 ± 1.00	5.5 ± 0.12	5.2 ± 0.45
	3	16.3 ± 0.94	14.1 ± 0.73	8.1 ± 0.27	9.0 ± 0.50	9.3 ± 0.29
	1	9.3 ± 0.62	4.7 ± 1.90	3.7 ± 1.59	4.4 ± 1.52	5.5 ± 1.94
22:6	2	8.0 ± 0.69	4.6 ± 0.66	6.6 ± 2.38	6.4 ± 0.40	7.1 ± 0.69
	3	8.1 ± 1.12	2.9 ± 0.52	6.6 ± 2.79	5.4 ± 0.40	6.1 ± 0.46

*The common names of fatty acids as per description in Table 3.1

acid composition on day 1 post-hatch, were high levels of stearic, arachidonic and docosahexaenoic acids and lower levels of oleic and linoleic acids. Substantial changes in the fatty acid composition with post-hatching age under all dietary treatments were observed. In spite of a downward trend, the proportions of arachidonic and docosahexaenoic acids remained high compared to the proportions found in the triglyceride and cholesterol ester lipid fractions. There were increases in both oleic and linoleic acids with age; palmitic acid remained fairly constant throughout the experimental period. There were no apparent dietary effects on phospholipid fatty acid composition.

The major fatty acids of the liver free fatty acid fraction are presented in Tables 4.14 and 4.15. As in the case of the phospholipid fraction, high levels of arachidonic and docosahexaenoic were noted on day 1 post-hatch. On day 3 post-hatch the level of linoleic acid in chicks receiving the soyabean oil based diet was significantly higher (24 percent) compared to levels of about 15 percent observed in chicks receiving both the Dalgety chick starter and the tallow oil based diet; these differences were maintained throughout the experimental period. During the 12 day post-hatch period, substantial increases in palmitoleic, oleic and linoleic acids and decreases in stearic, arachidonic and docosahexaenoic acids were observed under all dietary treatments.

In general the major changes in fatty acid distributions in most of the liver lipid fractions resulting from either age or diet occurred between the first and sixth day after hatching.

Table 4.15: The effect of age and dietary fat sources on the fatty acid composition (major long fatty acids, weight percentage of total present) of liver free fatty acids

		Age (days)				
		1	3	6	9	12
fatty acid	Diet					
16:0*	1	-	22.6 ± 1.73	26.5 ± 0.81	27.1 ± 0.22	28.3 ± 1.29
	2	16.2 ± 2.28	20.2 ± 1.80	17.9 ± 1.29	17.5 ± 1.24	17.9 ± 1.67
	3	16.1 ± 1.33	15.4 ± 0.75	14.0 ± 0.10	14.0 ± 0.74	13.5 ± 2.10
16:1	1	-	10.0 ± 1.21	8.0 ± 0.85	5.9 ± 0.21	5.9 ± 0.89
	2	0.4 ± 0.10	3.5 ± 0.26	3.3 ± 1.70	5.1 ± 1.63	6.6 ± 0.67
	3	10.9 ± 0.52	3.4 ± 1.03	6.1 ± 2.02	4.4 ± 0.39	3.9 ± 1.30
18:0	1	-	9.4 ± 0.89	9.6 ± 0.14	11.4 ± 0.21	11.3 ± 0.66
	2	13.7 ± 0.62	11.6 ± 1.09	11.6 ± 1.87	11.0 ± 1.23	9.9 ± 1.18
	3	13.3 ± 0.33	13.9 ± 0.54	9.9 ± 0.83	10.4 ± 0.18	9.2 ± 2.11
18:1	1	-	32.5 ± 1.64	35.1 ± 1.30	36.0 ± 0.26	32.4 ± 1.83
	2	21.1 ± 1.12	29.2 ± 2.34	37.1 ± 2.69	46.6 ± 1.30	46.6 ± 3.56
	3	24.0 ± 2.17	24.7 ± 0.85	37.9 ± 0.79	38.2 ± 1.65	42.6 ± 1.61
18:2	1	-	15.3 ± 0.36	14.6 ± 0.80	12.1 ± 0.19	13.5 ± 0.88
	2	15.9 ± 0.47	15.8 ± 1.09	17.3 ± 1.51	13.8 ± 1.20	12.5 ± 0.25
	3	15.7 ± 1.03	24.1 ± 1.23	22.5 ± 2.34	23.5 ± 0.86	24.2 ± 0.74

*The common names of fatty acids as per description in Table 3.1

4.3.4 Gall bladder bile changes in lipid and fatty acid compositions

Lipid changes

Table 4.16 shows the proportions of the major lipid fractions in the gall bladder bile of chicks receiving diets containing different dietary fat sources. On day 1 post-hatch, the phospholipid fraction was by far the major lipid component but was also accompanied by substantial levels of free cholesterol. Low levels of other lipid components were present. Although in general terms there were high levels of phospholipid and free cholesterol throughout the 12 day period of the experiment, substantial dietary effects on the levels of the other lipid components were observed with associated changes to levels of both phospholipid and free cholesterol. The phospholipid fraction remained as the major lipid component after day 3 post-hatch in chicks receiving the tallow oil based diet, whereas, it was fluctuating in chicks receiving both Dalgety chick starter and the soyabean oil based diets. The low triglyceride proportions of less than 5 percent in chicks receiving the tallow and soyabean oil based diets after day 3 post-hatch were unexpected. In contrast significantly higher triglyceride levels of more than 30 percent were observed in chicks receiving the Dalgety chick starter diet. The level of free fatty acid was not affected by either age or dietary fat source.

Fatty acid changes

The major fatty acid distributions in the bile phospholipid are presented in Table 4.17. The major fatty acids in the phospholipid fraction of the gall bladder bile were palmitic, stearic, oleic, linoleic, arachidonic and docosahexaenoic. Following hatching there was a dramatic fall in the proportions of both arachidonic and docosahexaenoic acids and increases in oleic acids. Most of the fatty acids remained fairly constant during the experimental period, except for a drastic fall by about 50

Table 4.16: The effect of age and dietary fat sources on lipid composition (major fraction, weight percentage of total lipid present) in chick gall bladder bile

		age (days)				
		1*	3	6	9	12
lipid group:	diet					
cholesterol ester	1	-	23.2 ± 1.41	7.4 ± 1.45	1.7 ± 0.22	1.5 ± 0.67
	2	4.7 ± 1.02	61.3 ± 1.39	<0.1	6.5 ± 1.95	2.9 ± 0.12
	3	-	1.5 ± 0.50	<0.1	2.1 ± 0.34	<0.1
triglyceride	1	-	7.0 ± 1.17	23.1 ± 2.95	51.0 ± 2.07	47.2 ± 3.57
	2	1.8 ± 0.09	2.4 ± 0.56	1.0 ± 0.32	9.3 ± 1.31	3.0 ± 0.79
	3	-	0.8 ± 0.09	<0.1	1.5 ± 0.89	3.4 ± 0.62
free fatty acid	1	-	3.4 ± 0.32	5.0 ± 0.60	3.2 ± 0.45	3.3 ± 0.59
	2	1.0 ± 0.31	3.1 ± 0.54	4.5 ± 0.59	5.9 ± 0.78	2.5 ± 0.22
	3	-	3.5 ± 0.45	7.5 ± 0.89	6.5 ± 0.54	2.2 ± 0.12
phospholipid	1	-	39.0 ± 2.41	49.7 ± 1.50	31.0 ± 0.75	33.4 ± 1.36
	2	68.6 ± 1.32	25.7 ± 0.32	76.8 ± 1.74	58.5 ± 1.33	70.7 ± 1.47
	3	-	77.4 ± 1.67	44.2 ± 0.78	52.3 ± 1.98	78.8 ± 1.76
free cholesterol	1	-	28.5 ± 1.82	28.8 ± 1.25	12.9 ± 1.04	12.6 ± 1.60
	2	24.6 ± 1.52	7.5 ± 0.27	16.9 ± 1.33	19.8 ± 0.61	20.9 ± 1.23
	3	-	16.0 ± 1.89	47.9 ± 1.93	37.2 ± 1.76	15.2 ± 1.55

* Samples from all treatments were pooled together

Table 4.17: The effect of age and dietary fat sources on fatty acid composition (major long chain fatty acids, weight percentage of total present) in phospholipid of the chick gall bladder bile

		age (days)				
		1	3	6	9	12
fatty acid:	diet					
16:0*	1	23.1 ± 0.42	24.9 ± 0.67	25.9 ± 0.68	22.7 ± 0.61	28.4 ± 0.79
	2	-	23.4 ± 0.98	28.7 ± 0.71	31.8 ± 0.74	28.1 ± 1.10
	3	-	21.4 ± 1.78	25.2 ± 0.61	23.3 ± 0.56	27.5 ± 0.69
18:0	1	19.9 ± 0.27	22.2 ± 0.17	18.5 ± 0.33	19.7 ± 0.32	19.1 ± 1.14
	2	-	15.7 ± 0.98	19.0 ± 0.76	20.3 ± 0.83	19.5 ± 0.15
	3	-	21.4 ± 1.56	20.2 ± 0.73	19.6 ± 0.34	18.2 ± 0.45
18:1	1	7.4 ± 0.16	12.8 ± 0.62	18.7 ± 0.90	21.2 ± 0.56	18.0 ± 0.92
	2	-	26.6 ± 0.79	20.5 ± 0.99	23.1 ± 0.63	27.1 ± 0.71
	3	-	12.7 ± 0.32	20.2 ± 0.94	18.9 ± 0.61	18.2 ± 0.11
18:2	1	15.7 ± 0.02	18.5 ± 0.48	17.7 ± 0.73	17.6 ± 0.13	17.6 ± 0.67
	2	-	16.0 ± 0.87	16.8 ± 0.54	15.2 ± 0.59	16.8 ± 0.54
	3	-	22.6 ± 0.24	28.4 ± 0.43	25.4 ± 0.50	22.0 ± 0.91

* The common names of the fatty as per description in Table 3.1

percent in both arachidonic and docosahexaenoic and increases in oleic acid. There was no apparent effect of diet on the bile phospholipid fatty acid composition.

Concentrations of cholesterol ester, triglyceride and free fatty acid from chick bile were too low to enable any meaningful comparisons to be performed. However, in the case of cholesterol ester analysis of pooled samples from all three dietary treatments showed that oleic acid accounted for about 75 percent of the total fatty acids present. Between the first and sixth day post-hatch an increase in oleic acid was observed, but underwent a significant decrease thereafter.

4.4 DISCUSSION

The use of substantial incorporations of fat in poultry rations for all ages of stock is widely practised. Apart from being a source of energy, its role in providing a range of essential tissue components in particular the polyunsaturated fatty acids and fat soluble vitamins are well known (Ewing, 1963; Scott *et al.*, 1982; Brue and Latshaw, 1985; McDonald *et al.*, 1988; Phelps, 1989). The nutritional and to some extent the physical properties of dietary fat sources are influenced by the fatty acid composition of the major lipid fractions. Variations in the fatty acid composition and sometimes lipid composition between fat sources are not uncommon (Ketels and De Groote, 1987). The differences in fatty acid composition among the major fat sources and consequently in diets have varying effects on the performance of chickens. Hence due to the wide variations observed in lipid and fatty acid compositions in most of the dietary fat sources and the compounded diets, advance konowledge on the composition of the components is vital for the feed manufacturers and the animal producers (Whitehead and Fisher, 1975; Whitehead, 1981; Wiseman, 1984; Akinyemi, 1989).

The lipid compositions for the tallow and soyabean oils used in the present study were comparable to those used by others (Renner and Hill, 1960; Hakansson, 1974; Ketels and De Groote, 1987). However, all the dietary fat sources used contained high proportions of triglycerides. These high levels were anticipated since triglycerides form the major part within the total lipid content of many commercially important fats and oils of both plant and animal origin (Enser, 1984; Gurr, 1984; Noble, 1987; McDonald *et al.*, 1988). In contrast, the complete diets manufactured either on a small scale or large industrial scale displayed substantially high levels of free fatty acids. Similar observations were also reported by Akinyemi (1989). The likely source of such levels of free fatty acid in the diet is through hydrolysis of the feed during compounding, promoted by heat and contaminating constituents (Noble, 1984; Christie, 1989; Wiseman, 1990). The major total fatty acids of the dietary fat sources and the compounded diets obtained in the present study conformed to those expected from the constituents used. Thus, the soyabean oil based diet displayed a high level of linoleic acid whilst the tallow oil based diet contained high levels of palmitic and stearic acids (Enser, 1984; Gurr, 1984). The saturated:unsaturated fatty acid ratios in the complete diets also reflected the lipid composition of the major fat sources used. Compositional differences between the diets used were expected to result in differing performances in the chicks. Ketels and De Groote (1989) showed that about 75 percent of the variations observed in fat utilization and metabolizable energy by chickens was due to differences in the chemical composition of the fat fraction in the diet, as measured by the ratio between saturated and unsaturated fatty acids.

The body weight of chicks and the weight of the residual yolk sac materials on day 1 post-hatch was within the range reported by others (Romanoff, 1960; Noble and Ogunyemi, 1989; Daly and Peterson, 1990). Furthermore, the proportion

observed during the first 5 days post-hatch underlined the importance of the yolk lipids being the sources of energy during this transitional period. However, the increased levels of lipid within the remnant yolk material which was observed beyond day 5 post-hatch was unexpected although similar observations were also reported by Ogunyemi (1987). It is possible that this increase might have resulted from differential uptake of the major yolk components or due to lipid deposition from the exogenous sources into the yolk sac during the early post-hatch period (Romanoff, 1960). The other probable explanation could be that the onset of feeding by the chick reduces the role of the yolk as an energy and nutrient supplier.

The lipid composition of the yolk sac obtained in the present study were generally consistent with findings in other studies. The level of cholesterol esters presently observed in the yolk sac lipid on day 1 post-hatch was low compared to that reported by Noble and Ogunyemi (1989). The subsequent increase in the proportion of cholesterol esters after hatching agrees with the findings of (Romanoff, 1960; Noble and Moore, 1967; Noble and Ogunyemi, 1989). Romanoff (1960) attributed this to a slow absorption rate of cholesterol esters in comparison with other lipid fractions. Triglycerides formed the largest lipid fraction of the residual yolk sac materials on day 1 post-hatch but declined drastically thereafter. Although earlier investigations by Romanoff (1960) showed that the decline of triglycerides and other lipid constituents were not affected by diet, slight differences were observed in the present study between chicks receiving the different diets. The rate of triglyceride decline was slightly faster in chicks receiving the Dalgety chick starter and the soyabean oil based diets, both diets containing high levels of unsaturated fatty acids and least in chicks receiving the tallow oil based diet. The reason for this is unclear.

The insignificant changes in the fatty acid composition within the major lipid fractions of the residual yolk sac materials accord with findings reported by Noble and Ogunyemi (1989). This indicates that unlike lipid uptake there seems to be no preferential absorption of the fatty acids.

The precise mechanisms by which yolk lipids are distributed in the body during the early post-hatch days are not known. However, it has been suggested that the liver (Svanberg, 1971) and possibly the GIT (Esteban *et al.*, 1991) serves as the major routes of yolk lipid distribution. Several evidences are currently available to substantiate that the liver is the major site through which the yolk materials are distributed to other parts of the body during the early post-hatch period. The similarity in fatty acid compositions of the cholesterol ester lipid fraction between the yolk sac materials, the liver and the gall bladder bile on day 1 post-hatch, is an indication of an interrelationship which exists amongst these tissues (Svanberg, 1971; Noble and Ogunyemi, 1989). Entenman *et al.* (1940) showed that most of the cholesterol in the liver was derived from the yolk with the rate of accumulation being high during the last 2-3 days of incubation. The liver cholesterol during incubation and the early post-hatch period is thought to have an important role associated with lipid transport. The high level of cholesterol esters observed in the liver on day 1 post-hatch in the present study conformed to previous observations of liver lipid composition in the neonatal chick (Noble and Connor, 1984; Noble *et al.*, 1984; Noble and Ogunyemi, 1989). The rapid decline in the levels of cholesterol ester in the liver with post-hatching age observed presently was also reported by Svanberg (1971) and Noble and Ogunyemi (1989). Svanberg (1971) showed that, the concentrations of cholesterol ester in the liver were high between 12 hours and 6 days after hatching; levels declining gradually thereafter. It was also shown by Svanberg (1971) that the level of radioactivity associated with C14 radiolabelled

cholesterol ester in the liver was similar to that of other tissues in the body after 9 days, indicating that most of this lipid fraction previously derived from the yolk prior to hatching had already been distributed to other parts of the body or excreted. On the other hand, such changes in the levels of liver cholesterol ester during the early post-hatch period might have been a result of the changing metabolic role of the liver with respect to lipid metabolism after hatching; as the liver becomes the major site of fat synthesis (Leveille *et al.*, 1975; Annison, 1983; Hill, 1983; Noble and Ogunyemi, 1989). The decline of liver cholesterol ester concentrations was accompanied by differential increases in concentrations of triglycerides and phospholipids. Similar observations have been made by other workers (Entenman *et al.*, 1940; Noble and Ogunyemi, 1989). The decline of the total lipid content per unit weight of liver observed in the present study after day 6 post-hatch conforms with other reports (Entenman *et al.*, 1940; Noble and Ogunyemi, 1989).

The variations in liver lipid compositions as a result of dietary treatment obtained in the present study were to be expected since it is known that liver lipid composition is influenced among other factors by diet (Marion, 1965; Privetti, 1965; Sim *et al.*, 1973; Shapira *et al.*, 1978; Rogel and Watkins, 1987; Watkins, 1987). The present observations whereby triglyceride levels were lowest in chicks receiving the soyabean oil based diet and highest in chicks receiving the tallow oil based diet, accord to the findings of Klopfenstein and Clegg (1980) who showed that the level of liver triglycerides was higher in chicks fed a diet containing 5 percent palmitic acid and lower in chicks fed a diet containing 5 percent oleic acid. In contrast the amount of liver triglyceride increased when the dietary level of oleic acid was increased to 10 percent and decreased when the level of palmitic acid was increased to 10 percent. These differences were attributed to the positioning of the fatty acids during the synthesis of triglycerides by the liver, position *sn*-1 of the

glycerol being preferentially esterified with a saturated fatty acid whilst *sn*-2 was preferentially esterified with an unsaturated fatty acid. Hence, it is likely that at lower levels of dietary unsaturated fatty acids, triglyceride synthesis might be inhibited by the lack of requisite acids needed for the esterification at the *sn*-1 position, due to the efficient uptake of unsaturated fatty acids from the digestive tract (Klopfenstein and Clegg, 1980). The presence of higher levels of oleic acid within the diet may satisfy not only the metabolic requirements but also a wider role in triglyceride synthesis than are performed either by the saturated and polyunsaturated fatty acids. The higher phospholipid levels observed in the livers of chicks receiving the soyabean oil based diet conforms with previous findings of Giordani *et al.* (1988) in which it was shown that an increase in dietary unsaturated fatty acids led also to a decrease in triglycerides; the effects on liver lipid composition were reversed when saturated fatty acids were fed.

The distribution of the major fatty acids in the lipid fractions of the liver and the gall bladder bile, particularly within the cholesterol ester were similar to those previously observed in the yolk sac lipids (Noble and Ogunyemi, 1989). The presence of high oleic acid levels in cholesterol ester of these tissues was shown to be associated with the distribution of the yolk lipids during the early post-hatch days (Jain *et al.*, 1972; Noble, 1984). However, as shown in this study the fatty acid composition of the triglyceride and the phospholipid fractions of the liver and gall bladder bile differed extensively from those of the residual yolk sac lipid. Such differences would indicate that apart from the cholesterol ester which plays an important role in lipid transport, the sources of these two lipid fractions in the liver are independent. The changes in the fatty acid compositions of the major lipid fractions in the liver and the gall bladder bile with age conforms to previous findings (Entenman *et al.*, 1940; Noble and Connor, 1984; Noble *et al.*, 1988; Noble and

Ogunyemi, 1989). Most of the fatty acid compositional changes were observed during the first six days post-hatch conforming with the suggestion that the physiological changes connected with lipid metabolism in the young chick is particularly extensive during this time. Noble and Ogunyemi (1989) attributed such changes to the alterations in the role of the liver, from being mainly a depository organ during the embryonic period to one of synthesizing fat for both structural and functional purposes.

The variations in fatty acid composition of the liver and the gall bladder bile between the different dietary fat sources observed in the present study is in agreement with other reported work (Abraham, 1970; Sim *et al.*, 1973; Shapira *et al.*, 1978). Abraham (1970) pointed out that dietary fat differences had more effect on the distribution of the major fatty acids within the major liver lipid fractions than the total amount of lipid present. This underlines the specificity of the individual fatty acid requirements by the liver during the process of lipid synthesis. In the present study the tallow oil based diet resulted in the establishment of high levels of both oleic and palmitic acids within the liver after day 6 post-hatch. It has been suggested that this effect is probably the result of a combination of processes involving the elongation of dietary palmitic acid to form stearic acid followed by desaturation via a delta-9 desaturase in the liver giving rise to oleic acid and the direct desaturation of dietary stearic acid to oleic acid (Watkins, 1987). In accordance with observations in other animal species Sim *et al.* (1973) showed that chickens were not able to synthesize dienoic acids from non fat precursors or from saturated fatty acids derived from the diet. The decrease of linoleic acid in the liver lipid fractions with age in chicks that received the tallow oil based diet may have been a result of a specific mechanism aimed at conserving essential fatty acids (Guenter *et al.*, 1971). It has previously been shown that decreased levels of oleic

acid, slight increases in linoleic and stearic acids obtained when diets high in unsaturated fatty acids are fed may be due to homeostatic mechanisms in the liver which inhibit the de novo synthesis of oleic acid (Sim *et al.*, 1973). This mechanism appears to promote the maintenance of saturated fatty acids levels in order to conserve a specific ratio of unsaturated to saturated fatty acids under conditions of high polyunsaturated fatty acid ingestion. It has also been reported that inhibition of fatty acid synthesis is greater during ingestion of unsaturated triglyceride and lower when saturated triglyceride are ingested (Sim *et al.*, 1973). This led to the assumption that dietary linoleic acid was a major factor in the feedback of hepatic fatty acid synthesis of long chain monoenoic acids.

In the present investigation the fatty acid distribution of the liver phospholipids on day 1 post-hatch was characterised by high levels of polyunsaturated fatty acids in particular arachidonic acid. Noble and Moore (1967) showed that the phosphatidyl ethanolamine and phosphatidyl choline accounted for about 30 and 44 percent of the total liver phospholipids in the yolk on the last day of incubation. As the liver is a depository organ of the yolk lipids during the incubation period with deposition being particularly high over the last days of incubation, it is most probable that liver phospholipid fatty acid composition at hatching would be greatly influenced by this. Hence, since arachidonic and docosahexaenoic acids are high in both phospholipid classes, it may account for the high levels observed in the liver phospholipid at hatching. The declining influence of the yolk lipids on the liver lipid composition and other body tissues, in conjuction with the changing role of the liver after 6-9 days post-hatch as previously shown by Entenman *et al.* (1940), Svanberg (1971) and Noble and Ogunyemi (1989) was also accompanied by drastic changes in the fatty acid composition of the phospholipid fraction. These changes mainly characterised by a dramatic decline of arachidonic

acid and a more gradual decline of docosahexaenoic acid (Noble and Ogunyemi, 1989). The lower levels of both arachidonic and docosahexaenoic observed after one week post-hatch was a reflection of the fatty acid composition of the dominant phospholipid classes (Glen and Dam, 1965). Unlike the yolk, the liver phospholipid fraction at that stage contained about 30 percent phosphatidyl ethanolamine and about 40-50 percent phosphatidyl choline, whereby the latter in particular contained lower levels of arachidonic acid, thus influencing the fatty acid composition of the phospholipid fraction as a whole.

The concentration of cholesterol ester in the lipid fraction of the gall bladder bile of less than 5 percent obtained in the present study on day 1 post-hatch was unexpected; it was anticipated that large concentrations of this lipid fraction present in the liver would have been reflected in the gall bladder bile. Noble and Connor (1984) showed that the gall bladder bile of the chick embryo contained about 30 percent cholesterol ester on the nineteenth day of incubation. As a consequence it was anticipated that the chick might have emerged with substantial levels of cholesterol ester in the gall bladder bile. Svanberg (1971) showed that radiolabelled ^{14}C cholesterol first appeared in the gall bladder bile some 12 hours after injection when administered into the invaginated yolks of a 4 hour post hatch chick. Following this initial appearance the intensity of the radioactivity increased rapidly to reach comparable levels to that of the liver after 24 hours. The level of radioactivity in the gall bladder bile remained higher than all other tissues up to the ninth day of age. Under the present circumstances therefore it may be suggested that distinct differences may exist between the rates of cholesterol excretion into the bile by the embryo just prior to hatching and the post-hatch chick. Therefore it may have been possible that the distribution of the cholesterol from the liver to gall bladder bile was still in its initial stages when the samples were collected on the first day of

age, since high bile cholesterol esters levels were later observed on day 3 post-hatch in chicks receiving the Dalgety chick starter and the tallow oil based diets. The large quantities of phospholipid and free cholesterol in the gall bladder bile conformed to the levels obtained for the chick embryo (Noble and Connor, 1984). The triglyceride levels observed presently were in agreement with findings reported elsewhere (Cross *et al.*, 1987).

The similarity in fatty acid composition of the cholesterol esters in the yolk sac, liver and gall bladder bile up to day 6 post-hatch indicated that the liver was to some extent still intimately involved in the assimilation of the yolk reserves during the early post-hatch days. The high levels of saturated fatty acids within the phospholipid on day 1 post-hatch were similar to levels within the gall bladder bile of the chick embryo (Noble and Connor, 1984). Glen and Dam (1965) attributed the high levels of saturated fatty acids to the presence of very high levels of phosphatidyl choline, about 75-80 percent of the total phospholipid. Additionally in the present study both arachidonic and docosahexaenoic acids formed a major proportion of the fatty acids present in the phospholipid fraction; similar results were also reported by Noble and Connor (1984) with chick embryos. It was suggested that the high levels of arachidonic acid in the bile of the chick embryo were associated in particular, with increasing importance of polyunsaturated fatty acid in lipid metabolism during the incubation period. Thus the rapid decline in the levels of both arachidonic and docosahexaenoic acids during the early post-hatch days indicated further the changing role played by the liver and the gall bladder with respect to lipid metabolism. The results obtained in the present study for fatty acid composition of cholesterol esters in the gall bladder bile conform with already established patterns for the chick embryo (Noble and Connor, 1984). The distribution of the fatty acids within the phospholipid fraction of the gall bladder bile

observed after day 3 post-hatch were similar to previous observations (Noble *et al.*, 1988). Glen and Dam (1965) showed that the distribution of the fatty acids within the phospholipid fraction of the chicken bile was significantly affected by diet. The major differences noted in the present work involved higher levels of palmitic and oleic acids in the chicks receiving the tallow oil based diet. Glen and Dam (1965) obtained similar results when butter fat was added to the diet and observed low levels of oleic acid when increasing amounts of cotton seed and linseed oils were added. The underlying reason for this feature was equivocal. It can be speculated that the chick embryo and the newly hatched chick reacts rapidly to highly unsaturated diets by suitable adjustment of various metabolic parameters and tissue lipid composition.

CHAPTER 5

THE EFFECTS OF AGE AND DIETARY FAT SOURCES ON LIPID AND FATTY ACID COMPOSITIONS OF THE GASTROINTESTINAL TRACT

5.1 INTRODUCTION

The short life span of the broiler bird calls for sound management and feeding practices to start as soon as the chick is hatched (Gyles, 1989). In order to obtain rapid growth the newly hatched broiler chick is in most cases immediately introduced to a high energy feed, the fat content of which is normally very high (Ewing, 1963; Ferket, 1991). The presence of high levels of dietary fat coupled with large amounts of yolk lipid reserves at hatching (Noble and Ogunyemi, 1989) exerts enormous pressure on the physiological mechanisms of the young chick, in particular those associated with lipid metabolism. Furthermore the situation is complicated by the incomplete development of most of the enzyme and digestive systems (Krogdahl, 1985; Sell *et al.*, 1986; Akiba *et al.*, 1988). It has been widely reported that the development of most of the enzymic systems is influenced among other factors by the type of diet fed during the early post-hatch period (Escribano *et al.*, 1988; Sell *et al.*, 1991). Thus feeding improper diets during this period, especially those containing high fat levels may interfere with the normal growth and development of the chick. In view of these facts the present experiment was carried out to investigate the following:

(i) to establish the relationship between the yolk reserve lipids and the GIT lipids during the early post-hatch period

(ii) to study the changes in lipid and fatty acid compositions along the GIT and their implications on fat utilization by the young broiler chick

(iii) to assess the impact of different dietary fat sources on the overall performance of the broiler chick

5.2 MATERIALS AND METHODS

5.2.1 Treatments

As in experiment 1 there were three dietary treatments. They were designated diets 1, 2 and 3. The major fat components and basic ingredients were as in experiment 1. Lipid and fatty acid compositions were as listed in Tables 4.2-4.5.

5.2.2 Rearing of chicks

One hundred and ninety five Ross Breeder day old male broiler chicks obtained from the Scottish Agricultural College (Auchincruive) were randomly allocated to the three dietary treatments. The management practices and feeding programmes followed were similar to those described in Experiment 1: section 4.2.2. Additionally weighing and recording of all feed consumptions and residues were performed throughout the experimental period.

5.2.3 Sampling

Samples from the different sections of the GIT were obtained on day 1, 3, 6, 9 and 12 post-hatch. Following weighing, 12 chicks from each dietary treatment were randomly selected and killed by neck dislocation. Immediately after killing the GIT was revealed by laparatomy. The GIT was then carefully stretched and divided by excision into five parts namely the duodenum, jejunum, upper and lower ileum and the large intestine. The excised samples were temporarily stored at approximately $5^{o}C$ in a suitable sample bottle containing 0.85 percent saline solution. For each GIT section samples from three chicks were pooled; each dietary treatment was replicated four times.

The contents from each section of the GIT were individually squeezed using forceps into a preweighed round bottomed flask also maintained at 5°C. Efforts were made to avoid handling the samples by hand so as to prevent contamination and throughout the general working area was wiped clean using chloroform and covered with a clean tissue paper. The weight of the of the GIT contents were determined in each case.

5.2.4 Lipid extraction and analyses

Lipid moiety determinations and fatty acid analyses were performed using standard procedures as described in Chapter 3.0 Materials and Methods: sections 3.1-3.3.

5.2.5 Statistical analyses

All the data obtained were subjected to a one way analysis of variance using the Minitab Version of 1989.

5.3 RESULTS

5.3.1 Body weight changes

The mean body weights of chicks and statistical analyses are shown in Table 5.1. As in experiment 1, the differences in the initial hatching weight of the chicks were not significant. The average body weights of the chicks receiving the Dalgety chick starter diet were significantly higher than their counterparts throughout the experimental period whilst no significant differences were observed between chicks receiving the tallow and soyabean oils based diets. The relative weight gains of chicks over the whole experimental period were similar to those observed in experiment 1. The average feed consumed per gram weight gain was highest in chicks that received the tallow based diet.

Table 5.1: The effect of different dietary fat sources on body weight gain of chicks during the early post-hatch days

Diets	weight (gm) 1	2	3
age (days):			
1	38.3 ± 0.37	38.9 ± 0.35	39.0 ± 0.33
3	55.4 ± 0.66	51.5 ± 0.72	51.0 ± 0.65
6	96.4 ± 1.36	82.1 ± 1.15	80.2 ± 1.16
9	155.5 ± 2.85	130.3 ± 2.70	127.9 ± 2.18
12	317.5 ± 4.99	275.3 ± 4.87	277.5 ± 4.19
g feed/g wt gain	1.26	1.38	1.38
statistical analyses on weight gain	1 vs 2	1 vs 3	2 vs 3
1	N.S	N.S	N.S
3	**	**	N.S
6	***	***	N.S
9	***	***	N.S
12	**	**	N.S

N.S = not significant; *, ** and *** siginificant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively

5.3.2 Lipid changes

Total lipid changes

The proportions of the total lipid in the different sections of the GIT from day 1 to 12 post-hatch in chicks receiving the different dietary treatments are presented in Figures 5.2(i)-(iii) for chicks receiving the Dalgety chick starter, tallow oil and the soyabean oil based diets, respectively. The amount of lipid in the duodenum was more than 25 percent of the total GIT lipid throughout the experimental period under all dietary treatments. During the first 3 days post-hatch there was a substantial increase of the duodenal lipid content in chicks receiving the soyabean oil based diet; by comparison only a slight increase occurred in chicks receiving the tallow oil based diet, whilst no apparent change was observed in chicks receiving the Dalgety chick starter diet. The lowest level in the duodenal lipid content was observed on the ninth day post-hatch in all the dietary treatments. More than 75 percent of the total lipid of the GIT was found between the duodenum and the upper ileum the proportion remaining relatively constant whilst, the large intestine accounted for 10 and 15 percent of the total lipid. In overall terms all the three dietary groups showed a cyclic pattern of change in their duodenal lipid contents following hatching. This was characterised by increasing levels reaching maximum between days 3 and 6 post-hatch and declining on day 9 and increasing rapidly thereafter. Between days 1 and 3 post-hatch the changes in the duodenal lipid contents were more pronounced in chicks that received the soyabean oil based diet, whereas, between days 6 and 12 post-hatch these changes were more pronounced in chicks that received the Dalgety chick starter diet.

Figure 5.2(i-iii): The effect of age and dietary fat sources on total lipid content within the different sections of the GIT
D= duodenum; J= Jejunum; UI= Upper ileum;
LI= Lower ileum; Lt= Large intestine

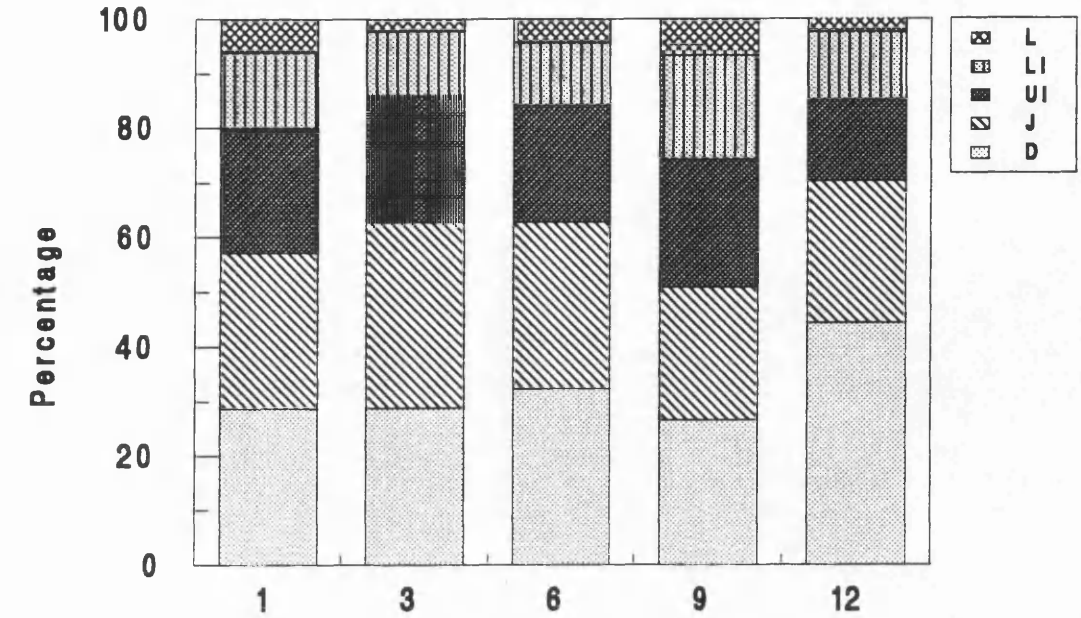

Fig. 5.2(I)

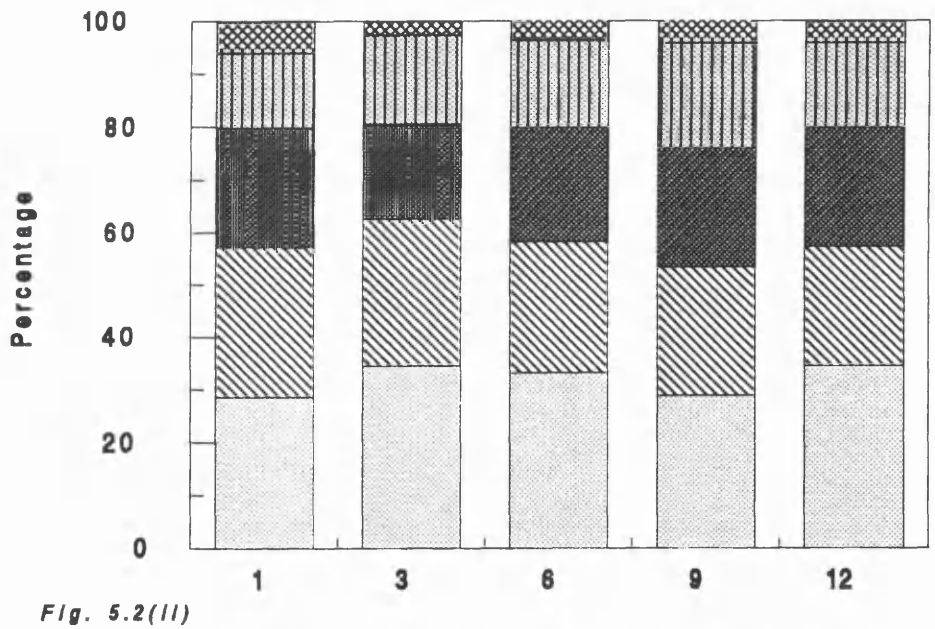

Fig. 5.2(II)

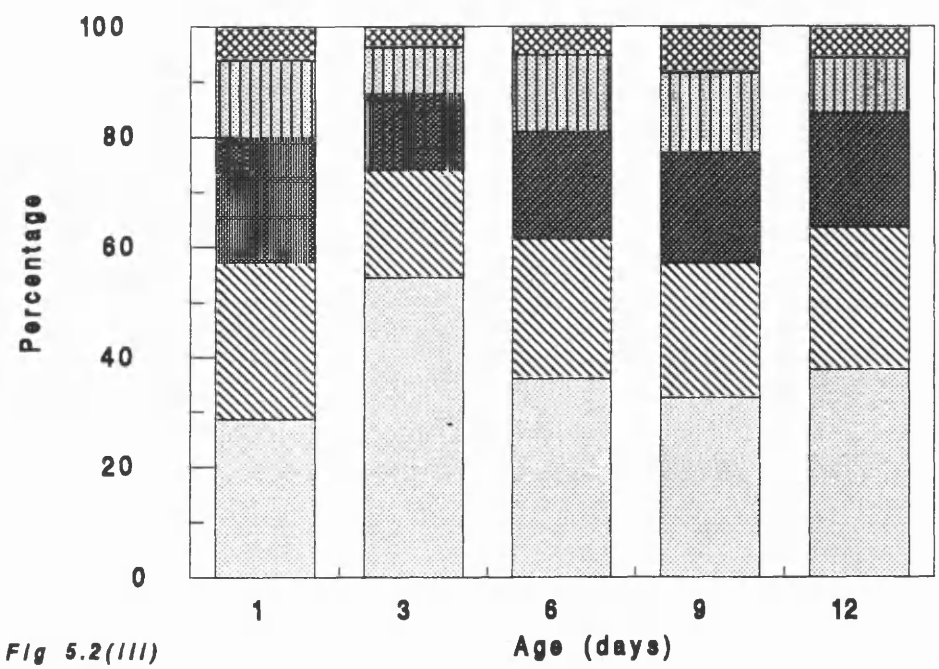

Fig 5.2(III)

Lipid moiety changes

(A) *GIT lipid compositions in chicks receiving the Dalgety chick starter diet*

The proportions of the major lipid moieties i.e cholesterol esters, triglycerides, free fatty acids, phospholipids and free cholesterol in the different sections of the GIT in chicks receiving the Dalgety chick starter diet during the 12 day post-hatch period are shown in Table 5.2(i). The statistical comparisons between the sequential sections of the GIT are shown in Table 5.2(ii) and 5.2(iii) shows the effects of chick age on the lipid compositions of the GIT.

Cholesterol ester

The increases in the level of cholesterol ester between the sequential sections of the GIT observed on days 1 and 3 post-hatch were not significant. However, on day 12 post-hatch, the increases in cholesterol ester levels between the duodenum and jejunum and between the jejunum and upper ileum were significant. Cholesterol ester levels in the large intestine were significantly higher than those found in the duodenum throughout the experimental period. Compared with day 1 post-hatch, the levels of cholesterol ester were significantly lower in almost all the GIT sections at day 3, but between days 3 and 12 post-hatch, significant differences were only observed within the duodenum and lower ileum.

Triglycerides

Levels of triglycerides showed a declining trend along the sequential sections of the GIT. The differences in the level of triglyceride between the sequential sections of the GIT over the experimental period were insignificant. The exception to this were between the duodenum and large intestine and between the

Table 5.2(i): Lipid compositions (major lipid fraction, weight percentage of total lipid present) within the different sections of the GIT in chicks receiving the Dalgety chick starter diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
lipid group:	age (days)					
cholesterol ester	1	7.2 ± 0.80	8.4 ± 0.70	9.0 ± 1.19	9.9 ± 1.40	14.5 ± 1.83
	3	1.7 ± 0.31	2.0 ± 0.18	3.0 ± 0.80	5.6 ± 1.81	8.6 ± 1.67
	12	0.7 ± 0.06	1.9 ± 0.43	4.7 ± 0.91	2.7 ± 0.20	4.3 ± 1.44
triglyceride	1	5.0 ± 0.96	9.2 ± 0.78	6.5 ± 0.13	6.1 ± 0.61	1.8 ± 0.04
	3	15.6 ± 0.59	17.5 ± 1.67	18.5 ± 1.06	17.1 ± 1.35	23.3 ± 0.87
	12	15.9 ± 1.98	24.8 ± 1.45	19.0 ± 0.45	18.9 ± 0.54	19.3 ± 1.43
free fatty acid	1	2.4 ± 0.16	5.2 ± 0.89	14.4 ± 1.80	10.8 ± 1.35	8.5 ± 0.22
acid	3	4.6 ± 0.78	7.3 ± 0.84	22.3 ± 1.71	33.2 ± 1.81	13.3 ± 1.14
	12	7.0 ± 1.07	31.1 ± 1.95	43.3 ± 0.91	39.6 ± 1.08	36.1 ± 1.31
phospholipid	1	65.2 ± 0.05	50.8 ± 1.40	45.3 ± 1.66	37.2 ± 1.13	43.6 ± 1.93
	3	65.3 ± 1.98	61.2 ± 3.31	34.4 ± 1.55	23.6 ± 0.67	29.6 ± 1.69
	12	63.9 ± 1.73	27.3 ± 1.39	13.6 ± 0.81	21.9 ± 1.66	25.5 ± 1.75
free cholesterol	1	16.0 ± 0.56	34.7 ± 1.89	24.5 ± 1.76	35.8 ± 1.45	28.1 ± 1.05
	3	12.8 ± 1.66	10.4 ± 2.08	16.2 ± 3.79	19.0 ± 3.38	33.2 ± 2.34
	12	10.7 ± 0.29	8.8 ± 2.04	13.8 ± 0.45	13.5 ± 1.32	20.1 ± 2.59

Table 5.2(ii): Statistical analyses for the lipid fractions in the different sections of the GIT from chicks receiving the Dalgety chick starter diet

		+D vs J	J vs Ui	Ui vs Li	Li vs Lt	D vs Lt
lipid group:	age					
cholesterol	1	N.S	N.S	N.S	N.S	*
ester	3	N.S	N.S	N.S	N.S	*
	12	***	*	N.S	N.S	*
triglycerides	1	N.S	N.S	N.S	***	*
	3	N.S	N.S	N.S	N.S	N.S
	12	N.S	N.S	N.S	N.S	N.S
free fatty	1	*	**	N.S	N.S	***
acids	3	N.S	*	*	**	*
	12	**	N.S	N.S	N.S	***
phospholipids	1	***	N.S	N.S	N.S	*
	3	N.S	***	**	N.S	**
	12	***	***	N.S	N.S	***
free	1	***	**	*	*	***
cholesterol	3	N.S	N.S	N.S	**	*
	12	N.S	N.S	N.S	N.S	*

N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively

+ D= doudenum, J= jejunum, Ui= upper ileum, Li= lower ileum, Lt= large intestine

Table 5.2(iii): Statistical analyses of age effects on lipid utilization along the GIT in chicks receiving the Dalgety chick starter diet

		+D	J	Ui	Li	Lt
lipid group:	age					
cholesterol ester	1 vs 3	***	***	**	N.S	*
	3 vs 12	*	N.S	N.S	*	N.S
	1 vs 12	***	***	*	**	*
triglycerides	1 vs 3	**	N.S	**	**	*
	3 vs 12	N.S	N.S	N.S	N.S	N.S
	1 vs 12	***	*	**	***	*
free fatty acids	1 vs 3	*	N.S	N.S	***	N.S
	3 vs 12	*	**	**	N.S	**
	1 vs 12	*	**	***	***	***
phospholipid	1 vs 3	N.S	*	N.S	***	N.S
	3 vs 12	N.S	*	*	N.S	N.S
	1 vs 12	N.S	**	**	***	*
free cholesterol	1 vs 3	N.S	***	N.S	*	N.S
	3 vs 12	N.S	N.S	N.S	N.S	*
	1 vs 12	***	***	**	*	N.S

N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively

+ = Full names as per description in Table 5.2(ii)

lower ileum and the large intestine at day 1 post-hatch. Significant increases in the levels of triglycerides within most sections of the GIT were observed between day 1 and 3 post-hatch and no further changes were observed thereafter.

Free fatty acids

Free fatty acid levels increased along the sequential sections of the GIT. This increase was significantly different only between the duodenum and upper ileum on day 1 post-hatch and between the sequential sections beyond the duodenum on day 3 post-hatch. On day 12 post-hatch the only significant differences observed were between the duodenum and jejunum and the jejunum and upper ileum. The levels of free fatty acids in the duodenum were significantly lower than those present in the large intestine throughout the experimental period. Increases in free fatty acid levels with age within the duodenum were observed. Beyond the duodenum the changes observed between days 1 and 3 post-hatch were limited to the large intestine, whilst after day 3 post-hatch significant increases in the levels of free fatty acids were observed within most sections of the GIT.

Phospholipid

As can be seen in Table 5.2(i) phospholipid comprised by far the major lipid component in the duodenal contents throughout the experimental period, accounting for about 60 percent of the total lipid present in the GIT. Phospholipid fraction was also a major lipid component remaining sections of the GIT although levels were lower than those present in the duodenum at day 1 post-hatch. Significant decreases in the levels of the phospholipid between the sequential sections of the GIT were observed in the upper sections of the GIT only. The level of phospholipid in the

large intestine was significantly lower than that present in the duodenum at all ages. The proportion of phospholipid in the duodenum remained unchanged but decreased with post-hatching age in the remaining sections of the GIT.

Free cholesterol

Free cholesterol accounted for about 15 percent of total lipid present in the duodenum at day 1 post-hatch and increased significantly between the sequential sections. This trend was not observed on day 3 and 12 post-hatch. The free cholesterol levels were significantly higher in the large intestine compared to the duodenum. A decline in the levels of free cholesterol with post-hatching age was observed within most sections of the GIT.

(B) *Comparison of lipid changes in the GIT between chicks receiving different diets*

The proportions of the major lipid moieties in the different sections of the GIT in chicks receiving the tallow and the soyabean oils based diets during the 12 day post-hatch period are shown in Tables 5.3 and 5.4, respectively. The statistical comparisons for the lipid changes on days 3 and 12 post-hatch in the different sections of the GIT between chicks receiving the different diets are shown in Table 5.5.

Cholesterol ester

There were no apparent differences in the levels of cholesterol ester within the GIT sections at both days 3 and 12 post-hatch between chicks receiving the different dietary treatments.

Table 5.3: The distribution of the major lipid fractions (weight of lipid, weight percentage of total lipid present) within the diffrent sections of the GIT in chicks receiving the tallow oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
lipid group:	age (days)					
cholesterol ester	1	7.2 ± 0.80	8.4 ± 0.70	9.0 ± 1.19	9.9 ± 1.40	14.5 ± 1.83
	3	1.4 ± 0.16	1.8 ± 0.21	2.5 ± 0.37	3.3 ± 0.17	5.2 ± 1.95
	12	0.8 ± 0.38	1.1 ± 0.21	1.9 ± 0.37	1.6 ± 0.39	4.7 ± 1.25
triglyceride	1	5.0 ± 0.96	8.2 ± 0.78	6.5 ± 0.13	6.1 ± 0.61	1.8 ± 0.04
	3	15.4 ± 2.40	23.8 ± 0.35	9.1 ± 0.85	5.4 ± 0.90	8.3 ± 0.82
	12	17.6 ± 0.48	14.1 ± 1.05	9.2 ± 1.28	6.5 ± 1.24	5.6 ± 0.91
free fatty acid	1	2.4 ± 0.16	5.2 ± 0.89	14.4 ± 1.80	10.8 ± 1.35	8.5 ± 0.22
	3	6.5 ± 0.50	27.0 ± 0.78	56.1 ± 2.17	59.2 ± 1.53	54.3 ± 1.48
	12	9.2 ± 1.25	53.8 ± 1.68	64.6 ± 2.43	70.1 ± 1.63	59.6 ± 1.80
phospholipid	1	65.2 ± 0.02	50.8 ± 1.40	45.3 ± 1.66	37.2 ± 2.13	43.6 ± 1.93
	3	64.3 ± 1.13	34.3 ± 1.66	19.1 ± 2.00	17.8 ± 1.67	9.6 ± 1.64
	12	60.3 ± 1.72	18.7 ± 1.15	10.5 ± 1.66	9.3 ± 0.93	9.6 ± 1.71
free cholesterol	1	16.0 ± 0.56	34.7 ± 1.89	24.5 ± 1.76	35.8 ± 1.45	28.1 ± 1.05
	3	10.9 ± 1.41	8.9 ± 0.23	15.2 ± 1.48	17.0 ± 1.65	24.9 ± 1.22
	12	10.9 ± 0.90	10.5 ± 0.94	12.4 ± 0.84	11.1 ± 0.52	17.5 ± 1.93

Table 5.4: The distribution of major lipid fractions (weight of lipid, weight percentage of total lipid present) within the different sections of the GIT in chicks receiving the soyabean oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
lipid group:	age (days)					
cholesterol ester	1	7.2 ± 0.80	8.4 + 0.70	9.0 + 1.19	9.9 + 1.40	14.5 + 1.83
	3	1.8 ± 0.50	5.5 + 0.38	2.6 + 0.24	<0.1	<0.1
	12	0.4 + 0.03	1.4 + 0.10	3.2 + 0.52	2.2 + 0.41	1.8 + 0.16
triglyceride	1	5.0 + 0.96	9.2 + 0.78	6.5 + 0.13	6.1 + 0.61	1.8 + 0.04
	3	17.8 + 1.65	22.3 + 1.03	18.6 + 1.09	18.2 + 1.11	18.8 + 1.12
	12	23.3 + 1.68	22.2 + 1.24	10.9 + 0.96	12.0 + 0.86	6.0 + 0.64
free fatty acid	1	2.4 + 0.16	5.2 + 0.89	14.4 + 1.80	10.8 + 1.35	8.5 + 0.22
	3	5.0 + 0.91	18.7 + 2.98	38.9 + 1.50	42.1 + 1.38	21.2 + 1.85
	12	7.9 + 0.22	37.5 + 1.03	63.9 + 2.56	57.4 + 1.71	66.6 + 2.17
phospholipid	1	65.5 + 0.22	50.8 + 1.40	45.3 + 1.66	37.2 + 2.13	43.6 + 1.93
	3	63.1 + 2.53	36.6 + 2.64	11.1 + 1.31	5.8 + 0.75	5.1 + 0.45
	12	57.1 + 1.87	25.3 + 0.68	8.6 + 0.30	9.1 + 0.42	7.3 + 1.08
free cholesterol	1	16.0 + 0.56	34.7 + 1.89	24.5 + 0.76	35.8 + 1.45	28.1 + 1.05
	3	14.1 + 0.80	16.6 + 0.62	22.3 + 1.60	30.6 + 1.36	53.9 + 1.60
	12	9.8 + 0.70	9.7 + 0.50	11.3 + 0.63	17.2 + 0.91	18.3 + 0.32

Table 5.5: Statistical comparisons of lipid utilization along the GIT between chicks receiving different dietary treatments

			D	J	Ui	Li	Lt
lipid group:	age (days)	diets					
triglycerides	3	1 vs 2	N.S	N.S	**	*	**
		1 vs 3	N.S	N.S	N.S	N.S	N.S
		2 vs 3	N.S	N.S	*	**	*
	12	1 vs 2	N.S	N.S	***	**	**
		1 vs 3	N.S	N.S	**	**	**
		2 vs 3	N.S	**	N.S	*	N.S
free fatty acids	3	1 vs 2	N.S	***	*	**	***
		1 vs 3	N.S	*	**	N.S	N.S
		2 vs 3	N.S	N.S	***	N.S	***
	12	1 vs 2	N.S	*	***	***	*
		1 vs 3	N.S	*	***	**	***
		2 vs 3	N.S	N.S	*	**	N.S
phospholipid	3	1 vs 2	N.S	**	*	N.S	*
		1 vs 3	N.S	*	*	***	**
		2 vs 3	N.S	N.S	*	**	N.S
	12	1 vs 2	N.S	N.S	N.S	**	**
		1 vs 3	N.S	N.S	**	***	***
		2 vs 3	N.S	N.S	N.S	N.S	N.S

N.S = not significant; *, ** and *** significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively

Diet names as per description in Table 4.1

+ = Full names as per description in Table 5.2(ii)

Triglycerides

The triglyceride levels within the upper ileum, lower ileum and the large intestine were significantly lower in chicks receiving the tallow oil based diet compared to those receiving either the Dalgety chick starter or soyabean oil based diets at day 3 post-hatch; but on day 12 post-hatch such differences were only observed between chicks receiving the tallow oil and soyabean oil based diets only.

Free fatty acids

As in the case of triglycerides the levels of free fatty acids within the duodenal contents were not affected by diet on both days 3 and 12 post-hatch. The free fatty acids levels in the remaining sections of the GIT were significantly higher in chicks receiving the tallow oil based diet compared to those of chicks receiving the Dalgety chick starter diet. Differences were also observed between chicks receiving the Dalgety chick starter and those receiving the soyabean oil based diet in all the sections of the GIT on day 12 post-hatch. The levels of free fatty acids within the different sections of the GIT at both days 3 and 12 post-hatch though insgnificant were higher in chicks receiving the tallow oil based diet compared to chicks receiving the soyabean oil based diet.

Phospholipid

The levels of phospholipid within the duodenum was not affected by the diet. However, in the remaining sections of the GIT, chicks receiving the Dalgety chick starter diet showed significantly higher levels at day 3 post-hatch than those receiving either the tallow or soyabean oil based diets. On day 12 post-hatch such a

trend was only confined to the lower ileum and the large intestine. With few exceptions the level of phospholipid in most sections of the GIT was lowest in chicks receiving the soyabean oil based diet.

Free cholesterol

The proportions of free cholesterol within the different sections of the GIT were not affected by the diet at both days 3 and 12 post-hatch.

5.3.3 Fatty acid composition and changes

Triglyceride

(A) *Fatty acid composition and changes in GIT contents from chicks receiving the Dalgety chick starter diet*

The fatty acid compositions of the triglyceride fraction in different sections of the GIT from chicks receiving the Dalgety chick starter diet are shown Table 5.6(i). The statistical analyses on fatty acid composition between the sequential sections of the GIT and the effects of post-hatching age on fatty acid compositions are presented in Tables 5.6(ii) and 5.6(iii), respectively.

Palmitic acid was by far the major component (30 percent) of the fatty acids present in the duodenum on day 1 post-hatch. A significant decline in the level of palmitic acid occurred between the duodenum and the jejunum, but increased between the remaining sections of the GIT on day 1 post-hatch. At days 3 and 12 post-hatch no significant changes were observed in the levels of palmitic acid between the GIT sections. However, the proportions of palmitic acid in the large intestine were consistently higher than levels found in the duodenum throughout the

Table 5.6(i): The fatty acid composition (major long chain fatty acids, weight perecentage of total present) of the triglyceride fraction of the GIT contents from chicks receiving the Dalgety chick starter diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	30.7 ± 0.92	25.7 ± 0.89	34.2 ± 0.92	37.1 ± 1.03	39.2 ± 1.25
	3	15.0 ± 0.50	17.3 ± 1.75	15.8 ± 1.01	17.3 ± 1.88	19.6 ± 0.61
	12	16.6 ± 1.54	16.8 ± 1.66	17.3 ± 1.43	16.6 ± 1.11	20.1 ± 1.11
16:1	1	2.7 ± 0.27	0.7 ± 0.10	3.2 ± 1.67	<0.1	<0.1
	3	1.9 ± 0.18	1.8 ± 0.08	1.6 ± 0.40	1.6 ± 0.20	1.5 ± 0.12
	12	1.9 ± 0.06	1.5 ± 0.01	1.8 ± 0.36	2.0 ± 0.42	0.4 ± 0.02
18:0	1	19.3 ± 0.85	15.4 ± 0.40	18.2 ± 1.21	14.8 ± 0.17	20.0 ± 0.89
	3	5.1 ± 0.19	5.1 ± 0.27	5.7 ± 0.72	4.4 ± 0.18	4.7 ± 0.30
	12	4.8 ± 0.03	3.8 ± 0.14	3.9 ± 0.41	4.9 ± 0.79	6.4 ± 0.34
18:1	1	23.2 ± 1.86	30.9 ± 0.59	23.6 ± 0.02	29.7 ± 0.11	29.8 ± 0.67
	3	19.3 ± 0.93	21.0 ± 0.66	22.0 ± 1.12	20.7 ± 0.46	18.8 ± 0.59
	12	19.8 ± 0.57	21.9 ± 0.25	22.1 ± 0.62	22.4 ± 0.49	20.2 ± 1.06
18:2	1	13.5 ± 0.52	15.3 ± 0.10	11.0 ± 0.62	15.2 ± 0.38	11.0 ± 0.73
	3	49.1 ± 1.13	34.9 ± 1.12	46.1 ± 0.91	49.7 ± 1.72	44.6 ± 1.15
	12	48.3 ± 0.42	49.0 ± 0.92	49.0 ± 1.19	49.2 ± 3.20	47.2 ± 1.46

* The common names of the fatty acids as per description in Table 3.1

Table 5.6(ii): Statistical analyses for the fatty acid compositions of the triglyceride lipid fraction in the GIT contents from chicks receiving the Dalgety chick starter diet

		+D vs J	J vs Ui	Ui vs Li	Li vs Lt	D vs Lt
fatty acid:	age					
16:0*	1	*	**	N.S	N.S	**
	3	N.S	N.S	N.S	N.S	**
	12	N.S	N.S	N.S	N.S	N.S
16:1	1	***	**	-	-	-
	3	N.S	N.S	N.S	N.S	N.S
	12	***	N.S	N.S	*	***
18:0	1	**	***	***	**	N.S
	3	N.S	N.S	N.S	N.S	N.S
	12	***	N.S	N.S	N.S	**
18:1	1	***	***	**	N.S	**
	3	N.S	N.S	N.S	N.S	N.S
	12	*	N.S	N.S	N.S	N.S
18:2	1	*	***	***	***	*
	3	*	N.S	N.S	*	*
	12	N.S	N.S	N.S	N.S	N.S

The common names of the fatty acids as per description in Table 3.1
N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively
+ = Full names as per description in Table 5.2(ii)

Table 5.6(iii): Statistical analyses of age effects on the fatty acid composition of the triglyceride lipid fraction in the GIT contents from chicks receiving the Dalgety chick starter diet

		+D	J	Ui	Li	Lt
fatty acid:	age					
16:0*	1 vs 3	***	**	***	***	***
	3 vs 12	N.S	N.S	N.S	N.S	N.S
	1 vs 12	***	**	***	**	***
16:1	1 vs 3	*	*	*	-	-
	3 vs 12	N.S	**	N.S	N.S	***
	1 vs 12	*	***	N.S	-	-
18:0	1 vs 3	***	***	***	***	***
	3 vs 12	N.S	**	N.S	N.S	**
	1 vs 12	***	***	***	***	**
18:1	1 vs 3	**	***	*	*	***
	3 vs 12	N.S	N.S	N.S	**	N.S
	1 vs 12	*	**	N.S	***	**
18:2	1 vs 3	***	***	***	***	**
	3 vs 12	N.S	*	N.S	N.S	N.S
	1 vs 12	***	***	***	***	***

* The common names of the fatty acids as per description in Table 3.1
N.S= not significant; *, **, and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively
+ = Full names as per description in Table 5.2(ii)

12 day post-hatch period. Furthermore, the proportion of palmitic acid observed on day 1 post-hatch were significantly higher than levels observed in all the GIT sections on days 3 and 12 post-hatch.

The proportion of palmitoleic acid in the duodenal contents was less than 3 percent on day 1 post-hatch. Significant increase in the level of palmitoleic acid between the jejunum and the upper ileum. The lower sections of the GIT contained only traces of this acid. On day 3 post-hatch there were no significant differences in the proportions of the palmitoleic acid between the sequential sections of the GIT. and changes observed on day 12 post-hatch showed no definitive trend.

Levels of stearic acid differed significantly between the sequential sections of the GIT on day 1 post-hatch, although no definitive trend was observed. Furthermore no differences in stearic levels were observed between the duodenum and the large intestine. On day 3 post-hatch no differences were observed in the proportions of stearic acid between the sequential sections of the GIT. A significant increase in the level of stearic acid between the duodenum and the large intestine was observed on day 12 post-hatch. The proportions of stearic acid observed on day 1 post-hatch along the entire GIT were significantly higher than those observed on days 3 and 12 post-hatch. The major reduction in the proportions of stearic acid in all sections of the GIT occurred between days 1 and 3 post-hatch. Subsequent changes between days 3 and 12 were relatively small.

Oleic acid accounted for about 23 percent of the total fatty acid present within the duodenum on day 1 post-hatch. Changes in the proportions of oleic acid observed between the sequential sections of the GIT on day 1 post-hatch did not reveal any clear pattern. However a significant increase in the proportion of oleic acid was observed between the duodenum and large intestine. The levels oleic acid between sequential sections of the GIT remained relatively constant at days 3 and 12

post-hatch. Significant decreases in the proportions of oleic acid were observed in all the GIT sections between days 1 and 3 post-hatch particularly within the jejunum and the large intestine. Between days 3 and 12 post-hatch there were some increases in the proportions of oleic acid within all the sections of the GIT.

Differences in the proportions of linoleic acid between the sequential sections of the GIT were observed on both days 1 and 3 post-hatch did not show any obvious pattern. The proportion of linoleic acid in the large intestine was significantly lower than the level present in the duodenum between days 1 and 3 post-hatch. On day 12 post-hatch levels of linoleic acid were constant along the GIT on day 12 post-hatch. Significant increases in the proportions of linoleic acid within the GIT sections occurred between days 1 and 3 post-hatch and no further changes were observed thereafter.

(B) *Comparison of the fatty acid changes of the triglyceride fraction in the GIT contents of chicks receiving the different diets*

The triglyceride fatty acid composition of the different sections of the GIT from chicks receiving the tallow and soyabean oils based diets are shown in Tables 5.7 and 5.8, respectively. The statistical comparisons of the fatty acid compositions within the triglyceride fraction in the contents obtained from different sections of the GIT between chicks receiving different diets are shown in Table 5.9.

The proportions of palmitic acid in all the GIT sections from chicks receiving the tallow oil based diet were significantly higher than in those receiving either the Dalgety chick starter or the soyabean oil based diets at days 3 and 12 post-hatch. There were no differences in the levels of palmitic within the GIT contents between chicks receiving the Dalgety chick starter and the soyabean oil based diets.

Table 5.7: The fatty acid composition (major long chain fatty acids, weight precentage of total present) of the triglyceride fraction of the GIT contents from chicks receiving the tallow oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid	age (days)					
16:0*	1	30.7 ± 0.92	25.7 ± 0.89	34.2 ± 0.92	37.1 ± 1.03	39.2 ± 1.25
	3	23.5 ± 1.18	23.7 ± 0.48	19.7 ± 0.16	22.6 ± 0.29	37.1 ± 0.60
	12	21.3 ± 0.53	21.7 ± 0.18	23.2 ± 1.15	22.3 ± 1.05	18.4 ± 1.01
16:1	1	2.7 ± 0.26	0.7 ± 0.10	3.2 ± 0.67	<0.1	<0.1
	3	3.4 ± 0.71	2.9 ± 0.40	2.5 ± 0.84	2.7 ± 0.82	<0.1
	12	4.1 ± 0.19	4.3 ± 0.11	4.4 ± 0.56	1.6 ± 0.24	<0.1
18:0	1	19.3 ± 0.85	15.4 ± 0.40	18.2 ± 1.21	14.8 ± 0.17	20.0 ± 0.89
	3	12.8 ± 0.87	10.1 ± 0.90	13.5 ± 0.48	14.7 ± 1.14	14.7 ± 1.46
	12	11.7 ± 0.34	13.6 ± 1.36	11.4 ± 1.14	13.2 ± 1.58	7.2 ± 0.04
18:1	1	23.2 ± 1.86	30.9 ± 0.59	23.8 ± 0.20	29.7 ± 0.11	29.8 ± 0.67
	3	28.9 ± 1.94	35.6 ± 1.22	29.3 ± 1.62	27.1 ± 0.84	24.2 ± 1.38
	12	30.2 ± 0.86	35.5 ± 0.90	36.7 ± 1.87	37.1 ± 0.27	28.5 ± 1.78
18:2	1	13.5 ± 1.52	15.3 ± 1.10	11.0 ± 1.62	15.2 ± 1.38	11.0 ± 0.73
	3	24.4 ± 0.95	22.8 ± 0.78	26.2 ± 2.73	31.2 ± 1.47	22.5 ± 1.55
	12	27.0 ± 0.31	20.7 ± 1.05	22.1 ± 1.41	31.6 ± 1.01	37.4 ± 1.52

* The common names of the fatty acids as per description in Table 3.1

Table 5.8: The fatty acid composition (major long chain fatty acids, weight percentage of total present) of the triglyceride fraction of the GIT contents from chicks receiving the soyabean oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	30.7 ± 0.92	25.7 ± 0.89	34.2 ± 0.92	37.1 ± 1.03	39.2 ± 1.25
	3	15.4 ± 0.43	16.3 ± 0.98	12.0 ± 1.30	12.6 ± 1.37	20.3 ± 1.21
	12	16.4 ± 1.71	10.0 ± 0.72	14.8 ± 1.08	16.5 ± 0.53	21.8 ± 1.78
16:1	1	2.7 ± 0.27	0.7 ± 0.10	3.2 ± 0.67	<0.1	<0.1
	3	1.0 ± 0.24	0.7 ± 0.01	<0.1	<0.1	<0.1
	12	1.9 ± 0.29	1.1 ± 0.29	0.2 ± 0.01	<0.1	<0.1
18:0	1	19.3 ± 0.85	15.5 ± 0.40	18.2 ± 1.21	14.7 ± 0.17	20.0 ± 0.89
	3	6.4 ± 0.44	4.7 ± 0.08	5.3 ± 0.54	4.7 ± 0.22	6.4 ± 0.35
	12	5.6 ± 0.26	5.4 ± 0.17	6.7 ± 0.83	4.7 ± 0.83	7.3 ± 1.51
18:1	1	23.2 ± 1.86	30.9 ± 0.59	23.6 ± 0.20	29.7 ± 0.11	29.8 ± 0.67
	3	21.5 ± 1.09	23.7 ± 0.25	25.0 ± 0.64	23.8 ± 1.18	20.6 ± 0.91
	12	22.3 ± 0.30	25.4 ± 0.29	28.6 ± 0.38	25.8 ± 0.66	27.5 ± 0.63
18:2	1	13.5 ± 1.52	15.3 ± 1.10	11.0 ± 1.62	15.2 ± 1.38	11.0 ± 0.73
	3	49.5 ± 0.33	48.9 ± 0.43	49.9 ± 1.26	53.0 ± 1.85	49.0 ± 1.98
	12	46.9 ± 1.22	46.3 ± 0.09	45.7 ± 1.51	47.5 ± 1.02	41.3 ± 1.22

* The common names of the fatty acids as per description in Table 3.1

Table 5.9: Statistical comparisons of the triglyceride fatty acid composition along the GIT between chicks receiving different dietary treatments

			+D	J	Ui	Li	Lt
fatty acid:	age (days	diets					
16:O*	3	1 vs 2	***	*	**	*	***
		1 vs 3	N.S	N.S	N.S	N.S	N.S
		2 vs 3	***	*	**	**	***
	12	1 vs 2	*	*	*	*	N.S
		1 vs 3	N.S	N.S	N.S	N.S	*
		2 vs 3	*	***	*	**	N.S
18:0	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	N.S	N.S	N.S	N.S
		2 vs 3	***	***	**	*	N.S
	12	1 vs 2	***	*	*	**	*
		1 vs 3	N.S	*	N.S	N.S	N.S
		2 vs 3	***	*	*	**	N.S
18:1	3	1 vs 2	**	***	**	***	*
		1 vs 3	N.S	**	N.S	*	N.S
		2 vs 3	*	**	*	N.S	N.S
	12	1 vs 2	**	***	*	***	N.S
		1 vs 3	N.S	***	***	**	**
		2 vs 3	***	***	*	***	N.S
18:2	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	*	N.S	N.S	N.S
		2 vs 3	***	***	**	***	**
	12	1 vs 2	***	***	***	**	**
		1 vs 3	N.S	N.S	N.S	*	*
		2 vs 3	**	***	**	*	N.S

* The common names of the fatty acids as per descrption in Table 3.1

N.S = not significant; *, ** and *** = significant at P<0.05, P<0.01 and P<0.001, respectively

+ = Full names as per description in Table 5.2(ii).

Diets names as per description in Table 4.1

The proportions of stearic acid in the GIT contents from chicks receiving the tallow oil based diet were significantly higher when compared to levels of chicks receiving either the Dalgety chick starter or the soyabean oil based diets on day 3 and 12 post-hatch. Similar to palmitic acid, the differences in the proportions of stearic acid between chicks receiving the tallow oil based diet and the other diets were larger on day 3 post-hatch than on day 12 post-hatch.

Compared with the Dalgety chick starter diet, the contents from all sections of the GIT in chicks receiving the tallow oil based diet contained significantly higher levels of oleic acid on both days 3 and 12 post-hatch. Differences in the proportions of oleic acid between chicks receiving either the Dalgety chick starter or the soyabean oil based diets were only observed on day 12 post-hatch. Between days 1 and 12 post-hatch increases in the proportions of oleic acid within all sections of the GIT were observed in chicks receiving the tallow or the soyabean oils based diets as opposed to decreases observed in chicks receiving the Dalgety chick starter diet.

Linoleic acid was the major acid in all the GIT sections from chicks receiving either the Dalgety chick starter or the soyabean oil based diets and levels were significantly higher throughout the entire GIT in comparison to those of chicks receiving the tallow oil based diet during the 12 day post-hatch period. Apart from the lower sections of the GIT, on day 12 post-hatch there were no differences in linoleic acid levels between chicks receiving the Dalgety chick starter and the soyabean oil based diets.

Free fatty acid

(A) *Fatty acid composition and changes in the GIT contents from chicks receiving the Dalgety chick starter diet*

The fatty acid composition of free fatty acid in the different sections of the GIT from chicks receiving the Dalgety chick starter diet are shown in Tables 5.10 and 5.13. The statistical analyses of the fatty acid compositions between the sequential sections of the GIT and the effects of post-hatching age on fatty acid compositions are presented in Tables 5.10(i) and 5.10(ii), respectively.

Palmitic acid was by far the major component (35 percent) of the total fatty acids present in the duodenum on day 1 post-hatch. Sequential significant decreases in the level of palmitic acid occurred up to the lower ileum. A significant increase in palmitic acid was observed between the lower ileum and the large intestine on day 1 post-hatch. At days 3 and 12 post-hatch the only differences in palmitic acid levels between the sequential sections of the GIT were observed between the lower ileum and the large intestine. The levels of palmitic acid were significantly higher in all the sections of the GIT on day 1 than on days 3 and 12 post-hatch.

The level of palmitoleic acid within the duodenal contents on day 1 post-hatch was 4 percent and did not alter significantly between the sequential sections of the GIT. This feature was also observed within the upper sections of the GIT on both days 3 and 12 post-hatch. Inconsistent but significant changes in palmitoleic acid levels were observed within the lower sections of the GIT. Significant decreases in the proportions of palmitoleic acid within sections of the GIT occurred between days 1 and 3 whilst between days 3 and 12 post-hatch such differences only occurred within the lower sections of the GIT.

Table 5.10(i): The fatty acid composition (major long chain fatty acids, weight percentage of total present) of the free fatty acid fraction of the GIT contents from chicks receiving the Dalgety chick starter diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age					
16:0*	1	35.1 ± 0.84	31.8 ± 0.89	27.6 ± 0.91	23.9 ± 0.41	41.5 ± 1.05
	3	12.2 ± 0.38	11.7 ± 0.35	12.4 ± 0.87	14.2 ± 0.81	25.4 ± 0.47
	12	12.4 ± 0.27	13.6 ± 0.69	15.1 ± 0.21	15.4 ± 0.26	19.7 ± 0.12
16:1	1	4.0 ± 0.11	3.5 ± 0.51	3.0 ± 0.56	4.7 ± 0.54	3.0 ± 0.89
	3	1.8 ± 0.18	1.4 ± 0.17	1.6 ± 0.17	1.5 ± 0.13	3.5 ± 0.67
	12	1.4 ± 0.06	1.4 ± 0.08	1.1 ± 0.06	1.3 ± 0.24	0.4 ± 0.01
18:0	1	28.0 ± 0.42	36.9 ± 0.98	21.8 ± 0.91	14.2 ± 0.90	23.3 ± 0.34
	3	7.7 ± 0.63	4.8 ± 0.14	4.8 ± 0.62	6.4 ± 0.47	12.6 ± 0.81
	12	6.0 ± 0.12	4.2 ± 0.25	5.3 ± 0.32	5.6 ± 0.92	14.8 ± 0.82
18:1	1	10.6 ± 0.25	9.0 ± 0.48	10.8 ± 0.50	20.5 ± 0.30	21.0 ± 0.67
	3	17.9 ± 0.23	17.7 ± 0.55	19.6 ± 0.41	20.3 ± 0.45	23.7 ± 0.88
	12	12.9 ± 0.60	20.0 ± 0.33	21.2 ± 0.87	21.0 ± 1.00	24.1 ± 0.57
18:2	1	6.9 ± 0.78	8.3 ± 0.86	12.0 ± 0.65	11.7 ± 0.65	5.1 ± 0.54
	3	48.4 ± 0.95	53.4 ± 0.65	51.0 ± 0.29	46.0 ± 0.17	30.3 ± 1.31
	12	52.3 ± 0.18	50.9 ± 0.79	48.6 ± 0.69	44.3 ± 0.87	38.4 ± 1.70
18:3	3	4.8 ± 0.24	6.3 ± 0.96	5.1 ± 0.16	4.8 ± 0.20	2.9 ± 0.42
	12	5.7 ± 0.79	5.2 ± 0.23	5.1 ± 0.91	5.2 ± 0.67	2.9 ± 0.56

* The common names of the fatty acids as per description in Table 3.1

Table 5.10(ii): Statistical analyses for the fatty acid composition of the free fatty acid fraction along the GIT in chicks receiving the Dalgety chick starter diet

		+D vs J	J vs Ui	Ui vs Li	Li vs Lt	D vs Lt
fatty acid:	age (days)					
16:0*	1	*	*	**	***	**
	3	N.S	N.S	N.S	***	***
	12	N.S	N.S	N.S	***	***
16:1	1	N.S	N.S	N.S	N.S	N.S
	3	N.S	N.S	N.S	*	*
	12	N.S	*	N.S	**	***
18:0	1	***	***	***	***	***
	3	**	N.S	N.S	***	***
	12	***	*	N.S	***	***
18:1	1	*	*	***	N.S	***
	3	N.S	*	N.S	*	***
	12	***	N.S	N.S	*	***
18:2	1	N.S	*	N.S	***	N.S
	3	**	*	***	***	***
	12	N.S	N.S	**	*	***
18:3	3	N.S	N.S	N.S	**	**
	12	N.S	N.S	N.S	**	**
20:4	1	*	*	*	***	***
	3	N.S	N.S	N.S	N.S	-

* The common names of the fatty acids as per description in Table 3.1
N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.001$ and $P<0.001$ respectively
+ = Full names as per description in Table 5.2(ii)

Table 5.10(iii): Statistical analyses of age effects on the fatty acid composition of the free fatty acid fraction along the GIT in chicks receiving the Dalgety chick starter diet

		+D	J	Ui	Li	Lt
fatty acid:	age (days)					
16:0*	1 vs 3	***	***	***	***	***
	3 vs 12	N.S	*	*	N.S	***
	1 vs 12	***	***	***	***	***
16:1	1 vs 3	***	**	*	***	N.S
	3 vs 12	N.S	N.S	**	N.S	***
	1 vs 12	***	**	**	***	***
18:0	1 vs 3	***	***	***	***	***
	3 vs 12	*	N.S	N.S	N.S	N.S
	1 vs 12	***	***	***	***	***
18:1	1 vs 3	***	***	***	N.S	*
	3 vs 12	***	*	N.S	N.S	N.S
	1 vs 12	*	***	***	N.S	**
18:2	1 vs 3	***	***	***	***	***
	3 vs 12	**	*	*	N.S	*
	1 vs 12	***	***	***	***	***
18:3	3 vs 12	N.S	N.S	N.S	N.S	N.S
20:4	1 vs 3	***	***	***	***	-
	3 vs 12	***	-	***	-	-
	1 vs 12	***	-	***	-	-
22:6	1 vs 3	***	N.S	N.S	N.S	-
	3 vs 12	N.S	N.S	N.S	N.S	-
	1 vs 12	*	*	*	N.S	-

* The common names of the fatty acids as per description in Table 3.1

N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively

+ = Full names as per description in Table 5.2(ii)

Stearic acid accounted for about 28 percent of the total fatty acid present within the duodenum at day 1 post-hatch. Significant increases in the levels of stearic acid were observed between the duodenum and the jejunum and between the lower ileum and large intestine. In contrast significant decreases were observed between the jejunum and upper ileum and between the upper ileum and lower ileum. On days 3 and 12 post-hatch there were significant decreases in the proportions of stearic acid between the duodenum and jejunum; whilst increases occurred between the remaining sequential sections of the GIT. The level of stearic acid declined significantly within all sections of the GIT between days 1 and 3 and only with the duodenum after day 3 post-hatch.

Oleic acid accounted for only 10 percent of the total fatty acids present within the duodenum at day 1 post-hatch. Whereas, a reduction was observed between the duodenum and the upper ileum at day 1 post-hatch, there were increases in the levels of oleic acid between the remaining sequential sections of the GIT. This latter feature was also observed during days 3 and 12 post-hatch. A significant increase in the level of oleic acid between days 1 and 3 post-hatch was observed within all sections of the GIT other than the lower ileum. Between days 3 and 12 post-hatch increases in the levels of oleic acid were only observed within the duodenum and the upper ileum.

Linoleic acid accounted for only 7 percent of the total fatty acids present within the duodenum at day 1 post-hatch. Differences in the levels of linoleic acid between the sequential sections of the GIT on day 1 post-hatch were not consistent. By comparison at both days 3 and 12 post-hatch an overall decline in the levels of linoleic acid along the GIT was observed. The levels of linoleic acid within all the sections of the GIT increased significantly between days 1 and 3 post-hatch, whilst decreases were observed within most sections after day 3 post-hatch.

Levels of linolenic acid were negligible within all sections of the GIT on day 1 post-hatch but increased to about 4-6 percent of the total fatty acids present on day 3 and 12 post-hatch. The level of linolenic acid remained relatively constant between the sequential sections of the GIT, the exception being a significant decline observed between the lower ileum and the large intestine.

Arachidonic acid accounted for about 15 percent of the total fatty acids present in the duodenum on day 1 post-hatch. There was no discernable pattern in the differences of arachidonic acid levels between the sequential sections of the GIT. The levels of arachidonic acid within the GIT showed significant reductions between days 1 and 3 and between days 3 and 12 post-hatch.

Low levels only of docosahexaenoic were present within the GIT throughout the 12 day post-hatch period.

(B) *Comparison of the fatty acid and composition changes in free fatty acid fraction in GIT contents between chicks receiving different diets*

The fatty acid compositions of the free fatty acid fraction within different sections of the GIT from chicks receiving the tallow oil and the soyabean oil based diets are shown in Tables 5.11 & 5.13 and Tables 5.12 & 5.13, respectively. The statistical comparisons for the fatty acid composition of the free fatty acid fraction in the different sections of the GIT between chicks receiving different diets at days 3 and 12 post-hatch are shown in Table 5.14.

The palmitic acid levels within the GIT contents from chicks receiving the tallow oil based diet were significantly higher in chicks receiving either the Dalgety chick starter or the soyabean oil based diets at both days 3 and 12 post-hatch. Similar but less dramatic differences were also observed between chicks receiving the Dalgety chick starter and the soyabean oil based diet on days 3 and 12 within

Table 5.11: The fatty acid composition (major long chain fatty acids, weight percentage of total present) of the free fatty acid fraction in the GIT contents from chicks receiving the tallow oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	35.1 ± 0.84	31.9 ± 0.99	27.6 ± 0.91	23.9 ± 0.41	41.5 ± 1.05
	3	22.1 ± 0.26	20.0 ± 0.43	25.0 ± 0.72	29.9 ± 0.45	38.7 ± 0.42
	12	20.4 ± 0.97	26.2 ± 0.36	29.2 ± 0.54	30.8 ± 0.54	27.7 ± 0.87
16:1	1	4.0 ± 0.11	3.5 ± 0.51	3.0 ± 0.56	4.7 ± 0.54	3.0 ± 0.89
	3	2.0 ± 0.54	3.4 ± 0.24	2.0 ± 0.42	1.9 ± 0.12	2.1 ± 0.23
	12	2.8 ± 0.36	3.3 ± 0.06	2.6 ± 0.48	2.7 ± 0.34	3.1 ± 0.45
18:0	1	28.0 ± 0.42	36.2 ± 0.98	21.8 ± 0.91	14.2 ± 0.90	23.3 ± 0.34
	3	14.0 ± 0.73	14.9 ± 0.57	23.9 ± 0.39	30.6 ± 0.93	32.9 ± 1.25
	12	15.3 ± 0.74	21.5 ± 0.48	23.2 ± 0.89	23.3 ± 0.87	18.6 ± 0.81
18:1	1	10.6 ± 0.25	9.0 ± 0.48	10.8 ± 0.50	20.5 ± 0.30	21.0 ± 0.67
	3	24.0 ± 1.01	31.8 ± 0.70	22.0 ± 0.64	21.2 ± 0.86	20.4 ± 0.37
	12	27.4 ± 0.43	28.0 ± 0.35	27.0 ± 0.96	29.4 ± 0.94	32.3 ± 1.30
18:2	1	6.9 ± 0.78	8.3 ± 0.86	12.0 ± 0.18	11.7 ± 0.65	5.1 ± 0.54
	3	29.0 ± 0.61	25.6 ± 1.17	20.1 ± 1.18	12.8 ± 0.53	3.5 ± 0.78
	12	27.8 ± 0.81	15.3 ± 0.31	14.4 ± 0.51	10.8 ± 0.48	14.1 ± 1.37
18:3	3	4.0 ± 0.62	1.6 ± 0.16	1.7 ± 0.34	0.88 ± 0.24	1.6 ± 0.46
	12	3.6 ± 0.14	5.8 ± 0.45	2.4 ± 0.12	<0.1	4.1 ± 0.67

* The common names of the fatty acids as per description in Table 3.1

Table 5.12: The fatty acid compositions (major long chain fatty acids, weight percentage of total present) of the free fatty acid fraction in the GIT contents from chicks receiving soyabean oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	35.1 ± 0.84	31.8 + 0.99	27.6 + 0.91	23.9 + 0.41	41.5 + 1.05
	3	12.3 ± 0.46	19.1 + 0.20	16.8 + 0.86	23.5 + 0.97	26.0 + 0.95
	12	13.6 ± 0.62	17.4 + 0.42	16.3 + 0.78	23.6 + 2.73	20.0 + 1.01
16:1	1	4.0 ± 0.11	3.5 ± 0.51	3.0 ± 0.56	4.7 ± 0.54	3.0 ± 0.89
	3	0.2 ± 0.01	<0.1	<0.1	<0.1	<0.1
	12	0.9 ± 0.08	1.0 ± 0.06	0.9 ± 0.06	0.3 ± 0.02	0.6 ± 0.04
18:0	1	28.0 ± 0.42	36.2 ± 0.51	21.8 ± 0.91	14.2 ± 0.90	23.2 ± 0.34
	3	10.1 ± 0.17	8.7 ± 0.65	8.7 ± 0.49	13.1 ± 0.62	22.5 ± 0.87
	12	7.9 ± 0.44	6.9 ± 0.28	7.4 ± 0.37	8.0 ± 0.29	9.0 ± 0.35
18:1	1	10.6 ± 0.25	9.0 ± 0.48	10.8 ± 0.50	20.5 ± 0.30	21.0 ± 0.67
	3	21.6 ± 0.52	26.9 ± 0.88	24.6 ± 0.43	27.4 ± 0.42	24.8 ± 0.28
	12	22.1 ± 0.28	26.2 ± 0.36	26.5 ± 0.75	27.5 ± 1.01	28.9 ± 0.76
18:2	1	6.9 ± 0.78	8.3 ± 0.86	12.0 ± 0.18	11.7 ± 0.65	5.1 ± 0.54
	3	49.9 ± 0.90	39.9 ± 0.37	48.8 ± 1.37	33.7 ± 0.96	29.5 ± 0.63
	12	48.1 ± 0.88	42.5 ± 0.18	42.0 ± 0.50	37.0 ± 0.93	36.6 ± 0.73
18:3	3	4.6 ± 0.07	5.4 ± 0.47	4.9 ± 0.24	2.5 ± 0.35	0.7 ± 0.02
	12	4.4 ± 0.45	5.0 ± 0.23	4.8 ± 0.65	3.6 ± 0.12	3.9 ± 0.32

* The common names of the fatty acids as per description in Table 3.1

Table 5.13: The distribution of arachidonic and docosahexaenoic (fatty acid, weight percentage of total present) of free fatty acid fraction in the GIT contents from chicks receiving different dietary treatments

			Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)	diet					
20:4*	1		15.4 ± 0.83	11.6 ± 0.66	15.7 ± 1.00	22.9 ± 1.09	4.0 ± 0.27
	3	1	4.3 ± 0.31	2.6 ± 0.70	2.1 ± 0.16	2.9 ± 0.73	<0.1
		2	2.8 ± 0.13	1.4 ± 0.08	3.6 ± 0.30	1.2 ± 0.06	0.7 ± 0.15
		3	1.3 ± 0.12	<0.1	0.4 ± 0.07	<0.1	0.4 ± 0.03
	12	1	0.8 ± 0.05	<0.1	0.1 ± 0.01	<0.1	<0.1
		2	0.8 ± 0.17	0.3 ± 0.01	0.7 ± 0.09	0.6 ± 0.20	<0.1
		3	1.0 ± 0.23	<0.1	0.2 ± 0.01	<0.1	<0.1
22:6	1		0.9 ± 0.12	1.5 ± 0.23	2.9 ± 0.67	1.7 ± 0.15	<0.1
	3	1	1.9 ± 0.09	1.1 ± 0.43	1.8 ± 0.39	2.2 ± 0.09	<0.1
		2	1.2 ± 0.31	0.9 ± 0.20	1.2 ± 0.12	0.4 ± 0.1	<0.1
	12	1	1.6 ± 0.43	2.1 ± 0.22	1.6 ± 0.17	2.0 ± 0.34	
		3	0.4 ± 0.06	0.3 ± 0.04	0.6 ± 0.10	<0.1	0.3 ± 0.01

* The common names of the fatty acids as per description in Table 3.1
Diet names as per description in Table 4.1

Table 5.14: Statistical comparisons of fatty acid composition of the free fatty acid along the GIT between chicks receiving different dieatry treatments

			+D	J	Ui	Li	Lt
fatty acid:	age (days)	diets					
16:0*	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	***	*	***	N.S
		2 vs 3	***	N.S	***	***	***
	12	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	**	N.S	***	N.S
		2 vs 3	**	***	***	***	**
16:1	12	1 vs 2	**	***	*	*	***
		1 vs 3	**	*	*	**	*
		2 vs 3	**	***	***	***	***
18:0	3	1 vs 2	***	***	***	***	***
		1 vs 3	*	***	***	***	***
		2 vs 3	**	***	***	***	***
	12	1 vs 2	***	***	***	***	***
		1 vs 3	**	***	**	*	***
		2 vs 3	**	***	***	***	***
18:1	3	1 vs 2	***	***	*	N.S	*
		1 vs 3	*	***	**	**	N.S
		2 vs 3	N.S	**	N.S	**	**
	12	1 vs 2	***	***	**	**	***
		1 vs 3	***	***	**	***	*
		2 vs 3	***	*	N.S	N.S	*
18:2	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	***	N.S	***	N.S
		2 vs 3	***	***	***	***	***
	12	1 vs 2	***	***	***	***	***
		1 vs 3	**	***	***	*	N.S
		2 vs 3	***	***	***	***	***
18:3	3	1 vs 2	N.S	**	***	***	N.S
		1 vs 3	N.S	N.S	N.S	**	**
		2 vs 3	N.S	**	**	*	N.S
	12	1 vs 2	*	N.S	N.S	-	N.S
		1 vs 3	N.S	N.S	N.S	N.S	N.S
		2 vs 3	N.S	N.S	*	-	N.S

* The common names of the fatty acids as per description in Table 3.1
N.S = not significant; *, ** and *** = significant at P<0.05, P<0.01 and P<0.001, respectively
+ = Full names as per description in Table 5.2(ii); Diet names as per description in Table 4.1

some of the GIT sections.

The levels of palmitoleic acid in most sections of the GIT at day 12 post-hatch were highest in chicks receiving the tallow oil based diet.

The proportions of stearic acid in the GIT contents differed significantly between the three diets. Stearic acid levels were highest in the GIT contents from chicks receiving the tallow oil based diet, whilst, lowest levels were observed in chicks receiving the Dalgety chick starter diet at both days 3 and 12 post-hatch. The effect of age on stearic acid levels in chicks receiving either the tallow oil or the soyabean oil based diet did not show any definitive trend.

There was also no discernable trend in the levels of oleic acid between treatments although significant differences were observed amongst some of the GIT sections on day 3 post-hatch. However, on day 12 post-hatch the proportions of oleic acid in all the GIT sections from chicks receiving the Dalgety chick starter diet were significantly lower than those observed in chicks receiving either the tallow oil or the soyabean oil based diets.

Linoleic acid was the major acid in all the GIT sections from chicks receiving the Dalgety chick starter and the soyabean oil based diets at days 3 and 12 post-hatch and levels in each diet were significantly higher than those of chicks receiving the tallow oil based diet. Further differences in the levels of linoleic acid within most sections of the GIT were observed between chicks receiving the Dalgety chick starter and those receiving the soyabean oil based diet; levels were significantly higher in the former.

The levels of linolenic acid along the entire GIT of chicks receiving either the Dalgety chick starter or the soyabean oil based diets were significantly higher than in chicks receiving the tallow oil based diet on day 3 post-hatch. However no differences in the levels of linolenic acid were observed between the different diets on day 12 post-hatch.

Phospholipid

(A) *Fatty acid composition and changes in phospholipid fraction of GIT contents from chicks fed the Dalgety chick starter diet*

The fatty acid composition of the phospholipid fraction in the different sections of the GIT from chicks receiving the Dalgety chick starter diet are shown in Tables 5.15(i) & 5.18. The statistical analyses of the fatty acid compositions between the sequential sections of the GIT and the effects of age on fatty acid compositions are presented in Tables 5.15(ii) and 5.15(iii), respectively.

Palmitic acid accounted for about 19 percent of the total fatty acids present within the duodenum on day 1 post-hatch. The differences in the levels of palmitic acid between sequential sections of the GIT were insignificant throughout the 12 day post-hatch period. Small increases in most sections of the GIT were observed between days 1 and 3, whereas, decreases occurred between days 3 and 12 post-hatch.

The level of palmitoleic acid was less than 1 percent of the total fatty acids present in all sections of the GIT excluding the large intestine post-hatch. Relatively small increases in the proportion of palmitoleic acid in most sections of the GIT were observed between days 1 and 3 post-hatch but no further changes were observed thereafter. The level of palmitoleic acid remained unchanged between the upper sections whilst, significant increases were observed between the lower ileum

Table 5.15(i): The fatty acid composition (major long chain fatty acids, weight percentage of total present) of the phospholipid fraction in the GIT contents from chicks receiving the Dalgety chick starter diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0	1	19.3 ± 0.38	21.2 ± 0.67	23.2 ± 0.67	20.9 ± 0.55	21.1 ± 0.24
	3	21.0 ± 0.32	22.4 ± 0.76	24.9 ± 0.42	23.3 ± 0.31	22.3 ± 0.29
	12	21.2 ± 0.15	22.4 ± 0.67	17.3 ± 0.63	19.3 ± 0.15	17.7 ± 0.42
16:1	1	0.4 ± 0.06	0.8 ± 0.13	0.5 ± 0.08	0.3 ± 0.04	3.9 ± 0.89
	3	0.9 ± 0.12	0.7 ± 0.04	1.0 ± 0.20	1.1 ± 0.35	4.2 ± 0.42
	12	1.0 ± 0.07	1.0 ± 0.17	1.0 ± 0.15	1.5 ± 0.09	<0.1
18:0	1	25.7 ± 0.30	25.3 ± 0.72	25.8 ± 0.56	23.9 ± 0.07	19.0 ± 0.33
	3	19.2 ± 0.37	19.7 ± 0.49	17.7 ± 0.57	18.2 ± 0.43	14.7 ± 0.57
	12	20.8 ± 0.48	17.4 ± 0.50	9.4 ± 0.54	13.6 ± 0.60	15.7 ± 0.55
18:1	1	8.3 ± 0.50	9.1 ± 0.69	9.6 ± 0.53	9.4 ± 0.98	17.2 ± 0.96
	3	9.8 ± 0.27	9.1 ± 0.34	11.4 ± 0.85	12.7 ± 0.22	18.8 ± 0.66
	12	9.4 ± 0.16	10.9 ± 0.44	14.1 ± 0.32	14.9 ± 0.62	22.1 ± 0.63
18:2	1	15.9 ± 0.43	14.6 ± 0.07	15.2 ± 0.36	17.2 ± 0.88	20.7 ± 0.27
	3	39.8 ± 0.35	38.4 ± 0.95	37.7 ± 0.36	35.5 ± 0.15	25.9 ± 0.93
	12	40.0 ± 0.10	40.7 ± 0.66	48.3 ± 0.88	44.0 ± 0.92	46.2 ± 0.69

* The common names of the fatty acids as per description in Table 3.1

Table 5.15(ii): Statistical analyses for the fatty acid composition of the phospholipid fraction along the GIT in chicks receiving the Dalgety chick starter diet

		+D vs J	J vs Ui	Ui vs Li	Li vs Lt	D vs Lt
fatty acid:	age (days)					
16:0	1	*	N.S	*	N.S	**
	3	*	N.S	*	N.S	*
	12	N.S	**	*	*	***
16:1	1	*	N.S	N.S	**	**
	3	N.S	N.S	N.S	**	***
	12	N.S	N.S	N.S	-	-
18:0	1	N.S	N.S	*	***	***
	3	N.S	*	N.S	**	***
	12	**	***	**	*	**
18:1	1	N.S	N.S	N.S	***	***
	3	N.S	**	**	***	***
	12	*	***	N.S	***	***
18:2	1	*	*	*	**	***
	3	N.S	N.S	**	***	***
	12	N.S	**	*	N.S	***
18:3	3	**	N.S	N.S	**	***
	12	*	*	N.S	-	-
20:4	1	N.S	***	*	***	***
	3	N.S	**	**	-	-
	12	N.S	N.S	*	-	-
22:6	1	N.S	N.S	N.S	N.S	N.S
	3	***	N.S	N.S	-	-
	12	N.S	N.S	N.S	-	-

* The common names of the fatty acids as per description in Table 3.1
N.S = no significant; *, ** and *** = significant at P<0.05, P<0.01 and P<0.001, respectively
+ = full names as per description in Table 5.2(ii)

Table 5.15(iii): Statistical analyses of age effects on the fatty acid composition of the phospholipid fraction along the GIT in chicks receiving the Dalgety chick starter diet

		+D	J	Ui	Li	Lt
fatty acid:	age (days)					
16:0*	1 vs 3	*	N.S	*	**	*
	3 vs 12	N.S	N.S	***	***	***
	1 vs 12	*	N.S	***	***	***
16:1	1 vs 3	*	N.S	N.S	N.S	N.S
	3 vs 12	N.S	N.S	N.S	N.S	-
	1 vs 12	***	N.S	N.S	***	-
18:0	1 vs 3	***	***	***	***	***
	3 vs 12	*	*	***	***	N.S
	1 vs 12	***	***	***	***	***
18:1	1 vs 3	*	N.S	*	*	N.S
	3 vs 12	N.S	*	***	*	*
	1 vs 12	N.S	N.S	***	*	*
18:2	1 vs 3	***	***	***	***	**
	3 vs 12	N.S	N.S	***	***	***
	1 vs 12	***	***	***	***	***
18:3	3 vs 12	N.S	N.S	***	N.S	*
20:4	1 vs 3	***	***	***	***	-
	3 vs 12	***	***	***	***	-
	1 vs 12	***	***	***	***	-
22:6	1 vs 3	*	***	*	*	-
	3 vs 12	N.S	N.S	N.S	N.S	-
	1 vs 12	***	**	**	*	-

* The common names of the fatty acids as per description in Table 3.1
N.S = not significant; *, ** and *** = significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively
+ = Full names as per description in Table 5.2(ii)

and upper ileum on day 1 and 3 post-hatch.

Stearic acid was the major component accounting for about 25 percent of the total fatty acids present in the duodenum at day 1 post-hatch. The level of stearic acid did not alter significantly between the duodenum and jejunum and between the jejunum and upper ileum; whereas decreases occurred between the remaining sections of the GIT. The changes in stearic acid levels between the different sections of the GIT observed on day 3 post-hatch showed no obvious pattern. In contrast, on day 12 post-hatch significant decreases were observed between the duodenum and the jejunum and between the jejunum and upper ileum. A decline in the level of stearic acid with post-hatching age was observed within most sections of the GIT.

The proportion of oleic acid in the duodenum was only 8 percent at day 1 post-hatch. Other than increases between the lower ileum and large intestine no obvious changes in the levels of oleic acid were observed between the remaining sections of the GIT at day 1 post-hatch. However, increases in the levels of oleic acid occurred between most of the sequential sections of the GIT at both days 3 and 12 post-hatch and within some sections of the GIT, particularly beyond the upper ileum between days 1 and 12 post-hatch.

Linoleic acid accounted for about 15 percent of the total fatty acids in the duodenum on day 1 post-hatch. An overall increase in the levels of linoleic acid in the sequential sections of the GIT were observed on day 1 post-hatch. On day 3 linoleic acid levels did not alter significantly between the duodenum and upper ileum but decreased significantly beyond the upper ileum. The differences observed between the sequential sections of the GIT on day 12 post-hatch did not show any consistency. Over the 12 day post-hatch period, but particularly between days 1 and 3 post-hatch significant increases in the levels of linoleic acid were observed within most sections of the GIT.

The levels of linolenic acid were very low within all the GIT sections on the first day of age, but showed a slight increase with post-hatching age. On day 3 post-hatch significant increases occurred between the duodenum and the jejunum and between the lower ileum and large intestine, whilst on day 12 post-hatch similar increases the lower ileum only.

Arachidonic acid was amongst the major fatty acid within the duodenum accounting for about 20 percent on day 1 post-hatch. Significant increases in the levels of arachidonic acid along the GIT up to the lower ileum were observed on the first day post-hatch and no consistent trend along the GIT was observed thereafter. There were significant reductions, by about 80 percent, in the levels of arachidonic acid within most sections of the GIT during the first 3 days. Further significant declines were observed between days 3 and 12 post-hatch.

The level of docosahexaenoic was only 3 percent within the duodenum on day 1 post-hatch and did not alter significantly along the GIT. There were no changes in the level of docosahexaenoic along the GIT on days 3 and 12 post-hatch. Significant decreases were observed within all the GIT sections between days 1 and 3 post-hatch and the levels remained unchanged between days 3 and 12 post-hatch.

(B) *Comparison of fatty acid composition and changes in phospholipid fraction of the GIT contents between chicks receiving different diets*

The fatty acid compositions of the phospholipid fraction within different sections of the GIT from chicks receiving the tallow oil and the soyabean oil based diets are shown in Tables 5.16 & 5.18 and Tables 5.17 & 5.18, respectively. The statistical comparisons for the fatty acid compositions of the phospholipid fraction in the different sections of the GIT between chicks receiving the different diets at days 3 and 12 post-hatch are presented in Table 5.19.

Table 5.16: The fatty acid composition (major long chain fatty acids, weight percentage of total present) of the phospholipid fraction in the GIT contents from chicks receiving the tallow oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	19.3 ± 0.38	21.2 ± 0.67	23.2 ± 0.53	20.9 ± 0.55	21.1 ± 0.24
	3	19.6 ± 0.72	23.1 ± 0.12	26.5 ± 0.54	22.4 ± 0.64	31.2 ± 0.43
	12	20.5 ± 0.29	28.7 ± 0.11	26.9 ± 0.44	25.9 ± 0.27	24.7 ± 0.61
16:1	1	0.4 ± 0.06	0.8 ± 0.13	0.5 ± 0.08	0.3 ± 0.04	3.9 ± 0.88
	3	1.0 ± 0.06	2.0 ± 0.04	2.7 ± 0.16	2.1 ± 0.27	5.6 ± 0.42
	12	1.6 ± 0.11	2.6 ± 0.23	0.7 ± 0.05	1.0 ± 0.06	0.9 ± 0.12
18:0	1	25.7 ± 0.30	25.3 ± 0.73	25.8 ± 0.56	23.9 ± 0.07	19.0 ± 0.33
	3	24.7 ± 0.50	23.6 ± 0.21	25.8 ± 0.18	25.0 ± 0.18	14.4 ± 0.91
	12	22.9 ± 0.53	23.0 ± 0.56	19.2 ± 0.50	17.5 ± 0.38	13.0 ± 0.05
18:1	1	8.3 ± 0.50	9.1 ± 0.69	9.6 ± 0.53	9.4 ± 0.98	17.2 ± 0.96
	3	13.2 ± 0.13	18.4 ± 0.21	18.7 ± 0.80	20.9 ± 0.48	24.2 ± 0.42
	12	15.2 ± 0.10	17.4 ± 0.04	24.7 ± 0.50	23.9 ± 0.42	30.5 ± 0.81
18:2	1	15.9 ± 0.43	14.6 ± 0.07	15.2 ± 0.36	17.2 ± 0.88	20.7 ± 0.27
	3	33.3 ± 0.27	26.1 ± 0.72	20.7 ± 0.55	22.9 ± 0.21	18.3 ± 0.41
	12	34.8 ± 0.74	22.1 ± 0.70	24.4 ± 0.75	24.9 ± 0.31	27.3 ± 0.36

* The common names of the fatty acids as per description in Table 3.1

Table 5.17: The fatty acid composition (major long chain fatty acids, weight percentage of total) of the phospholipid fraction in the GIT contents from chicks receiving the soyabean oil based diet

		Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age (days)					
16:0*	1	19.3 ± 0.38	21.2 ± 0.67	23.2 ± 0.53	20.9 ± 0.55	21.1 ± 0.24
	3	18.7 ± 0.45	21.3 ± 0.62	22.4 ± 0.13	23.6 ± 0.30	28.0 ± 0.13
	12	22.0 ± 0.36	20.7 ± 0.97	18.0 ± 0.57	16.7 ± 0.60	20.2 ± 0.52
16:1	1	0.4 ± 0.06	0.8 ± 0.13	0.5 ± 0.08	0.3 ± 0.04	3.9 ± 0.88
	3	0.6 ± 0.04	<0.1	<0.1	<0.1	1.8 ± 0.04
	12	1.0 ± 0.18	0.9 ± 0.08	0.7 ± 0.03	0.2 ± 0.01	<0.1
18:0	1	25.7 ± 0.30	25.3 ± 0.72	25.8 ± 0.56	23.9 ± 0.07	19.0 ± 0.33
	3	22.0 ± 0.50	21.3 ± 0.30	16.6 ± 0.49	15.4 ± 0.18	14.1 ± 0.87
	12	21.3 ± 0.83	19.3 ± 0.43	10.6 ± 0.65	7.9 ± 0.32	8.6 ± 0.18
18:1	1	8.3 ± 0.50	9.1 ± 0.69	9.6 ± 0.53	9.4 ± 0.98	17.2 ± 0.96
	3	10.3 ± 0.30	12.5 ± 0.27	16.1 ± 0.63	17.5 ± 0.58	22.8 ± 0.85
	12	9.3 ± 0.22	12.4 ± 0.36	17.4 ± 0.49	17.9 ± 0.76	21.1 ± 0.27
18:2	1	15.9 ± 0.43	14.6 ± 0.07	15.2 ± 0.36	17.2 ± 0.88	20.7 ± 0.27
	3	41.0 ± 0.39	40.7 ± 1.08	40.3 ± 0.48	38.9 ± 0.30	29.3 ± 0.30
	12	41.4 ± 0.34	41.4 ± 0.70	47.4 ± 0.47	48.2 ± 0.62	42.4 ± 0.54

* The common names of the fatty acids as per description in Table 3.1

Table 5.18: The distribution of minor fatty acids (long chain fatty acid, weight percentage of total present) of the phospholipid fraction in the GIT contents of chicks receiving different dietary treatments

			Duodenum	Jejunum	Upper ileum	Lower ileum	Large intestine
fatty acid:	age	Diet					
18:3*	3	1	1.5 ± 0.70	2.8 ± 0.21	2.3 ± 0.21	2.1 ± 0.18	4.3 ± 0.64
		2	1.4 ± 0.43	1.6 ± 0.28	3.5 ± 0.44	0.7 ± 0.02	4.1 ± 0.38
		3	1.4 ± 0.04	2.1 ± 0.21	3.9 ± 0.24	4.6 ± 0.17	3.4 ± 0.52
	12	1	1.6 ± 0.12	2.3 ± 0.31	4.4 ± 0.17	3.2 ± 0.44	2.5 ± 0.19
		2	2.4 ± 0.03	6.2 ± 0.15	3.4 ± 0.41	5.9 ± 0.77	3.9 ± 0.19
		3	1.9 ± 0.21	5.2 ± 0.12	8.5 ± 0.35	7.4 ± 0.29	7.7 ± 0.41
20:4	1		20.0 ± 0.27	20.6 ± 0.11	17.4 ± 0.18	19.1 ± 0.52	13.1 ± 0.68
	3	1	4.0 ± 0.12	4.1 ± 0.37	2.7 ± 0.06	4.7 ± 0.22	<0.1
		2	5.4 ± 0.06	2.9 ± 0.51	0.5 ± 0.12	4.7 ± 0.70	1.5 ± 0.44
		3	4.6 ± 0.16	1.9 ± 0.53	0.4 ± 0.01	<0.1	<0.1
22:6	1		3.8 ± 0.56	4.8 ± 0.42	3.8 ± 0.30	4.2 ± 0.93	2.2 ± 0.21
	3	1	2.3 ± 0.10	1.0 ± 0.11	1.9 ± 0.50	1.8 ± 0.03	<0.1
		2	1.3 ± 0.06	1.6 ± 0.11	<0.1	1.1 ± 0.06	<0.1
		3	1.3 ± 0.04	<0.1	0.7 ± 0.15	<0.1	<0.1

* The common names of the fatty acids as per description in Table 3.1

Table 5.19: Statistical comparisons of the phospholipid fatty acid composition along the GIT between chicks receiving different dietary treatments

			+D	J	Ui	Li	Lt
fatty acid:	age (days)	diets					
16:0*	3	1 vs 2	N.S	*	N.S	N.S	***
		1 vs 3	**	N.S	**	N.S	***
		2 vs 3	N.S	N.S	***	N.S	**
	12	1 vs 2	N.S	***	***	***	***
		1 vs 3	N.S	N.S	N.S	**	**
		2 vs 3	*	***	***	***	**
16:1	3	1 vs 2	N.S	***	***	N.S	N.S
		1 vs 3	N.S	-	-	-	***
		2 vs 3	**	-	-	-	***
	12	1 vs 2	**	***	N.S	**	-
		1 vs 3	N.S	N.S	N.S	N.S	-
		2 vs 3	*	***	N.S	**	-
18:0	3	1 vs 2	***	***	***	***	N.S
		1 vs 3	*	*	N.S	**	N.S
		2 vs 3	**	***	***	***	N.S
	12	1 vs 2	*	***	***	***	**
		1 vs 3	N.S	*	N.S	**	***
		2 vs 3	**	***	***	***	***
18:1	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	**	**	**	**
		2 vs 3	***	***	*	*	N.S
	12	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	*	**	**	N.S
		2 vs 3	**	***	***	***	***
18:2	3	1 vs 2	***	***	***	***	***
		1 vs 3	N.S	N.S	**	***	*
		2 vs 3	***	***	***	***	***
	12	1 vs 2	***	***	***	***	**
		1 vs 3	N.S	N.S	**	***	*
		2 vs 3	***	***	***	***	***
18:3	3	1 vs 2	N.S	**	*	***	N.S
		1 vs 3	N.S	N.S	N.S	*	N.S
		2 vs 3	N.S	N.S	N.S	***	N.S
	12	1 vs 2	***	***	N.S	*	**
		1 vs 3	N.S	N.S	*	**	***
		2 vs 3	N.S	**	**	**	***

* The common names of the fatty acids as per description in Table 3.1
N.S = not significant; *, ** and *** significant at P<0.05, P<0.01 and P<0.001 respectively. + = Full names as per description in Table 5.2(ii), diet names as per description in Table 4.1

Compared with chicks receiving the Dalgety chick starter and the soyabean oil based diets, the levels of palmitic acid on day 3 post-hatch were higher in some sections of the GIT in chicks receiving the tallow oil based diet . However, on day 12 post-hatch, other than the duodenum, palmitic acid levels were significantly higher in the GIT sections from chicks receiving the tallow oil based diet than those receiving either the Dalgety chick starter or the soyabean oil based diets. Increases in the level of palmitic acid between days 3 and 12 post-hatch within all the GIT sections were observed in chicks receiving the tallow oil based diet; whereas a decline was observed in some sections of the GIT in chicks receiving either the Dalgety chick starter or the soyabean oil based diets.

The proportion of palmitoleic acid was higher in most of the GIT sections of chicks receiving the tallow oil based diet than those receiving either the Dalgety chick starter or the soyabean oil based diets. Between days 3 and 12 post-hatch no consistent differences were observed within the GIT sections from chicks receiving the different diets.

Apart from the large intestine, the levels of stearic acid were significantly higher within the GIT sections from chicks receiving the tallow oil based diet at both days 3 and 12 post-hatch compared with those from other diets. Differences in the level of stearic acid between some of the GIT sections were also observed between chicks receiving the Dalgety chick starter and the soyabean oil based diets, with levels being higher in the latter. A reduction in stearic acid levels with post-hatch age were observed within most sections of the GIT irrespective of diet.

The levels of oleic acid within the GIT sections varied significantly between chicks receiving the different diets. On both days 3 and 12 post-hatch oleic acid levels in most sections of the GIT were significantly higher in chicks receiving the tallow oil based diet than those receiving either the Dalgety chick starter or the

soyabean oil based diets. Increases in the level of oleic acid occurred between days 3 and 12 within most sections of the GIT in chicks receiving either the Dalgety chick starter or the tallow oil based diets, whereas, it remained unchanged in chicks receiving the soyabean oil based diet.

Linoleic acid was the major acid within the GIT contents from chicks receiving the Dalgety chick starter and the soyabean oil based diets, the levels being significantly higher than those from chicks receiving the tallow oil based diet on both days 3 and 12 post-hatch. Significant differences linoleic acid levels were also observed in some sections of the GIT between chicks receiving the Dalgety chick starter and the soyabean oil based diets, although no definitive trend was discernable. Increases in the proportions of linoleic acid between days 3 and 12 post-hatch from chicks receiving different diets occurred within most sections of the GIT.

In both linolenic and arachidonic acids, although some differences between diets were observed, no consistent trends were discernable between chicks receiving the different diets on both days 3 and 12 post-hatch.

The level of docosahexaenoic acid in most sections of the GIT in chicks receiving the tallow oil and the soyabean oil based diets were very much lower than for chicks receiving the Dalgety chick starter diet. The levels were such to preclude any meaningful comparisons.

5.4 DISCUSSION

The lipid composition of the GIT contents in the post-hatch chick is dependent upon a host of factors, which include the dietary fat composition, rate of absorption and post-hatching age. The differences in the amount of lipid within the different sections of the GIT observed in the present study in which the duodenum

contained the largest proportion of the overall lipid content are in agreement with previous findings (Freeman, 1976). The proportion of the duodenal lipid was lower on day 1 post-hatch compared with those of other days in all treatments. The duodenum contains substantial amount of lipid from the gall bladder (Hurwitz *et al.*, 1973; Inarrea *et al.*, 1989). The low duodenal lipid levels observed in the present study between days 1 and 3 post-hatch might have been associated with low biliary secretions, since it has been shown that bile production in very young chicks is lower when compared to adult chickens (Polin and Hussein, 1982; Hill, 1983). The increases within the duodenum of overall GIT lipid proportions after day 3 post-hatch is in agreement with the observation, that bile production and secretion into the duodenum in the chicken increases with age (Polin and Hussein, 1982; Hill, 1983). The gradual decline in the total lipid content along the GIT up to the lower ileum displayed by all three dietary treatments, is an indication that the major portion of the lipid was absorbed before reaching the large intestine. However, there were distinct variations in the rate of decline of overall lipid content along the GIT between the dietary treatments. Whereas, a sharp decline occurred between the duodenum and jejunum in chicks receiving the soyabean oil based diet, this feature was not observed in chicks receiving the tallow oil based diet in which large reduction in lipid levels occurred between the lower ileum and the large intestine. This difference between the soyabean oil and the tallow oil based diets may be due to ready absorption of dietary unsaturated fats within the upper parts of the GIT whilst saturated fats require far more intraluminal chemical processing before absorption can occur (Annison, 1983; Ketels and De Groote, 1989). Griminger (1986) reported that there was no lipid absorption beyond the ileum, which meant that all the unabsorbed fat associated with the large intestine was subsequently excreted in the faeces. In the present study the large intestine contained less than 15

percent of the total lipid extracted from the GIT contents under all dietary treatments throughout the study period. The underlying cause of the variation observed in lipid distribution along the GIT on the ninth day post-hatch when compared to other days in all the treatments is not known.

Esteban *et al.* (1991) suggested that the GIT might provide the major route through which a large proportion of yolk lipid is either made available for absorption or excreted from the body during the early post-hatch days. Such conclusions were made because of some common functional features observed between the small intestine and the vitelline stalk, which includes the continuous endoderm, basic absorptive function and comparable gross structure in both organs. However, the results obtained in experiment 1 and this study do not substantiate such a conclusion, since considerable differences were observed in lipid and fatty acid compositions between the residual yolk sac material and those in the GIT sections distal to the attachment of the yolk sac membrane. The present findings were similar to those of Romanoff (1960) which showed that no yolk material was traceable beyond the distal end of the yolk stalk. Thus, it would appear that the yolk sac undergoes involution and that the removal of yolk lipid over the immediate 5 days post-hatch, might be similar to that which occurs during late embryonic development involving an intimate metabolic relationship between the yolk contents and the yolk sac membrane (Noble and Moore, 1967; Noble, 1987).

The variations in the distribution of lipid moieties observed along the GIT in this study were a reflection of changes in lipid digestion and absorption processes occurring in the post-hatch chick. The wide differences observed between the lipid composition of the diets and the GIT contents showed that the lipid components underwent extensive degradation once they were ingested (Senior, 1964; Sklan *et al.*, 1975). The occurrence of such immediate changes have also been underlined by

Watkins (1987) who showed that in the chicken there appears to be extensive physical and chemical modifications (such as emulsification, enzymatic hydrolysis and micelle formation) of dietary fat prior to absorption. The mixing of the dietary lipids with bile and pancreatic secretions causes significant changes in lipid composition. The presence of high phospholipid levels within the duodenum throughout the 12 day post-hatch period in all the dietary treatments was clearly a reflection of biliary lipid secretion associated with this section. The substantial changes in the duodenal lipid composition observed after the first day post-hatch, may have an association with the extensive physiological and metabolical changes related to lipid metabolism which occur in the chick immediately post-hatch (Krogdahl, 1985; Akiba *et al.*, 1988).

The high triglyceride and low cholesterol ester levels in the duodenum obtained after the first day post-hatch were a reflection of the lipid compositional changes which occur in the liver and the gall bladder bile (Noble *et al.*, 1988) and the effects of increasing dietary fat intake (Carew *et al.*, 1972). However, after day 1 post-hatch the proportions of triglycerides within the duodenal contents were significantly lower than those present within the diets. Again this indicates the rapidity of the physical changes to which dietary fat is subjected once ingested (Sklan *et al.*, 1975). In the present study the free fatty acid levels in the duodenum was about 10 percent of the total lipid present. This accords with the findings of Freeman, (1976) showing that, following emulsification the level of free fatty acid in the duodenum in most instances accounted for less than 10 percent. Contrary to this Sklan *et al.* (1975) reported that over 95 percent of dietary triglyceride was broken down in the duodenum mostly to free fatty acids with some di- and mono-glycerides. Subsequent work by Sklan (1979) showed that under conditions where a high triglyceride based diet is fed to chicks, hydrolysis of the triglyceride is

extensive resulting in high levels of free fatty acid within the duodenum. The slight increases in free fatty acid levels in the duodenum with age observed in the present study is consistent with already existing theories that enzyme activities and bile production are low at hatching but improves markedly with age (Hurwitz *et al.*, 1973).

The relatively small changes observed in the lipid compositions along the GIT on day 1 post-hatch indicated that processes of lipid digestion and absorption were very limited at hatching. (Carew *et al.*, 1972; Krogdahl, 1985). The low fat utilization observed during the early post-hatch period could be due to a combination of many factors including low bile production, pancreatic secretions and enzymatic activities. The rapid lipid compositional changes observed within most of the GIT sections between days 1 and 3 post-hatch was an indication of the extensive increases in lipid metabolic activities following hatching which are influenced to some degree by dietary composition (Bjornhag, 1979; Akiba *et al.*, 1988; Krogdahl and Sell, 1989). The substantial increases in the proportions of free fatty acid and decreasing proportions of triglyceride and phospholipid beyond the duodenum between days 3 and 12 post-hatch, were evidences of extensive micelle formation (Hofmann, 1970) and also showed that most of the major activities associated with fat digestion and absorption occurred beyond the duodenum (Sklan *et al.*, 1975; Sklan, 1979; Krogdahl, 1985). In addition Freeman (1976) showed that the micellar lipid fraction of the post-hatch chick in comparison to human and rat contained apart from free fatty acid, higher proportions of phospholipid and to some extent triglycerides. It has been shown by various workers that, following hatching exposure of the GIT to increasing levels of dietary fat has a promotive effect on bile

effect on bile secretion and subsequent emulsification and fat digestion. Additionally secretions and absorptions into the duodenum were influenced by other dietary nutrients (Sklan *et al.*, 1973).

The differences in the proportions of the lipid moieties within the GIT sections between dietary treatments observed in the present study agrees with findings reported in other studies which showed that differing dietary fat compositions had an effect on lipid utilization by chicks (Ketels and De Groote, 1989). The similarity of the triglyceride levels within the duodenum and the jejunum obtained from chicks receiving different treatments observed in this study was probably due the influence of biliary secretions (Serafin and Neishem, 1970) and the low digestive activities within these sections (Freeman, 1976). The low proportions of triglyceride beyond the jejunum in chicks receiving the tallow oil based diet observed on the third day post-hatch in comparison with the high triglyceride levels in chicks receiving either the Dalgety chick starter or the soyabean oil based diets was unexpected. The probable causes for such differences may have been due to reduced bile secretion resulting from high levels of unsaturated fatty acids in the Dalgety chick starter and the soyabean oil based diets. High unsaturated fatty acid levels have a negative effect on hydrolytic abilities (Abraham, 1970). However, on day 12 post-hatch significant differences in the proportions of triglyceride observed beyond the jejunum between chicks receiving the Dalgety chick starter and the soyabean oil based diets indicated that, apart from dietary levels of saturated versus unsaturated fatty acids, there were other factors in the dietary fat composition which might have been responsible for the differences observed in fat utilization. Such factors may include the level of free fatty acid since high free fatty acid levels in the diet reduces bile secretion thus affecting micelle formation and fat digestion (Hurwitz *et al.*, 1973; Sklan, 1979).

The proportions of triglyceride along the GIT of chicks receiving the Dalgety chick starter and the soyabean oil based diets remained fairly constant. The findings observed in the present study are in agreement with the work reported by Garret and Young (1975) which showed that very little fat was absorbed as intact triglycerides in particulate form even in the presence of bile salts. Lower proportions of triglyceride in the large intestine of all the dietary treatments observed on day 12 post-hatch, as compared to those observed on day 3 post-hatch, was indicative of improvements in fat utilization with age (Renner and Hill, 1961; Fedde *et al.*, 1960; Carew *et al.*, 1972; Hakansson, 1974; Polin *et al.*, 1980; Watkins, 1987). Such an improvement in fat utilization with age is thought to be a result of the rapid growth of the GIT, in association with enzymic and endogenous secretions for example increased lipase concentrations Krogdahl and Sell (1989) and Sell *et al.* (1989) and increased bile secretion (Polin *et al.*, 1980; Watkins, 1987; Inarrea *et al.*, 1989).

Throughout this study the proportions of free fatty acid were higher within the GIT sections of chicks receiving the tallow oil based diet. Freeman (1976) and Ketels *et al.* (1986) attributed this to the formation of micelles and hydrolytic activities which is a more prominent step in the absorption of saturated fats, whilst a higher proportion of unsaturated fats could be absorbed directly into the intestine without being emulsified. On the other hand the low levels of free fatty acids observed in the GIT contents of chicks receiving the Dalgety chick starter diet as compared to levels in chicks receiving the soyabean oil based diet could have been in part due to the presence of high free fatty acid levels initially in the Dalgety chick starter diet. Sklan (1979) and Ketels *et al.* (1986) showed that there was a reduction in bile secretion and eventually emulsification when high levels of free fatty acids were incorporated in the diet. The high proportions of free fatty acids along the GIT in particular within the large intestine in all dietary treatments on day 12 post-hatch

observed in the present study may be associated with the lack of efficient free fatty acid solubilization in the intestinal lumen once the micelle products are formed (Senior, 1964). Additionally Sklan *et al.* (1975) showed that most of the absorption of free fatty acids occurred in the lower segments of the GIT.

The similarity in the proportions of phospholipid within the duodenum of chick in all dietary treatments throughout the 12 day period substantiates the contribution of biliary secretions to this section (Senior, 1964; Hurwitz et al., 1973; Freeman, 1976). Significant decreases in the proportions of phospholipid between the duodenum and the upper ileum, particularly on day 12 post-hatch, showed that most of the major activities associated with fat digestion and absorption of bile acids in particular were confined to the upper sections of the GIT (Sklan *et al.*, 1975; Sklan, 1979; Krogdahl, 1985). However, the proportions of phospholipid within most sections of the GIT in chicks receiving the Dalgety chick starter diet were higher in comparison with those from other diets probably due to the limited hydrolysis activities following the high free fatty acid levels initially present in the diet (Sklan, 1979; Ketels *et al.*, 1986).

The variations observed in the distribution of the fatty acids within the major lipid fractions between the dietary treatments after day 1 post-hatch was a reflection of the differences in dietary fatty acid composition. However, the findings obtained in the present study are in contrast to those reported by Sklan (1979) in which the fatty acid composition of lipid fractions from the different sections of the GIT were found to be similar irrespective of the dietary fatty acid composition. The fatty acid differences observed within the duodenum and in most of the other sections of the GIT in this study were confined mainly to the triglyceride and the free fatty acid fractions. This accords with the fact that triglyceride and free fatty acid were the major lipid fractions in the diets. On the other hand the similarity of phospholipid

fatty acid composition in the duodenal contents obtained from chicks receiving different diets illustrated further that the phospholipid component in this section was independent of the dietary influences. Clearly the contribution of phospholipid from the bile secretion outweighs any differences within the phospholipid components of the diet (Freeman, 1969; Sell *et al.*, 1991).

Apart from changes observed on day 1 post-hatch, the proportions of the majority of fatty acids associated with the triglyceride fraction in all dietary treatments remained fairly constant along the GIT. The reasons for these observations are unclear since previous workers had suggested that saturated triglyceride undergo hydrolysis more readily than unsaturated species. However, it has been reported previously by Hurwitz *et al.* (1973) that following rapid digestion and absorption of lipids within the jejunum, absorption along the remaining sections of the GIT is very gradual especially for components which have a high linoleic acid content. The significant differences in the fatty acid composition of the triglyceride fraction along the GIT in all dietary treatments observed between days 1 and 3 post-hatch were probably due to the commencement of feeding by the chick (Carew *et al.*, 1972; Hakansson, 1974). Unlike the fatty acid composition of the triglyceride fraction, there were significant changes in the composition of the free fatty acid fraction along the GIT, in all dietary treatments in particular between the lower ileum and the large intestine. The most likely cause of this is the combination of dietary free fatty acid release by digestive lipolysis of both dietary and endogenous components. Significant decreases in the proportions of linoleic acid along the GIT on both days 3 and 12 post-hatch, particularly in chicks receiving the tallow oil based diet, could have been a result of the preferential utilization of fatty acids of biliary origin during lipid digestion and absorption, since linoleic is a major fatty acid in the bile (Senior, 1964; Hurwitz *et al.*, 1973; Freeman, 1976; Krogdahl,

1975). However, in comparison with other lipid fractions, the changes in fatty acid composition including linoleic acid within the phospholipid fraction along the GIT in all dietary treatments were not extensive.

CHAPTER 6

THE EFFECT OF AGE AND DIETARY FAT SOURCES ON FAT INTAKE AND FAT EXCRETION BY GROWING BROILER CHICKS

6.1. INTRODUCTION

Fat supplementation to poultry diets is widely practised due to the nutritional and physical advantages that it gives under intensive production systems (Phelps, 1989). Experiments to determine the nutritive value of various dietary fat additions have shown that differences in dietary fat levels are associated with wide variations in the performance of both young chicks and adult chickens (Fedde *et al.*, 1960; Fuller and Rendon, 1979). Heavier body weights are normally observed when fat is added to the diet, although differences in weights resulting from differences in fat sources are in most cases small (Sim *et al.*, 1985). Nevertheless, differences in both feed intake and feed conversion are usually observed when dietary fat sources differing in their chemical composition are added to the diet (Sibbald and Krammer, 1980; Watkins, 1987; Wiseman and Salvador, 1991). The utilization of fat is relatively low during the early post-hatch periods a situation which under normal commercial regime may result in high fat excretion (Pattison, 1989). However, as the chick grows increased fat intake and decreased fat excretion are normally observed although both parameters are affected to a greater extent by the composition of dietary fat. The present study was therefore undertaken to investigate the influences of age and the dietary fat sources on the following:

(i) feed intake, growth rate and feed efficiency of broiler chicks during the early post-hatch period

(ii) fat intake and fat excretion

(iii) lipid and fatty acid compositions of the faecal fat

6.2 MATERIALS AND METHODS

6.2.1 Treatments

Three treatments with similar basic ingredients but differing considerably in their dietary fat sources and therefore fatty acid compositions were used. The treatments were designated as Diet 1, Diet 2 and Diet 3 in which Dalgety fat i.e the standard fat additive used in Dalgety feeds (Dalgety Company, Bristol, England) tallow and soyabean oils provided the major dietary fat sources, respectively. The basic description and estimated proximate composition are shown in Table 6.1.

6.2.2 Lipid and fatty acid compositions of the dietary fat sources and the compounded diets

The lipid composition of the fat sources and the experimental diets

The distributions of the major lipid moieties in the Dalgety fat, tallow and soyabean oils and the respective compounded diets used in experiment 3 are shown in Table 6.2. Triglyceride was by far the largest lipid group in both the tallow and soyabean oils accounting for more than 94 percent in each case; but was less than 50 percent in the Dalgety fat. Free fatty acid accounted for more than 50 percent of total lipid in the Dalgety fat, whereas in the tallow and soyabean oils it was negligible. The remaining lipid fractions i.e cholesterol ester, phospholipid and free cholesterol were very low in all the fat sources.

The distribution of the lipid moieties in the compounded diets, in particular the tallow and soyabean oil based diets differed slightly from that of the individual fat sources. The triglyceride fraction was still the major lipid moiety in the tallow and soyabean oils based diets, but its proportion was lower compared to that of the fat sources accounting for 60 percent of total lipid. In the Dalgety fat based diet the level of triglyceride was about 40 percent of total lipid. These differences in the

Table 6.1: Basic ingredients and estimated proximate composition of the experimental diets

	Diet 1	Diet 2	Diet 3
Ingredient, g/kg:			
barley	100	100	100
maize	250	250	250
wheat	245	245	245
herring meal	50	50	50
soyabean meal	220	220	220
grass meal	50	50	50
limestone	5.3	5.3	5.3
dicalcium phosphate	21.7	21.7	21.7
salt	2.5	2.5	2.5
vitamin mix	2.5	2.5	2.5
amprol mix	0.5	0.5	0.5
tallow oil	-	50	2.5
soyabean oil	-	-	47.5
Dalgety fat	50	-	-
Calculated analyses:			
ME (MJ/kg)	12.7	12.6	12.7
crude protein (%)	21.0	20.5	20.6
oil (%)	5.6	5.6	5.6

Diet 1 = diet containing Dalgety fat; Diet 2 = diet containing tallow oil and Diet 3 = diet containing soyabean oil

Table 6.2: The lipid compositions (major fractions, weight percentage of total lipid present) of the fat sources and compounded diets

	Dalgety fat	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
lipid group:						
cholesterol ester	<0.1	<0.1	<0.1	3.3	2.2	1.5
triglyceride	44.6	91.7	99.3	41.4	65.6	65.0
free fatty acid	51.3	7.5	0.5	31.6	11.9	20.8
partial glyceride	<0.1	<0.1	<0.1	6.7	6.5	4.3
phospholipid	4.1	0.8	0.8	17.3	13.7	8.5

Table 6.3: The fatty acid compositions (major long chain fatty acids, weight percentage of total present) of the total lipid in dietary fat sources and compounded diets

	Dalgety fat	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
fatty acid:						
16:0*	21.6	23.8	9.5	20.7	25.0	13.5
16:1	2.9	5.7	<0.1	2.8	4.2	0.9
18:0	5.6	23.4	3.1	9.3	16.2	4.1
18:1	40.1	41.2	30.4	31.1	32.4	27.5
18:2	21.6	3.6	48.6	30.6	16.7	43.5
18:3	5.3	2.0	8.2	3.8	3.0	7.6
20:3	0.2	<0.1	<0.1	0.4	0.1	0.3
20:4	0.2	<0.1	<0.1	<0.1	0.8	0.9
20:5	<0.1	<0.1	<0.1	0.6	0.8	0.9
22:5	1.8	<0.1	<0.1	<0.1	0.1	0.1
22:6	0.8	<0.1	0.1	0.6	0.7	0.8
S:U	1:2.68	1:1.11	1:6.96	1:2.32	1:1.42	1:4.68

* The common names as per description in Table 3.1

U:S = Ratio between total saturated and total unsaturated fatty acids

triglyceride levels between the tallow and soyabean oils and the compounded diets were balanced by comparable changes mainly in free fatty acid and to a lesser extent phospholipid and partial glyceride levels. The suprising high evolution of free fatty acids during compounding is discussed later.

Fatty acid distribution

The fatty acid composition, weight percent of total fatty acids present in total lipid and the two major lipid components i.e triglyceride and free fatty acid fractions of the Dalgety fat, tallow and soyabean oils and the compounded diets are shown in Tables 6.3, 6.4 and 6.5, respectively.

From Table 6.3 it can be seen that the Dalgety fat, soyabean oil and their corresponding compounded diets contained higher levels of unsaturated fatty acids, whilst, tallow oil and the tallow oil based diet contained higher levels of saturated fatty acids. The C20 and C22 polyunsaturated fatty acids in the triglyceride and free fatty acid fractions were extremely low in all the fat sources and the compounded diets. Apart from differences in palmitic acid levels in the soyabean oil and the soyabean oil based diet, close similarities in the fatty acid compositions between the triglyceride and free fatty acid fractions were observed in both the fat sources and the compounded diets.

6.2.3 Rearing of chicks

Three hundred day old Ross Breeder 1 chicks obtained from Scottish Agricultural College (Auchincruive) were used. The chicks were weighed and then randomly allocated to the three dietary treatments. The experiment was conducted under controlled environmental conditions for both temperature and humidity. The chicks were kept in metal cages, each cage with a collecting tray beneath it. The size

Table 6.4: The fatty acid compositions (major long chain fatty acids, weight percentage of total present) of the triglyceride fraction of the dietary fat sources and compounded diets

	Dalgety fat	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
fatty acid:						
16:0*	21.0	27.0	10.6	19.9	24.8	17.0
16:1	2.5	5.2	<0.1	1.3	4.9	2.2
18:0	6.6	19.5	2.0	3.7	13.2	6.7
18:1	44.9	43.4	30.5	31.9	34.8	28.9
18:2	19.8	3.6	48.3	38.9	18.9	40.1
18:3	5.2	1.0	8.4	4.2	2.1	3.5

Table 6.5: The fatty acid compositions (major long chain fatty acids, weight percentage of total present) of the free fatty acid fraction in dietary fat sources and the compounded diets

	Dalgety fat	Tallow oil	Soyabean oil	Diet 1	Diet 2	Diet 3
fatty acid:						
16:0*	25.1	24.2	21.2	17.1	25.9	18.5
16:1	3.3	4.6	1.1	3.7	3.3	2.7
18:0	5.1	23.1	5.9	4.0	12.4	5.2
18:1	36.8	42.8	25.5	22.8	28.9	25.9
18:2	24.7	3.2	40.1	46.4	21.5	42.6
18:3	4.5	2.2	5.2	3.7	4.4	5.0

* The common names of the fatty acids as per Table 3.1

of the individual cage was 1.5 x 2 m^2 and in each cage there were 2 feeding troughs each with 20 feeding spaces and 2 automatic drinkers. Each dietary treatment was replicated four times. A 23 hour lighting programme using about 20 lux fluorescent light was employed. The brooding temperature was 35 $\pm$ 0.5^{o}C during the first week and was reduced to 32 $\pm$ 0.5^{o}C and 30 $\pm$ 0.5^{o}C on week 2 and 3 respectively. To avoid environmental effects at 3 days intervals chicks were moved between cages after sampling. Feed were provided *ad libitum* but the feed added to the trough and the residue were weighed daily. Clean water was available at all times.

6.2.4 Sampling

Faecal sample collection began on day 3 post-hatch for a period of 21 days. During sample collection the chicks in each cage were group weighed. The faeces were sieved and using forceps other particles such as feed and feathers were removed before weighing. The faeces were then left to dry under room temperature for three days and the weights of the dry faecal samples were recorded. Representative samples from the dried faecal material were stored in suitable containers at -20^{o}C to await further analyses. All the calculations were based on the air dried weight of the faecal material.

6.2.5 Lipid extraction and analyses

These were conducted as per procedures described in detail in Chapter 3.0 Materials and Methods: Section 3.1 - 3.3.

6.2.6 Statistical analyses

All the data obtained were subjected to a two way analyses of variance using Minitab Release 7.1 Version of 1989.

6.3 RESULTS

6.3.1 Body weight changes and feed intake

The mean body weights of chicks receiving different dietary treatments during the experimental period are shown in Table 6.6. There were no significant effects due to diet on body weight changes between days 1 and 6 post-hatch. However, after day 6 significant differences in body weight changes between chicks receiving different dietary treatments were observed. Thus, between days 9 and 15 post-hatch, chicks receiving the Dalgety fat based diet were significantly heavier than those receiving either the tallow or the soyabean oil based diets. Similar differences were also observed between chicks receiving the Dalgety fat and tallow oil based diets on day 21 post-hatch. Additionally, significant differences between chicks receiving the tallow and the soyabean oils based diet were observed after day 12 post-hatch; the body weight being lower in chicks receiving the tallow oil based diet. The rate of body weight gain was lowest in chicks receiving the tallow oil based diet during the first 3 days post-hatch and lowest in chicks receiving the soyabean oil based diet between days 6 and 9 post-hatch. After week 2 post-hatch the rate of body weight gain was highest in chicks receiving the tallow oil based diet with an average weight gain of 23.4 g/day and lowest in chicks receiving the Dalgety fat based diet with an average weight gain of 20 g/day.

Feed intake (gram feed/ chick) over the various periods during the experiment are shown in Table 6.7. As in the case of body weight changes no significant differences were observed between the diets during the first 6 days post-hatch. With a few exceptions daily feed intake was lowest in chicks receiving the soyabean oil based diet between days 6 and 15 post-hatch, compared to those receiving either the Dalgety fat or the tallow oil based diets. No significant differences in feed intake were observed between the dietary treatments during days

Table 6.6: Mean body weight (gm) of chicks receiving diets containing different dietary fat sources

	body weight (gm)		
	Diet 1	Diet 2	Diet 3
age (days):			
1	37.9 ± 0.23	36.6 ± 0.30	37.2 ± 0.01
3	55.3 ± 1.17	52.9 ± 6.55	56.8 ± 0.48
6	101.5 ± 2.41	98.2 ± 12.61	96.7 ± 0.45
9	164.8 ± 5.74	156.7 ± 8.17	152.5 ± 5.35
12	274.5 ± 0.63	260.1 ± 9.76	262.7 ± 1.76
16	384.6 ± 0.64	350.3 ± 1.40	369.7 ± 1.83
21	484.8 ± 8.29	466.5 ± 13.95	480.2 ± 2.24

Table 6.7: Mean feed intake in chicks receiving diets containing different dietary fat sources

	feed intake (gm)		
	Diet 1	Diet 2	Diet 3
age (days)			
1 - 3	28.6 ± 6.80	25.4 ± 11.78	24.4 ± 2.05
3 - 6	66.0 ± 0.36	68.9 ± 9.06	72.5 ± 1.57
6 - 9	108.2 ± 1.08	105.7 ± 6.65	97.3 ± 2.53
9 - 13	206.4 ± 3.38	195.1 ± 3.44	197.1 ± 2.81
13 - 16	195.7 ± 4.37	177.8 ± 0.87	182.3 ± 5.05
16 - 21	273.4 ± 6.14	268.6 ± 8.22	269.3 ± 8.49
feed/gain ratio	1.97	1.97	1.90

16 and 21 post-hatch. Additionally feed efficiency, over the whole experimental period, measured as gram feed/ gram body weight gain was lowest in chicks receiving the soyabean oil based diet.

6.3.2 Fat intake and excretion

Figure 6.1 shows the effects of different dietary fat sources on fat intake per chick. Increase in fat intake with post-hatching age were observed in all dietary treatments as a result of increased feed intake. The level of fat intake in most cases was lowest in chicks receiving the Dalgety fat based diet and highest in chicks fed the soyabean oil based diet.

The total lipid content in the faeces are shown in Figure 6.2. With a few exceptions the total lipid content in the faeces was highest in chicks receiving the tallow oil based diet. Under all dietary treatments the amount of total lipid in the faeces increased with age up to day 9 post-hatch and decreased gradually thereafter. However, there were variations in the rate of decline between the dietary treatments, the rate being highest and lowest in chicks receiving the soyabean oil and the tallow oil based diets, respectively.

The level of excreted fat as a proportion of fat intake (see Figure 6.3) was exceptionally low during the first 3 days post-hatch. Large increases in percentage of excreted fat occurred in all dietary treatments between days 3 and 6 post-hatch. The level remained relatively unchanged in chicks receiving either Dalgety fat or tallow oil based diets between days 6 and 9 post-hatch. Increases were observed in chicks receiving the soyabean oil based diet over the same period. After day 9 post-hatch decreases in the percentage of excreted fat were observed in chicks receiving the Dalgety fat and the soyabean oil based diets. The rate of decline was however, higher in chicks receiving the soyabean oil based diet. In chicks receiving

Figure 6.1: Fat intake as influenced by post-hatching age and dietary fat sources

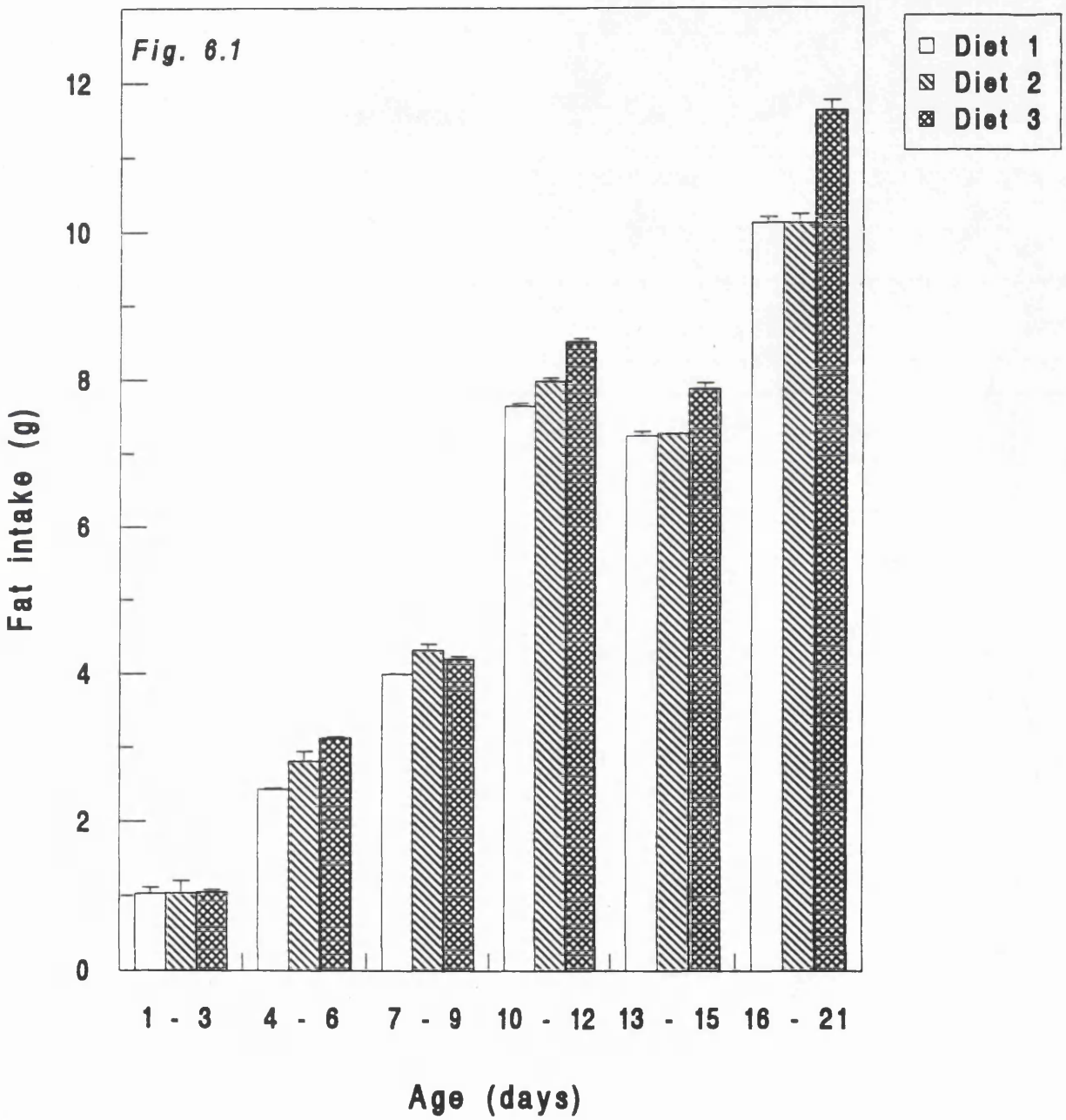
Fig. 6.1
Diet 1
Diet 2
Diet 3
Fat intake (g)
0
2
4
6
8
10
12
1 - 3
4 - 6
7 - 9
10 - 12
13 - 15
16 - 21
Age (days)

Figure 6.2: Total lipid content in the faecal material from chicks receiving different dietary fat sources

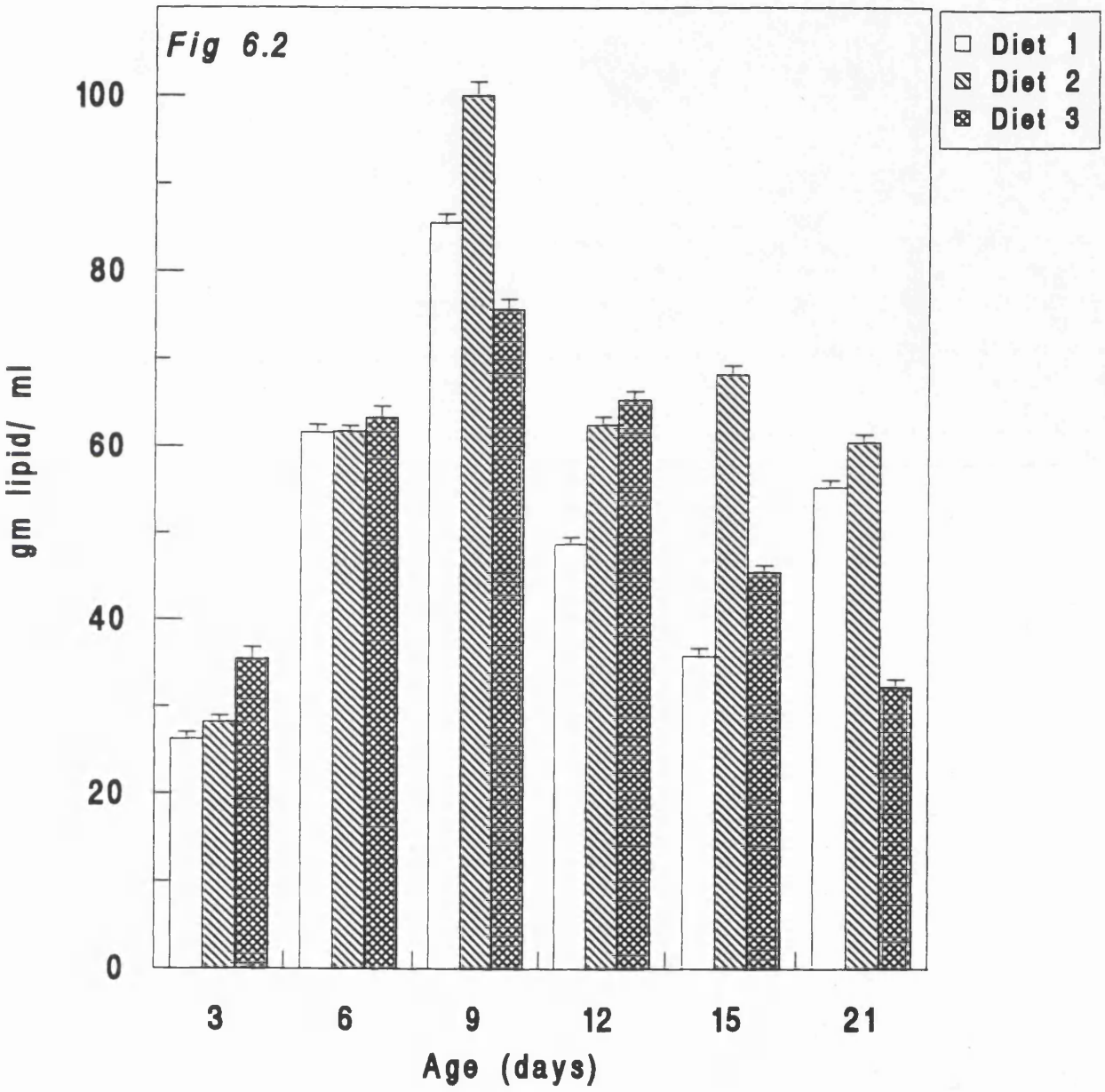
Fig 6.2
Diet 1
Diet 2
Diet 3
100
80
60
40
20
0
gm lipid/ ml
3
6
9
12
15
21
Age (days)

Figure 6.3: The proportion of excreted fat to fat intake as influenced by age and dietary fat sources

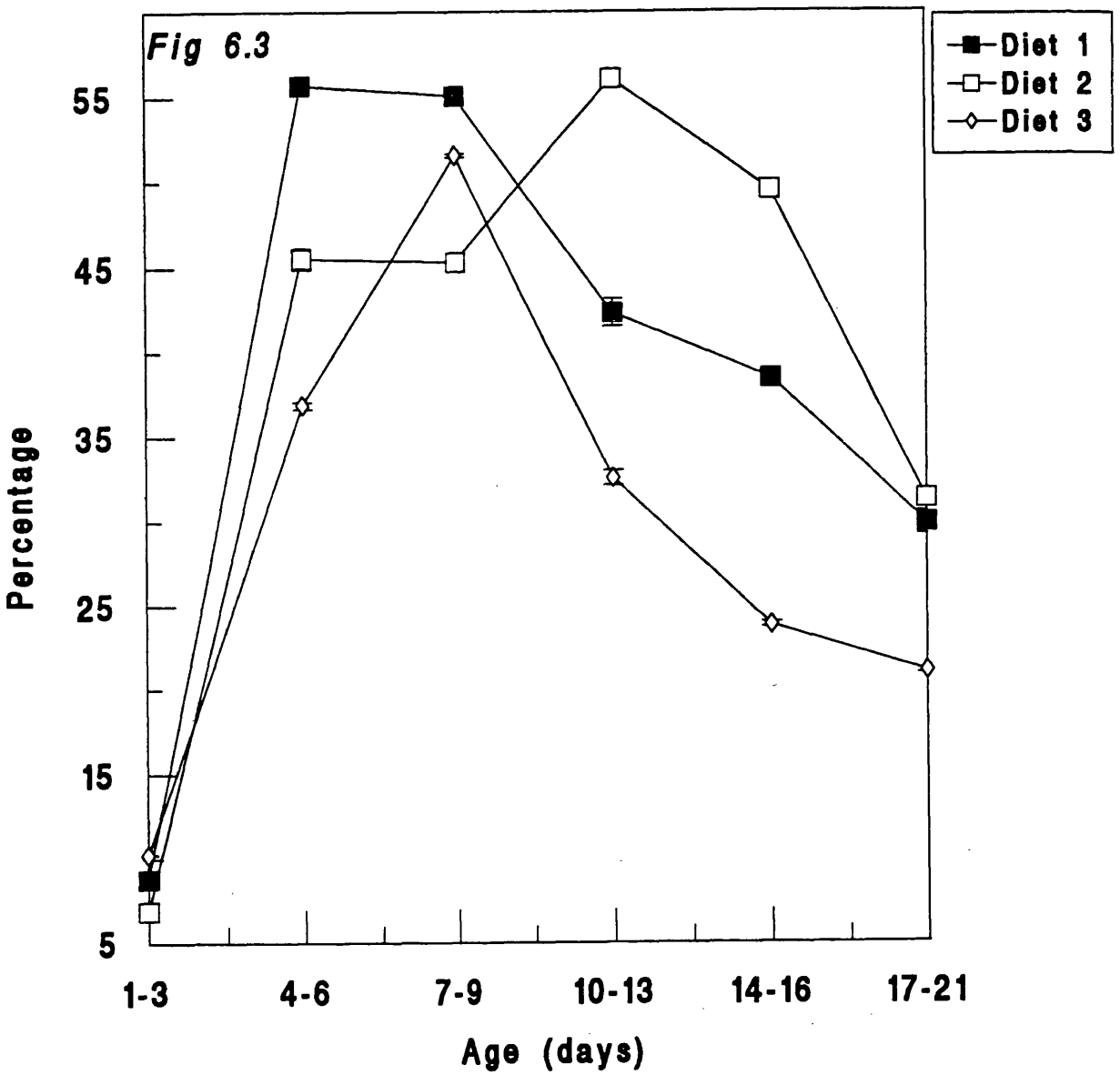
Fig 6.3
Diet 1
Diet 2
Diet 3
55
45
35
25
15
5
Percentage
1-3
4-6
7-9
10-13
14-16
17-21
Age (days)

the tallow oil based diet excreted fat increased further up to day 12 post-hatch before declining. By day 21 post-hatch the percentage of excreted fat was lowest in chicks receiving the soyabean oil based diet and no apparent differences in fat excretion were observed between chicks receiving the Dalgety fat and the tallow oil based diets.

6.3.3 Lipid and fatty acid composition changes

Lipid moiety changes

Table 6.8(i) shows the distribution of the major lipid fractions in the faeces from chicks receiving the different dietary treatments. The statistical analyses of dietary treatments and age effects on the distribution of the major lipid fractions in the faeces are shown in Tables 6.8(ii) and 6.8(iii) respectively. The level of cholesterol esters was significantly higher in chicks receiving the Dalgety fat based diet in comparison to those receiving either the tallow or the soyabean oil based diet on day 3 post-hatch. However, after day 3 post-hatch, levels were significantly higher in chicks receiving the soyabean oil based diet than those receiving either the Dalgety fat or the tallow oil based diets. The exception to this was observed on day 6 post-hatch . Significant decreases in the levels of cholesterol esters occurred in all dietary treatments between days 3 and 12 post-hatch, followed by increases thereafter.

The proportion of triglyceride was significantly lower in chicks receiving the Dalgety fat based diet on day 3 post-hatch and thereafter no significant differences were observed between the dietary treatments. With a few exceptions the level of triglyceride remained unchanged throughout the experimental period.

Free fatty acids was the major lipid component in the faeces in all dietary treatments throughout the experimental period. There were no obvious differences in

Table 6.8: The effect of age and dietary fat sources on the distiribution of the major lipid fractions (major lipid fraction, weight precentage of total lipid present) of the faeces

		Age (days)					
		3	6	9	12	15	21
lipid fraction:							
	Diet						
cholesterol	1	15.7 ± 2.52	5.2 ± 0.41	3.2 ± 0.13	1.5 ± 0.01	3.6 ± 1.13	2.4 ± 0.54
ester	2	9.9 ± 1.40	5.4 ± 1.47	3.4 ± 0.15	1.5 ± 0.12	3.6 ± 0.14	3.5 ± 0.48
	3	9.0 ± 1.39	7.9 + 0.21	1.9 ± 0.26	2.6 ± 0.82	4.1 ± 0.56	4.1 ± 0.50
	1	2.7 ± 1.42	3.3 + 0.06	2.2 ± 0.16	2.5 ± 1.50	3.0 ± 0.50	5.2 ± 0.92
triglyceride	2	5.9 ± 1.45	2.1 + 0.41	3.0 ± 0.29	2.1 ± 0.81	2.1 ± 0.17	2.5 ± 0.12
	3	7.0 ± 0.62	1.5 + 0.22	3.9 ± 0.29	2.8 ± 0.24	2.8 ± 0.88	7.2 ± 0.83
free fatty	1	70.2 ± 4.22	85.8 + 1.50	88.2 ± 1.98	85.5 ± 1.74	85.4 ± 3.21	84.4 ± 4.48
acid	2	75.4 ± 5.70	87.6 + 3.40	89.3 ± 2.51	89.6 ± 1.23	88.0 ± 0.43	87.1 ± 0.05
	3	75.3 ± 2.94	84.8 + 2.11	88.4 ± 1.47	89.4 ± 2.81	82.9 ± 5.76	76.9 ± 1.35
	1	7.2 ± 0.18	3.6 + 0.10	4.9 ± 0.07	6.6 ± 0.80	1.7 ± 0.89	4.2 ± 1.34
phospholipid	2	5.0 ± 0.56	4.2 + 0.16	1.8 ± 0.10	4.2 ± 0.39	3.9 ± 0.70	4.1 ± 0.18
	3	5.4 ± 0.65	4.6 + 0.11	3.8 ± 0.15	3.9 ± 0.71	6.4 ± 1.93	7.0 ± 0.19
free	1	4.4 ± 0.14	2.1 + 0.15	1.5 ± 0.13	3.9 ± 1.44	4.8 ± 2.12	3.9 ± 0.68
cholesterol	2	3.8 ± 0.69	0.8 + 0.35	2.5 ± 0.26	2.6 ± 0.11	2.4 ± 0.21	2.9 ± 0.51
	3	3.3 ± 0.12	1.3 ± 0.20	2.1 ± 0.17	3.0 ± 0.57	3.8 ± 1.48	4.9 ± 0.51

Table 6.8(ii): Statistical analyses on the effects of age and dietary fat sources on lipid composition in the faeces

lipid group	diets	age 3	6	9	12	15	21	diet	a	b	c	d	e
cholesterol	1 vs 2	**	N.S	N.S	N.S	N.S	*	1	***	*	N.S	*	***
ester	1 vs 3	N.S	*	*	*	N.S	*	2	**	*	N.S	N.S	**
	2 vs 3	**	*	*	*	N.S	N.S	3	*	**	**	N.S	**
triglyceride	1 vs 2	*	*	*	N.S	N.S	*	1	N.S	*	N.S	**	*
	1 vs 3	*	*	*	N.S	N.S	**	2	*	*	*	N.S	**
	2 vs 3	N.S	*	*	N.S	N.S	***	3	*	**	**	N.S	**
free fatty acid	1 vs 2	*	*	N.S	*	*	*	1	***	*	*	N.S	***
	1 vs 3	N.S	N.S	N.S	*	*	**	2	***	N.S	*	*	***
	2 vs 3	*	*	N.S	N.S	*	**	3	***	*	**	**	N.S
phospholipid	1 vs 2	*	*	**	*	*	N.S	1	**	*	**	**	**
	1 vs 3	*	*	*	*	**	*	2	N.S	**	**	N.S	N.S
	2 vs 3	N.S	N.S	**	N.S	**	*	3	N.S	**	**	N.S	*

free fatty acid levels between the dietary treatments during the first two weeks post-hatch. Significant declines were observed on days 15 and 21 post-hatch in chicks receiving the soyabean oil based diet compared with the other diets. The free fatty acid levels in all treatments on day 3 post-hatch were significantly lower in comparison to those observed during the rest of the experimental period, the exception to this occurred only on day 21 post-hatch in chicks receiving the soyabean oil based diet. The absolute amount of free fatty acid in the faeces under the different dietary regimes is presented in Figure 6.4. As can be seen the absolute amounts of free fatty acid followed a similar trend to that of total faecal lipid content. The absolute amount of free fatty acids were similar in all the dietary groups on day 6 post-hatch and variations were observed from day 9 post-hatch and onwards. In chicks receiving the Dalgety fat based diet, a sharp decline in the absolute amount of free fatty acid in the faeces occurred at days 12 and 15 post-hatch followed by an increase at day 21. In chicks receiving the soyabean oil based diet absolute amount of free fatty acid showed a distinctive and steady decline. In the case of chicks receiving the tallow oil based diet the decline was observed on day 12 post-hatch but thereafter remained relatively unchanged. By day 21 post-hatch the amount of free fatty acids was lowest in chicks receiving the soyabean oil based diet and highest in the chicks that received the tallow oil based diet.

Apart from the decreases observed between days 3 and 6 post-hatch the levels of both phospholipid and free cholesterol within the faeces (see Table 6.8(i)) were not significantly affected by either age or dietary treatment.

Figure 6.4: The effect of age and dietary fat sources on the level of free fatty acids in the faecal material

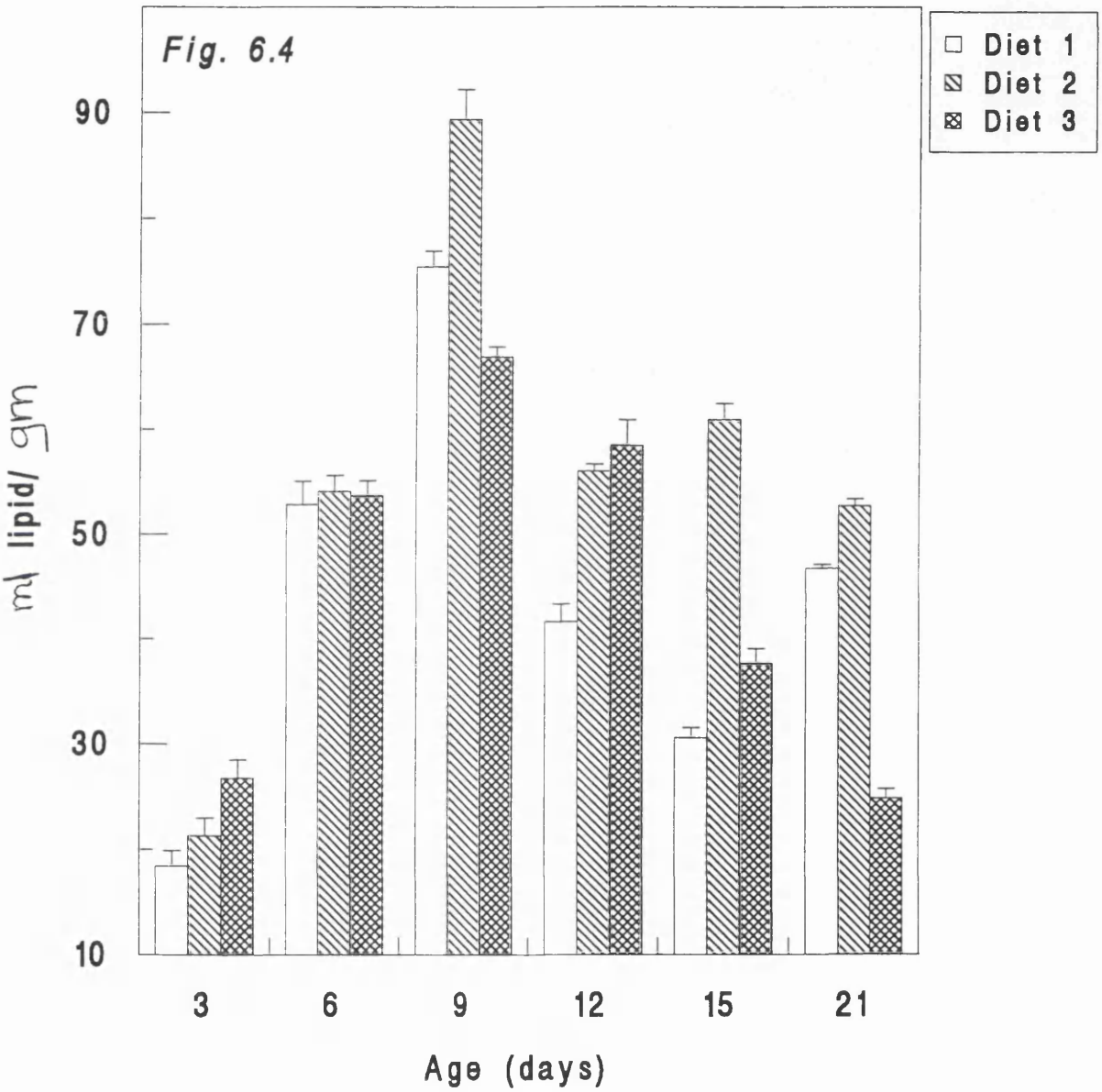
Fig. 6.4
Diet 1
Diet 2
Diet 3
90
70
50
30
10
ml lipid/ gm
3
6
9
12
15
21
Age (days)

6.3.4 Total fatty acids in the faecal lipid

The distribution of the fatty acids in the total lipid content of the faecal material from chicks under the different dietary treatments are presented in Tables 6.9 (i & ii). The statistical analyses of the dietary treatment and age effects on the total fatty acid composition of the faeces are shown in Table 6.10.

The level of palmitic acid in the faeces from chicks receiving the tallow oil based diet was significantly higher compared to levels observed in chicks receiving either the Dalgety fat or the soyabean oil based diets throughout the experimental period. Significant decreases palmitic acid levels were observed in all dietary treatments between days 3 and 6 post-hatch. Between days 6 and 9 post-hatch further reductions were observed in chicks receiving the Dalgety fat and soyabean oil based diets only. The level of palmitic acid remained unchanged after day 9 post-hatch in all the dietary treatments.

Palmitoleic acid levels were highest in chicks receiving the tallow oil based diet during the whole experimental period. Apart from differences observed on day 3 post-hatch there were no differences in the levels of palmitoleic acid between chicks receiving the Dalgety fat and soyabean oil based diets throughout the experiment. Levels of palmitoleic acid were to some extent affected by age in all dietary treatments, the pattern of change varying slightly between diets. In chicks receiving the Dalgety fat based diet decreases in palmitoleic acid levels occurred up to the ninth day post-hatch, then increased slightly thereafter. In chicks receiving the tallow oil based diet, there was no discernable trend although changes were occasionally observed. In chicks receiving the soyabean oil based diet a significant reduction in the level of palmitoleic acid occurred between days 3 and 6 post-hatch and no further changes were observed thereafter.

Table 6.9: Fatty acid composition (major long chain fatty acids, weight percentage of total present) in faecal lipid from chicks receiving different diets

		Age (days)					
		3	6	9	12	15	21
fatty acid:	Diet						
16:0*	1	17.3 ± 0.05	15.5 ± 0.32	13.7 ± 0.02	14.5 ± 0.68	15.4 ± 0.57	15.5 ± 0.27
	2	30.5 ± 0.13	28.8 ± 0.53	29.0 ± 0.24	27.1 ± 0.99	28.7 ± 0.27	28.9 ± 0.31
	3	17.7 ± 0.18	16.1 ± 0.40	14.2 ± 0.81	14.6 ± 0.38	14.8 ± 0.20	14.9 ± 0.48
16:1	1	2.3 ± 0.25	1.1 ± 0.10	0.8 ± 0.02	0.9 ± 0.06	1.1 ± 0.06	1.1 ± 0.04
	2	3.3 ± 0.12	3.7 ± 0.20	2.5 ± 0.23	3.1 ± 0.15	3.3 ± 0.01	2.8 ± 0.01
	3	1.4 ± 0.12	1.0 ± 0.03	0.9 ± 0.01	0.9 ± 0.02	0.9 ± 0.07	0.8 ± 0.05
18:0	1	6.4 ± 0.99	8.1 ± 0.27	6.2 ± 0.11	6.1 ± 0.31	5.8 ± 0.24	5.5 ± 0.30
	2	25.4 ± 0.29	22.8 ± 0.34	22.8 ± 0.53	20.2 ± 0.48	23.4 ± 0.32	23.0 ± 0.03
	3	6.3 ± 0.10	7.0 ± 0.02	6.8 ± 0.19	6.6 ± 0.40	7.0 ± 0.40	7.1 ± 0.11
18:1	1	31.0 ± 0.45	32.5 ± 0.50	31.7 ± 0.41	30.2 ± 0.27	28.9 ± 0.26	28.8 ± 0.48
	2	28.8 ± 0.56	26.1 ± 0.66	26.3 ± 0.06	29.1 ± 0.11	26.1 ± 0.21	24.1 ± 0.25
	3	26.7 ± 0.41	27.3 ± 0.22	27.5 ± 0.29	28.6 ± 0.21	28.4 ± 0.34	28.4 ± 0.89
18:2	1	36.7 ± 0.60	35.1 ± 0.30	39.9 ± 0.13	40.3 ± 0.70	40.1 ± 0.10	41.0 ± 0.65
	2	12.8 ± 0.81	13.7 ± 0.50	14.6 ± 0.13	15.4 ± 0.47	13.8 ± 0.70	15.3 ± 0.30
	3	37.9 ± 0.36	37.1 ± 1.11	38.6 ± 0.59	38.9 ± 0.76	38.5 ± 0.70	38.5 ± 0.68

* The common names of the fatty acids as per description Table 3.1

Table 6.9(ii): Statistical analyses on age and dietary fat sources effects on total fatty acid distribution in the faeces

fatty acid	diets	age 3	6	9	12	15	21	diet	a	b	c	d	e
16:0*	1 vs 2	***	***	***	***	***	***	1	*	*	*	N.S	*
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S	2	N.S	N.S	N.S	N.S	N.S
	2 vs 3	***	***	***	***	***	***	3	*	*	N.S	N.S	*
16:1	1 vs 2	*	**	***	***	***	***	1	*	*	*	N.S	*
	1 vs 3	*	N.S	N.S	N.S	N.S	N.S	2	N.S	N.S	*	*	*
	2 vs 3	**	**	***	**	***	***	3	*	N.S	N.S	N.S	*
18:0	1 vs 2	***	***	***	***	***	***	1	*	*	*	N.S	N.S
	1 vs 3	N.S	*	N.S	N.S	N.S	*	2	*	N.S	N.S	N.S	N.S
	2 vs 3	***	***	***	***	***	***	3	N.S	N.S	N.S	N.S	*
18:1	1 vs 2	**	*	**	**	**	**	1	N.S	N.S	*	N.S	*
	1 vs 3	*	*	**	**	N.S	N.S	2	*	N.S	N.S	*	N.S
	2 vs 3	**	N.S	*	N.S	**	**	3	N.S	N.S	N.S	N.S	*
18:2	1 vs 2	***	***	***	***	***	***	1	N.S	*	N.S	N.S	**
	1 vs 3	N.S	*	N.S	N.S	*	*	2	N.S	N.S	N.S	N.S	*
	2 vs 3	***	***	***	***	***	***	3	N.S	N.S	N.S	N.S	N.S
18:3	1 vs 2	***	***	***	***	***	***	1	N.S	N.S	N.S	N.S	N.S
	1 vs 3	N.S	*	*	N.S	N.S	**	2	N.S	N.S	N.S	N.S	N.S
	2 vs 3	***	***	***	***	***	***	3	N.S	N.S	N.S	N.S	N.S
20:4	1 vs 2	N.S	**	**	*	*	-	1	N.S	N.S	N.S	-	-
	1 vs 3	*	**	***	*	*	-	2	N.S	N.S	N.S	-	-
	2 vs 3	*	*	**	*	*	-	3	N.S	N.S	N.S	-	-

Table 6.10: The distribution of lilonenic and arachidonic acids in the total lipid and free fatty acid fraction of the faecal material from chicks receiving different diets

		Age (days)					
		3	6	9	12	15	21
fatty acid:	Diet						
total lipid							
18:3	1	6.1 ± 0.22	5.6 ± 0.13	6.0 ± 0.04	6.2 ± 0.10	6.2 ± 0.04	6.5 ± 0.09
	2	2.3 ± 0.04	2.5 ± 0.03	2.6 ± 0.07	2.7 ± 0.16	2.5 ± 0.09	2.8 ± 0.24
	3	6.4 ± 0.12	6.1 ± 0.20	6.5 ± 0.02	6.5 ± 0.08	6.0 ± 0.31	6.0 ± 0.05
20:4	1	0.7 ± 0.01	0.5 ± 0.01	0.5 ± 0.19	0.5 ± 0.02	0.6 ± 0.08	-
	2	0.7 ± 0.01	1.0 ± 0.09	1.1 ± 0.06	1.3 ± 0.03	1.2 ± 0.06	-
	3	1.5 ± 0.38	1.9 ± 0.16	2.0 ± 0.03	1.6 ± 0.06	1.6 ± 0.09	-
free fatty acid							
18:3	1	4.4 ± 0.75	3.2 ± 0.26	4.6 ± 0.08	3.5 ± 0.21	3.9 ± 0.09	4.2 ± 0.16
	2	1.9 ± 0.01	3.2 ± 0.40	3.1 ± 0.32	2.8 ± 0.30	2.8 ± 0.08	2.5 ± 0.01
	3	2.3 ± 0.10	4.2 ± 0.50	4.0 ± 0.15	3.7 ± 0.06	2.9 ± 0.52	3.0 ± 0.02

* The common names of the fatty acids as per description in Table 3.1

Stearic acid levels were highest in chicks receiving the tallow oil based diet in comparison to those receiving either the Dalgety fat or the soyabean oil based diets throughout the experimental period. The levels of stearic acid in most cases did not differ significantly between chicks receiving the Dalgety fat and soyabean oil based diets. Apart from a few exceptions in all dietary treatments the levels of stearic acid were not affected by age.

Oleic acid was a major fatty acid in the faeces comprising more than 20 percent of total fatty acids present in all dietary treatments. However, levels were highest in chicks receiving the Dalgety fat based diet between days 3 and 12 post-hatch and lowest in chicks receiving the tallow oil based diet throughout the experimental period. After the twelfth day post-hatch the proportions of oleic acid were similar between chicks receiving the Dalgety fat and the soyabean oil based diets. Significant changes in the proportion of oleic acid occurred between days 3 and 6 post-hatch in chicks receiving the Dalgety fat and the tallow oil based diets and between days 15 and 21 post-hatch in chicks receiving the tallow oil based diet.

The faecal material from chicks receiving either the Dalgety fat or the soyabean oil based diets contained significantly higher levels of linoleic acid than chicks receiving the tallow oil based diet throughout the experimental period. In chicks receiving the Dalgety fat and the soyabean oil based diets linoleic acid levels did not differ significantly during most of the experimental period. In general there were no apparent changes in the level of linoleic acid due to age in all dietary treatments.

As in the case of linoleic acid, linolenic acid was highest in chicks receiving either the Dalgety fat or the soyabean oil based diets throughout the experimental period. Levels of linolenic acid remained unchanged in all dietary treatments throughout the experimental period.

The apparent digestibilities i.e the difference in the absolute amount of fatty acid intake and excretion of the total lipid and the major fatty acids in total faecal lipid are shown in Figures 6.5(i-v). The apparent digestibility of total lipid was over 80 percent on day 3 in all the dietary treatments and a significant decline was observed in all treatments between day 3 and 6 post-hatch. However, increases in apparent digestibility were observed after day 9 post-hatch in chicks receiving either the Dalgety fat or the soyabean oil based diets whereas increases occurred after days 12 post-hatch in chicks receiving the tallow oil based diet.

The apparent digestibilities of palmitic, stearic, oleic and linoleic acids were over 80 percent in all dietary treatments on day 3 but declined significantly between days 3 and 6 post-hatch. After day 6, a gradual increase in the apparent digestibility of palmitic acid was observed in chicks receiving the Dalgety fat based diet whereas, further decreases occurred in chicks receiving the tallow oil and the soyabean oil based diets. However, after day 9 and 12 post-hatch increases in apparent digestibility were observed in chicks receiving the soyabean oil and the tallow oil based diets respectively. Throughout the experimental period the apparent digestibility of palmitic acid was highest in chicks receiving the soyabean oil based diet and lowest in chicks receiving the tallow oil based diet.

Between days 3 and 6 post-hatch there were also significant declines in apparent digestibilities for stearic, oleic and linoleic acids in all dietary treatments. After day 6 post-hatch different patterns were observed between chicks receiving the different diets. In chicks receiving the Dalgety fat based diet increase in the apparent digestibility of all acids was observed. In chicks receiving the tallow oil based diet the stearic acid levels remained relatively constant between days 6 and 15 post-hatch, followed thereafter by a drastic increase. Apparent digestibility for oleic and linoleic acids declined further between days 9 and 12 post-hatch, followed by

Figure 6.5(i): The apparent digestibility of total lipid

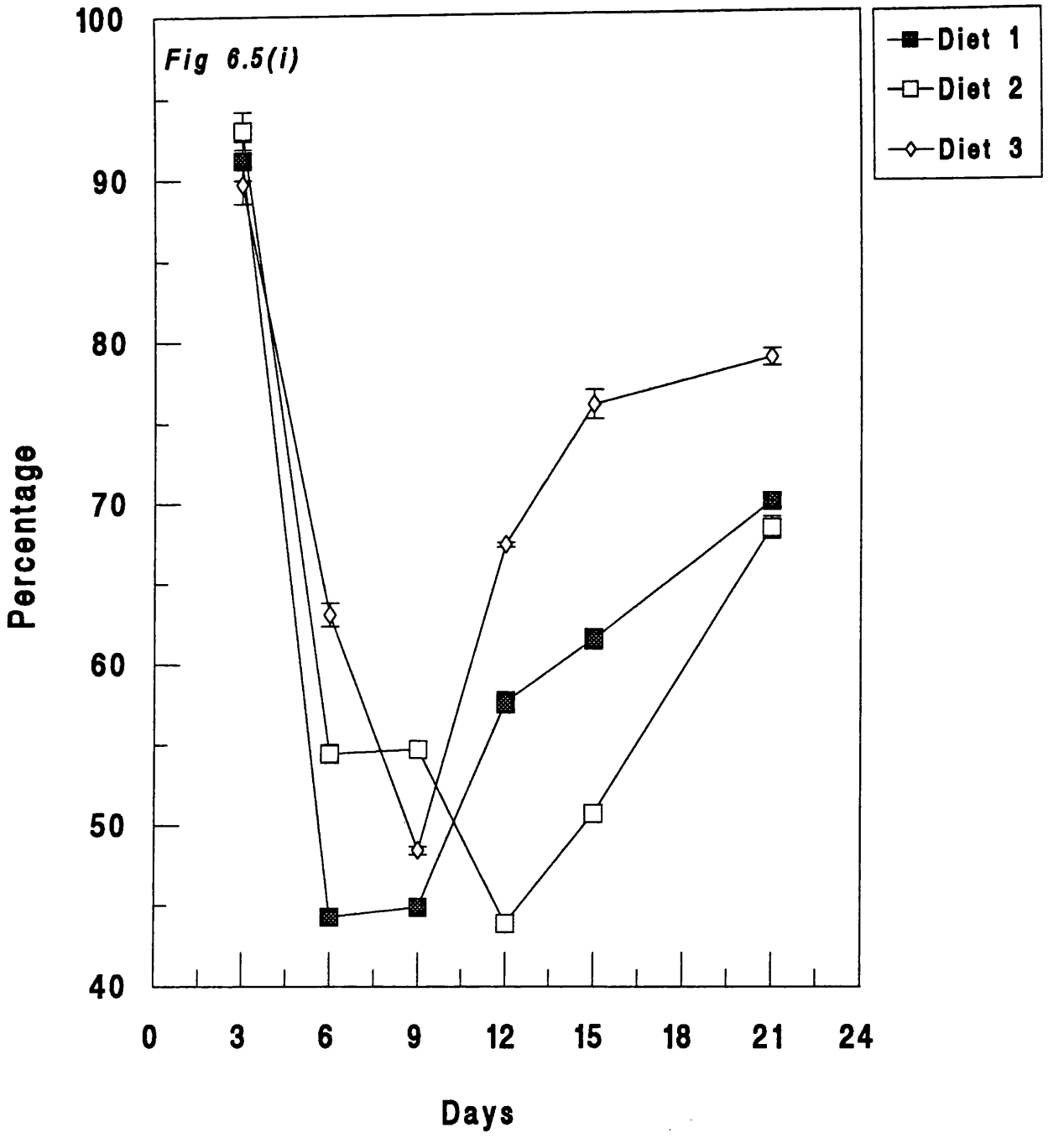
Fig 6.5(i)
Diet 1
Diet 2
Diet 3
Percentage
100
90
80
70
60
50
40
0
3
6
9
12
15
18
21
24
Days

Figure 6.5(ii-v): The apparent digestibility of the major long chain fatty acids

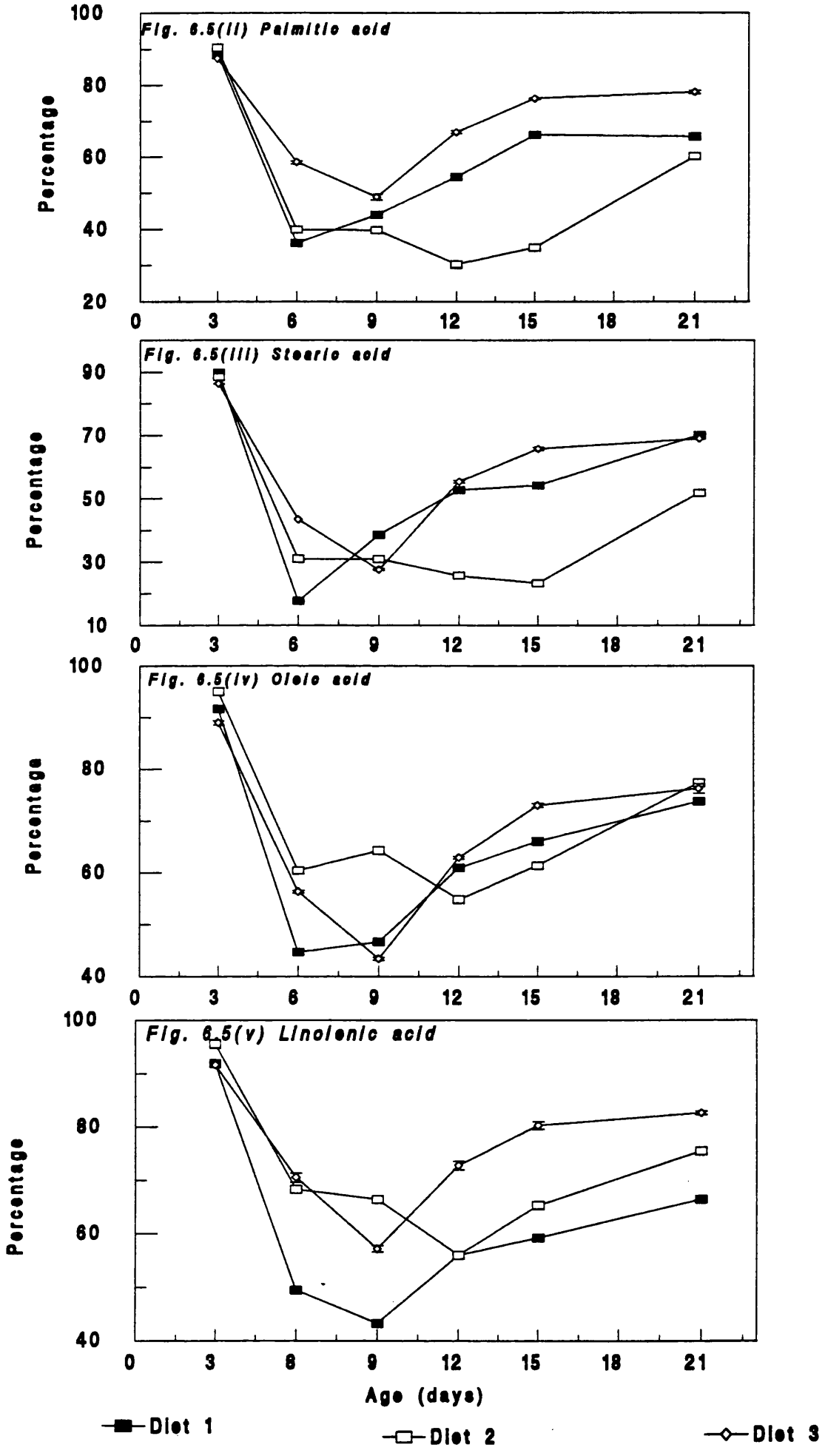
Fig. 6.5(ii) Palmitic acid
Percentage
100
80
60
40
20
0
3
6
9
12
15
18
21
Fig. 6.5(iii) Stearic acid
Percentage
90
70
50
30
10
Fig. 6.5(iv) Oleic acid
Percentage
100
80
60
40
Fig. 6.5(v) Linolenic acid
Percentage
100
80
60
40
Age (days)
Diet 1
Diet 2
Diet 3

gradual increases thereafter. In chicks receiving the soyabean oil based diet decreases in the apparent digestibilities for stearic, oleic and linoleic acids occurred between days 6 and 9 post-hatch afterwhich large increases were observed. Between days 9 and 21 post-hatch, the apparent digestibilities of most acids were highest in chicks receiving the soyabean oil based diet and lowest in chicks receiving the tallow oil based diet.

6.3.5 Fatty acid composition and changes in the free fatty acid fraction

The distribution of the major fatty acids in the free fatty acid fraction, i.e the major lipid fraction of the faecal material in chicks receiving the different dietary treatments are presented in Tables 6.11. The distributions of fatty acids in this fraction were to some extent similar to those observed for the total fatty acid distributions.

With few exceptions the levels of palmitic and palmitoleic acids were highest in chicks receiving the tallow oil based diet compared to levels in chicks receiving either the Dalgety fat or the soyabean oil based diets. Significant decreases in palmitic acid levels were observed between days 3 and 6 in chicks receiving the tallow oil based diet and between days 3 and 12 post-hatch in chicks receiving the soyabean oil based diet. Changes observed in chicks receiving the Dalgety fat based diet did not show any consistency. Increase in palmitic acid levels occurred between days 15 and 21 post-hatch in chicks receiving the tallow oil and the soyabean oil based diets. The level of palmitoleic remained unchanged throughout the experimental period in all the three dietary treatments.

Apart from day 6 post-hatch the proportions of stearic acid were highest in chicks receiving the tallow oil based diet and lowest in chicks receiving the Dalgety fat based diet throughout the experiment. Significant changes in levels of stearic

Table 6.11: Fatty acid composition (major long chain fatty acids, weight percentage of total present) of the free fatty acid in the faeces from chicks receiving different diets

		Age (days)					
		3	6	9	12	15	21
fatty acid:	Diet						
16:0*	1	23.7 ± 0.08	24.4 ± 0.15	20.9 ± 0.67	25.8 ± 0.92	27.4 ± 0.57	24.3 ± 0.12
	2	32.6 ± 0.23	24.5 ± 0.16	24.3 ± 0.42	28.2 ± 0.67	24.8 ± 0.19	29.8 ± 0.71
	3	27.4 ± 0.55	23.4 ± 0.61	22.4 ± 1.05	24.3 ± 0.54	27.7 ± 0.23	30.8 ± 0.51
16:1	1	1.6 ± 0.47	2.3 ± 0.56	1.5 ± 0.11	1.8 ± 0.35	1.5 ± 0.08	1.5 ± 0.06
	2	3.0 ± 0.26	1.8 ± 0.40	3.4 ± 0.15	3.1 ± 0.17	3.0 ± 0.12	2.9 ± 0.01
	3	2.0 ± 0.15	1.8 ± 0.28	1.7 ± 0.12	1.7 ± 0.54	1.9 ± 0.18	2.1 ± 0.02
18:0	1	11.9 ± 0.48	16.6 ± 0.85	6.1 ± 0.50	13.1 ± 0.40	10.7 ± 0.51	8.0 ± 0.51
	2	30.2 ± 1.15	17.0 ± 0.28	18.5 ± 0.89	21.8 ± 0.36	23.6 ± 0.15	24.5 ± 0.51
	3	18.0 ± 0.41	9.1 ± 0.71	8.6 ± 0.65	11.5 ± 0.21	19.9 ± 0.19	21.6 ± 0.72
18:1	1	28.7 ± 0.07	27.7 ± 0.15	29.9 ± 0.54	26.1 ± 0.13	26.6 ± 0.26	28.8 ± 0.10
	2	21.1 ± 0.89	28.2 ± 0.44	29.6 ± 0.90	26.4 ± 0.80	25.3 ± 0.62	23.4 ± 0.26
	3	24.6 ± 0.34	29.7 ± 0.67	30.3 ± 0.72	28.2 ± 0.22	21.9 ± 0.46	20.1 ± 0.64
18:2	1	29.8 ± 0.43	23.2 ± 0.40	33.2 ± 0.98	25.8 ± 0.79	26.8 ± 0.81	24.2 ± 0.33
	2	9.3 ± 1.00	22.3 ± 0.96	18.0 ± 0.51	15.0 ± 0.18	14.7 ± 0.64	14.5 ± 0.25
	3	21.8 ± 0.56	29.3 ± 0.27	29.6 ± 0.32	26.9 ± 0.01	21.1 ± 0.83	18.4 ± 0.51

* The common names of the fatty acids as per description in Table 3.1

Table 6.11(ii): Statistical analyses of the age and dietary fat sources on the distribution of fatty acids in the faeacl free fatty acids

fatty acid	diets	age						diet	a	b	c	d	e
		3	6	9	12	15	21						
16:0*	1 vs 2	***	N.S	*	*	*	*	1	N.S	**	**	*	N.S
	1 vs 3	**	N.S	*	*	N.S	N.S	2	***	N.S	N.S	*	*
	2 vs 3	**	N.S	*	*	*	*	3	*	N.S	*	*	*
16:1	1 vs 2	N.S	N.S	*	*	*	*	1	N.S	N.S	N.S	N.S	N.S
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S	2	*	*	N.S	N.S	N.S
	2 vs 3	N.S	N.S	*	*	*	*	3	N.S	N.S	N.S	N.S	N.S
18:0	1 vs 2	***	N.S	**	**	**	***	1	*	***	*	*	*
	1 vs 3	*	**	*	*	*	***	2	***	N.S	*	N.S	*
	2 vs 3	***	**	***	***	**	*	3	**	N.S	***	N.S	*
18:1	1 vs 2	**	N.S	N.S	N.S	N.S	*	1	N.S	*	*	N.S	N.S
	1 vs 3	*	N.S	N.S	*	*	**	2	**	N.S	*	*	N.S
	2 vs 3	*	N.S	N.S	*	*	*	3	*	N.S	**	N.S	*
18:2	1 vs 2	***	N.S	***	***	**	***	1	*	***	*	**	*
	1 vs 3	*	*	*	*	*	**	2	***	*	*	N.S	*
	2 vs 3	***	*	**	*	**	***	3	**	N.S	**	*	*
18:3	1 vs 2	*	N.S	*	N.S	*	*	1	N.S	*	*	N.S	N.S
	1 vs 3	*	*	*	N.S	*	*	2	*	N.S	*	N.S	*
	2 vs 3	N.S	*	*	*	N.S	*	3	*	N.S	*	N.S	N.S

acid with age occurred in all dietary treatments during the experimental period. In chicks receiving the Dalgety fat based diet, increases were observed between days 3 and 6 and 9 and 12 post-hatch whereas, decreases occurred between days 6 and 9 and 15 and 21 post-hatch. In chicks receiving the tallow oil and soyabean oil based diets decreases in stearic acid levels were observed between days 3 and 6 post-hatch whilst, increases occurred after day 9 post-hatch.

On day 3 post-hatch oleic acid levels were highest in chicks receiving the Dalgety fat based diet and lowest in chicks receiving the tallow oil based diet. No significant differences were observed between the dietary treatments during day 6 and 12 post-hatch. Between days 15 and 21 oleic acid levels were lowest in chicks receiving the soyabean oil based diet. Some changes in the levels of oleic acid due to age were observed during the experimental period. Increases were observed in chicks receiving the tallow oil and soyabean oil based diets between days 3 and 6 post-hatch. Significant decreases of oleic acid in all dietary treatments occurred between days 9 and 12 post-hatch. After day 12 post-hatch such decreases were confined to chicks receiving the tallow oil (between days 15 and 21 post-hatch) and in chicks receiving the soyabean oil based diet (between days 12 and 21 post-hatch).

The proportions of linoleic acid were lowest in chicks receiving the tallow oil based diet throughout the experimental period and highest in chicks receiving the Dalgety fat based diet on days 3, 9, 15 and 21 post-hatch. The changes due to age observed in chicks receiving the Dalgety fat based diet did not show any consistency. In chicks receiving the tallow oil based diet significant increases were observed between days 3 and 6 post-hatch whereas decreases occurred between days 6 and 9 post-hatch. No further changes were observed thereafter. In chicks receiving the soyabean oil based diet increases in linoleic acid levels occurred between days 3

and 6 whilst decreases occurred between days 9 and 21 post-hatch. Apart from differences observed on day 3 post-hatch the levels of linolenic acid were not significantly affected by either age or diet.

6.4 DISCUSSION

The effects of dietary fat sources and age on the overall utilization of fat by young chicks are discussed. The diets used in this study were similar to those used in experiments 1 and 2, except for Diet 1 which was compounded using Dalgety fat (Dalgety Agriculture Company LTD, Bristol, England) but in combination with ingredients similar to those used in the other diets.

The lipid and fatty acid compositions of dietary fat sources and compounded diets accord with those obtained in experiment 1. The high levels of free fatty acid present in Dalgety fat were unexpected. However, this accord with suggestions put forward by Akinyemi (1989) that apart from hydrolysis during feed compounding, dietary fat sources also contribute to the high free fatty acid levels associated with compounded diets. Such high levels of free fatty acids in the fat sources might be due to the extensive in the poultry industry of by-products from the fat/oil industry which are readily available and cheap as dietary fat sources (Watkins, 1987). Most of these by-products contain characteristically high free fatty acid levels. The observations of Akinyemi (1989) and those obtained in the present study accord such a suggestion. Watkins (1987) pointed out that most of the feed manufacturers are aware of the characteristics of the fat sources they use and their effect on the bird's performance. Nevertheless, the cost of production and the availability of cheaper materials is given greater importance.

Pattison (1989) showed that fat excretion was a major indicator of fat utilization and digestibility in the chick. In the present investigations there were unexpectedly high levels of apparent fat digestibilities on day 3 post-hatch. These high levels might have been due to low feed intake accompanied by low efficiency in intestinal emptying during the first 3 post-hatch days (Carew *et al.*, 1972). The lower feed intake observed during this period may have been due to the nutritional contribution arising of the residual yolk reserves towards the nutrient requirements of the chick, which is thought to be substantial during the early post-hatch days. Over the first three days post-hatch the yolk reserves may account for up to 29% energy and 42% protein of the dietary intakes, thereby reducing the required exogenous dietary intake of the chick (Murakami *et al.*, 1988). Carew *et al.* (1972) attributed lower feed intakes with the time required by the chick to initiate the process of eating and for the time necessary for the food to traverse the GIT. Various studies have shown that the utilization of fat by chicks improves markedly with post-hatch age (Renner and Hill, 1960; Hakansson, 1974; Polin *et al.*, 1980; Krogdahl, 1985; Krogdahl and Sell, 1989). The high apparent fat digestibilities observed during the first 3 days post-hatch and subsequent low digestibilities between days 6 and 9 post-hatch is in contradiction to such observations. The possible reasons underlying these discrepancies are equivocal. However, it is suggested that it may have been partly due to the physiological adjustments by the chick to exposure to both diets and the environment. The early stages of the chick life is accompanied by many changes in fat metabolism (Akiba *et al.*, 1988). Most of these changes are affected by the the sudden change in lipid source available to the chick i.e mainly from the yolk to exogenous fat sources (Krogdahl, 1985). However, the time span over which these changes can be accommodated is very difficult to determine. The high apparent fat digestibilities in chicks receiving the

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soyabean oil based diet observed in the present study were in agreement with findings reported elsewhere (Renner and Hill, 1961b; Ketels and De Groote, 1987). The rate of improvement in apparent fat digestibilities between day 9 and 21 post-hatch was highest in chicks receiving the tallow oil based diet than those receiving either the soyabean oil based diet or the Dalgety fat based diet, in-spite of the low digestibilities values observed in the former. These findings agree with other reported sudies which showed that the improvement with age in fat utilization by chicks was higher for saturated fats than the unsaturated fats (Fedde *et al.*, 1960; Carew *et al.*, 1972; Krogdahl, 1985). Again the underlying cause for this is unclear, although it is thought that it may be a consequence of a compensatory promotion of enzymic mechanisms in order to achieve normal levels of fat digestibility.

The apparent digestibility of total lipid and the major long chain fatty acids observed in the present study were consistent with the fact that unsaturated fatty acids are more digestible than saturated ones (Renner and Hill, 1961a; Freeman, 1969; Annison, 1983; Ketels *et al.*, 1986b; Wiseman and Lessire, 1987). The low digestibility of long chain saturated fatty acids is normally attributed to their lower polarity and difficulty in forming emulsions. In the present study the apparent digestibility of stearic acid was significantly lower compared to palmitic acid in all treatments. This would indicate that the utilization of saturated fatty acids is inversely related to increasing chain length of the fatty acid (Renner and Hill, 1961; Wiseman and Lessire, 1987). Differences observed in the present study being highest in chicks receiving the soyabean oil based diet and lowest in chicks receiving the tallow oil based diet are in agreement with findings reported by (Young 1961; Young and Garret, 1963; Ketels and De Groote, 1989). This might have been due to the synergistic effect in which the utilization of saturated fatty acids is improved by the presence of unsaturated ones. The apparent digestibilities

obtained in the present study of 46.3% and 69.0% for stearic acid in tallow and soyabean oil based diets, respectively were lower compared to those reported by Young (1961) of 57.1% and 89.2% when stearic acid was measured in beef tallow and maize oil, respectively. This might has been associated with the ratio between the unsaturated and saturated fatty acids which is an important factor influencing the utilization of the latter. Lall and Slinger (1973) suggested that ratios ranging from 2.15:1 to 2.62:1 of unsaturated and saturated fatty acid was optimum, whilst, Ketels and De Groote (1989) showed that the synergistic effect reached its maximum level at unsaturated:saturated ratio of 4:1. The high ratio of 5.2:1 between oleic and stearic acids in the soyabean oil based diet observed in the present study might have limited the improvement in the absorbability of stearic acid because higher ratios can induce deficiency of some nutrients which are vital in the digestion processes (McDonald *et al.*, 1988). Additionally it has also to be considered that the low levels of apparent digestibility observed in the present study could have been associated with other factors such as age of chicks. The physiological basis for the improvements observed in the utilization of saturated fatty acids when unsaturated fatty acids are added to the diet, include increases in the availability of the fatty acids to the enzymes (Wiseman and Lessire, 1987) and ease of micelle formation (Freeman, 1969; Garret and Young, 1975). Wiseman and Lessire (1987) showed that the availability of palmitic and stearic acids was relatively low in pure tallow but increased gradually when rape oil was added to the fat mixture. On the other hand (Freeman, 1969; Garret and Young, 1975) showed that entry into the micellar phase was mostly achieved by solutes that are polar. For example unsaturated fatty acids of chain length C16 and C18 may act as swelling amphiphiles thereby promoting the entry into micellar phase of non polar solutes e.g saturated fatty acids of chain length C16 and C18. It is clear from the present results that the digestibilities of

unsaturated fatty acids differed between diets, being highest in the soyabean oil based diet and lowest in the tallow oil based diet. This agrees with findings of Wiseman and Lessire (1987) who found out that both oleic and linoleic acids were poorly utilized by chicks when they were contained within predominantly saturated fats. The utilization of unsaturated polar fatty acids is thought to be impaired by the presence of large amounts of saturated non-polar fatty acids particularly when the bile concentration is limited, a feature normally observed in young chicks (Wiseman and Lessire, 1987). The cause of low fatty acid utilization in chicks that received the Dalgety fat based diet is equivocal since the levels of unsaturated fatty acids in this diet were similar to those of the soyabean oil based diet. However, it is possible that the presence of high free fatty acid levels in that diet may have played some role.

Many studies have shown that the young chick is unable to utilize fat efficiently especially saturated fat (Renner and Hill, 1961; Carew *et al.*, 1972; Ketels and De Groote, 1989; Wiseman and Lessire, 1987). However, a marked improvement in fat utilization normally occurs with age (Krogdahl, 1985; Wiseman and Lessire, 1987; Akiba *et al.*, 1988). The results obtained in the present study are in agreement with the existing ideas, although the apparent fat and fatty acid digestibilities were exceptionally high on day 3 day post-hatch. As already noted, reasons for such observations are unclear although it may have been due to low feed intake and the continuation of the "embryonic" enzymic activities. Improvements in fat and fatty acid utilization with age were observed in all dietary treatments, being most notable in saturated fats.

The probable causes for low fat absorption by young chicks include low bile production (Polin *et al.*, 1980) low enzyme activity (Akiba *et al.*, 1988; Sell *et al.*, 1988) and low dietary fat requirements during the early post-hatch days (Ewing, 1963; Fedde *et al.*, 1960). Polin *et al.* (1980) observed significant improvement in

the absorption of tallow during days 1-7 post-hatch when chenodeoxycholic acid was added to the chicks diet. This indicated that bile production might be the limiting factor in the absorption of fat, especially the saturates, during the first week of life. Additionally lack of response to fat supplementation during the early post-hatch days reported by Fedde *et al.* (1960) led to the assumption that the newly chick had a low requirement of exogenous fat probably due to the endogenous supply of nutrients from the yolk reserves (Murakami *et al.*, 1988). Feeding high levels of fat at an early age normally lead to high fat excretion which in some instances result in a deficiency of other nutrients due to shorter transit retention time in young chicks when compared to adult chickens (Mateos *et al.*, 1982; Golian and Polin, 1984). Similarly, Renner and Hill (1961a) showed that the absorbability of saturated fatty acids was lower in chicks when compared to other monogastric species mainly because of the relative differences in transit retention times; for instance whereas in the chick the transit retention time is 3 hours in the rat it is 16 hours. The changes in the utilization levels of fat observed in the present study on day 9 post-hatch seem to mark an important turning in with respect to fat digestion processes since following the 9^{th} day post-hatch considerable positive changes in fat uptake were observed irrespective of the fatty acid composition of the diet.

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CHAPTER 7

THE EFFECT OF DIETARY FAT SOURCES AND AGE ON THE ESTABLISHMENT OF MICRO-ORGANISMS IN BROILER CHICKS

7.1 INTRODUCTION

Most studies in the chicken have shown that the establishment of micro-organisms in the GIT begins soon after hatching and may be completed within a period of 2-3 weeks post-hatch (Shapiro and Sarles, 1949; Humbert *et al.*, 1989). The establishment, type and numbers of micro-organisms present in the GIT of chickens is significantly affected by both environmental and dietary conditions (Morishita *et al.*, 1982; Furuse and Yokota, 1985; Muramatsu *et al.*, 1988). Thus studies to evaluate the effects of environmental conditions on the establishment of micro-organisms and subsequent performance of chickens have been widely performed. Generally it has been shown that the performance of germ free chicks in terms of nutrient utilization, body weight gain and other parameters is higher than the performance observed in conventional chicks (Okumura *et al.*, 1976; Morishita *et al.*, 1982; Furuse and Yokota, 1984; Furuse and Yokota, 1985). The difference in performance between germ free and conventional chicks is more pronounced when dietary nutrients such as energy and protein are fed at marginal levels (Furuse and Yokota, 1985). This led to the assumption that dietary nutrients requirements may be higher in conventional chicks probably due to extra demands arising from the presence of large numbers of micro-organisms. Nevertheless, studies to evaluate the effects of different dietary nutrients on the microbial establishment in the chicken GIT are limited and in some cases results obtained are very variable. This is probably due to many factors amongst which is the large variety of micro-organisms present in the GIT. Whereas a few studies to assess the effects of dietary proteins and carbohydrates on the GIT flora of chickens have been performed (Harrison and Coates, 1972; Morishita *et al.*, 1982) limited work has been undertaken in regard to

the effect of different dietary fat sources on the establishment and growth of the GIT flora in young chicks. Therefore the present study was carried out to investigate the following:

(i) find out if different dietary fat sources affect the establishment of the GIT microflora in young broiler chicks

(ii) assess the changes in the GIT flora with respect to age and diet

7.2 MATERIALS AND METHODS

7.2.1 Treatments

Three dietary treatments used in this study were similar to those used in experiment 3. The treatments were designated as Diet 1, Diet 2, and Diet 3 in which Dalgety fat, tallow oil and soyabean oil provided the major fat sources respectively. The basic description and proximate composition of the diets have already been described (see Table 6.1.)

7.2.2 Preparation of media

The previous studies by Salanitro *et al.* (1978) have shown that the GIT of the young chick is mainly inhabited by Lactobacilli, Clostridia, Streptococci and Coliform species. Due to the wide diversity of the micro-organisms present in the GIT of chickens different media are normally used in the identification of different micro-organisms.

The following established media were used in the present study

Rogosa agar (Rogosa *et al.*, 1951)

The agar was used for isolating Lactobacilli spp.

82 gm of Rogosa mixture (Oxoid) was dissolved in 1 litre of distilled water and then boiled gently while shaking.

MacConkey agar (Fuller, 1973)

The agar was used to isolate Coliforms.

52 gm of MacConkey agar (Oxoid) mixture was dissolved in 1 litre of distilled water and then boiled gently while shaking. After boiling, 90 ml of molten Rogosa and MacConkey were each separately poured into medical flat bottles, capped lightly and then autoclaved at 121^{o}C for 15 minutes.

Thallous acetate tetrazolium glucose agar (TATG) Barnes (1956) adapted by Mead, (1978)

The agar was used for isolation of Streptococci species.

10 g of peptone and 8 g of Lab lemco were dissolved in 500 ml of distilled water. The pH value was adjusted to 6.0-6.1 using hydrochloric acid and sodium bicarbonate. Bacteriological agar was dissolved separately since it needed boiling. The two solutions were mixed and thoroughly shaken and 92 ml of the mixture was poured into medical flat bottles, lightly capped and then autoclaved at 121^{o}C for 15 minutes. After cooling the agar was stored at room temperature. Before pouring, the following solutions were added to each of the 92 ml of molten basal medium

(i) 5 ml of 20 % (w/v) filter sterilised glucose

(ii) 2 ml of 5 % (w/v) thallous acetate autoclaved at 115^{o}C for 15 minutes

(iii) 1 ml of 1 % (w/v) 2:3:5 tripheny- tetrazolium chloride.

7.2.3 Chick rearing

One hundred and twenty day old Ross Breeder chicks from the Scottish Agricultural College at Auchincruive were randomly distributed to each dietary treatment after weighing. The chicks were kept in floored pens (3m x 2.5m) covered with wood shavings to about 2.5 cm thick. Two hanging feed hoppers were placed in

each pen. The initial room temperature was 35 $\pm$ 0.5^{o}C and was lowered by 3^{o}C per week. Feed and clean water were provide *ad libitum*. The lighting programme used was the standard; 23 hours of lighting and 1 hour of darkness every 24 hours.

7.2.4 Sampling

As far as possible efforts were made to minimise environmental contaminations collection of samples was carried out in a microbiological cabinet and sterilised equipments were used. On the first day post-hatch 3 chicks were killed by neck dislocation followed by laparatomy. The GIT (the duodenum, both the upper and lower ileum and the two caecal pouches) sections were individually removed from the chick's body and put straight into a preweighed sterilised sample bottle. The bottle was weighed again. After weighing 10 g of the sample was mixed with 90 ml of sterile Reinforced Clostridial Medium and the mixture were homogenised for 1 minute. The resultant homogenate gave a concentration of 10^{-1}.

After homogenization, the homogenate was transferred to a sterilised bottle and then serial dilutions were made (i.e 1 ml of the homogenate was transferred to a universal bottle containing 9 ml of sterile Reinforced Clostridial Medium until a final concentration 10^{10} was obtained. To ensure that the organisms were evenly dispersed the bottle was shaken vigorously between each dilution.

7.2.5 Plating

Double layered plates were used to isolate the different micro-organisms. Dilutions used were 10^{-6}, 10^{-7} and 10^{-8} and each dilution was duplicated. 1 ml of the solution from each dilution containing the micro-organisms was placed on an empty sterile petri dish followed by about 18 mls of molten agar at 45^{o}C. After thorough mixing the contents were left to solidify at room temperature before

pouring another layer of the medium on top. The plates were incubated at 37°C depending on the recommended time for each medium as described below. After incubation the plates with colonies between 30 and 300 were divided into four quarters and direct counting was carried out. The description and colour of the individual colonies was also recorded. This was followed by gram staining.

Lactobacillal species

Plating procedures described in section 7.2.3 were followed using Rogosa agar. The inoculated plates were incubated at 37°C for 48 hours and thereafter the organisms were enumerated. All creamy to white hexagonal colonies were counted. Gram negative cocci clustered colonies were identified.

Streptococcal species

Plating procedures described in section 7.2.3 were also followed using Thallous Acetate Tetrazolium Agar. The plates were incubated at 37°C for 3 days. All pink and light red round colonies were counted. Gram positive cocci, mainly single but with few clustered cells were regarded as Streptococci.

Coliforms

Plating procedures described in section 7.2.3 were followed using MacConkey agar. The plates were incubated at 37°C for 1-2 days. Counting included only large deep red colonies. Gram negative red shaped cells were regarded as coliforms.

Clostridial species

These were identified by the Most Probable Number (MPN) method described by Hirsch and Grinsted (1954). In addition a black tube method using Differential Reinforced Clostridial Medium was used. In the MPN, method 1 ml of the sample was added gently to Bijoux bottles containing 4 ml of sterile Reinforced Clostridial Medium, care was undertaken to avoid air bubbles in the medium and no air space was left. 5 bottles were used for each dilution. The inoculated samples were incubated at 37°C for 1-2 days depending on the intensity of growth. White filaments varying in intensity seen after the incubation period in the bottles were regarded as positive.

For the Black tube method, 1 ml of the sample was added to the Differential Reinforced Clostridial Medium in the universal bottle, capped and incubated at 37°C for 3 days. The change in colour from green to black was taken as a positive result.

7.3 RESULTS

7.3.1 Body weight changes

The mean body weights of chicks during the experimental period are shown in Table 7.1. The overall average weight gain was highest in chicks receiving the soyabean oil based diet and lowest in chicks receiving the tallow oil based diet.

7.3.2 Micro-organisms in the different sections of the GIT

The GIT was divided into three sections namely, the duodenum; ileum and caecum. Figures 7.1, 7.2 and 7.3 shows the distribution of the different species of micro-organisms in in the duodenum, ileum and the caecum, respectively from chicks receiving different dietary treatments. The distribution of the Clostridial

species within different sections of the GIT is shown in Table 7.2.

(A) Duodenum

Clostridial species.

On day 1 post-hatch day there was no growth of Clostridia. After one week post-hatch the numbers of Clostridia were highest in chicks receiving the Dalgety fat based diet whereas no growth was observed in chicks receiving the tallow oil based diet. Reduction in the levels of Clostridia species with post-hatch age was observed from 10^8 on day 7 to less than 10^5 on day 26 were observed in all dietary treatments.

Lactobacillal species

In the present study Lactobacilli species were not detected in the duodenum in any of the dietary treatments at any time during the experimental period.

Streptococcal species

The Streptococci species were only found on day 26 day post-hatch and levels were slightly higher in chicks receiving the tallow oil based diet.

Coliform species

The Coliforms were only detected on day 1 post-hatch.

(B)Ileum

Clostridial species

The numbers of Clostridia spp. in the ileum (see Table 7.2) on the first day post-hatch were approximately 10^8 and remained relatively unchanged throughout the experimental period in chicks receiving the Dalgety fat based diet whereas

Table 7.1: The effect of different dietary fat sources on body weight gain in chicks

	diet		
	1	2	3
age (days):	body weight (gram)		
1	37.5	35.8	34.8
12	273.0 ± 11.7	254.4 ± 4.1	254.3 ± 16.7
25	964.4 ± 22.9	905.9 ± 23.7	1049.3 ± 27.8
average weight gain (g)	925.5	870.1	1011.8

Diet names as per description in Table 6.1

Table 7.2: The effect of age and dietary fat sources on the distribution of Clostridial along the GIT of growing broiler chicks

		gut section		
		Duodenum	Ileum	Caecum
age (days)	diet			
1		N.D	$<10^8$	$>10^9$
7	1	$<10^8$	$<10^{86}$	<10
	2	N.D	$>10^9$	$<10^8$
	3	$<10^6$	$<10^{68}$	<10
14	1	N.D	$<10^8$	$<10^8$
	2	N.D	$<10^7$	$<10^8$
	3	N.D	N.D	$>10^6$
26	1	$<10^5$	$<10^{86}$	<10
	2	$<10^5$	$<10^{97}$	<10
	3	$<10^5$	$<10^{86}$	<10

N.D= Not detected

Figure 7.1: The distribution of Streptococci spp. in the duodenal contents

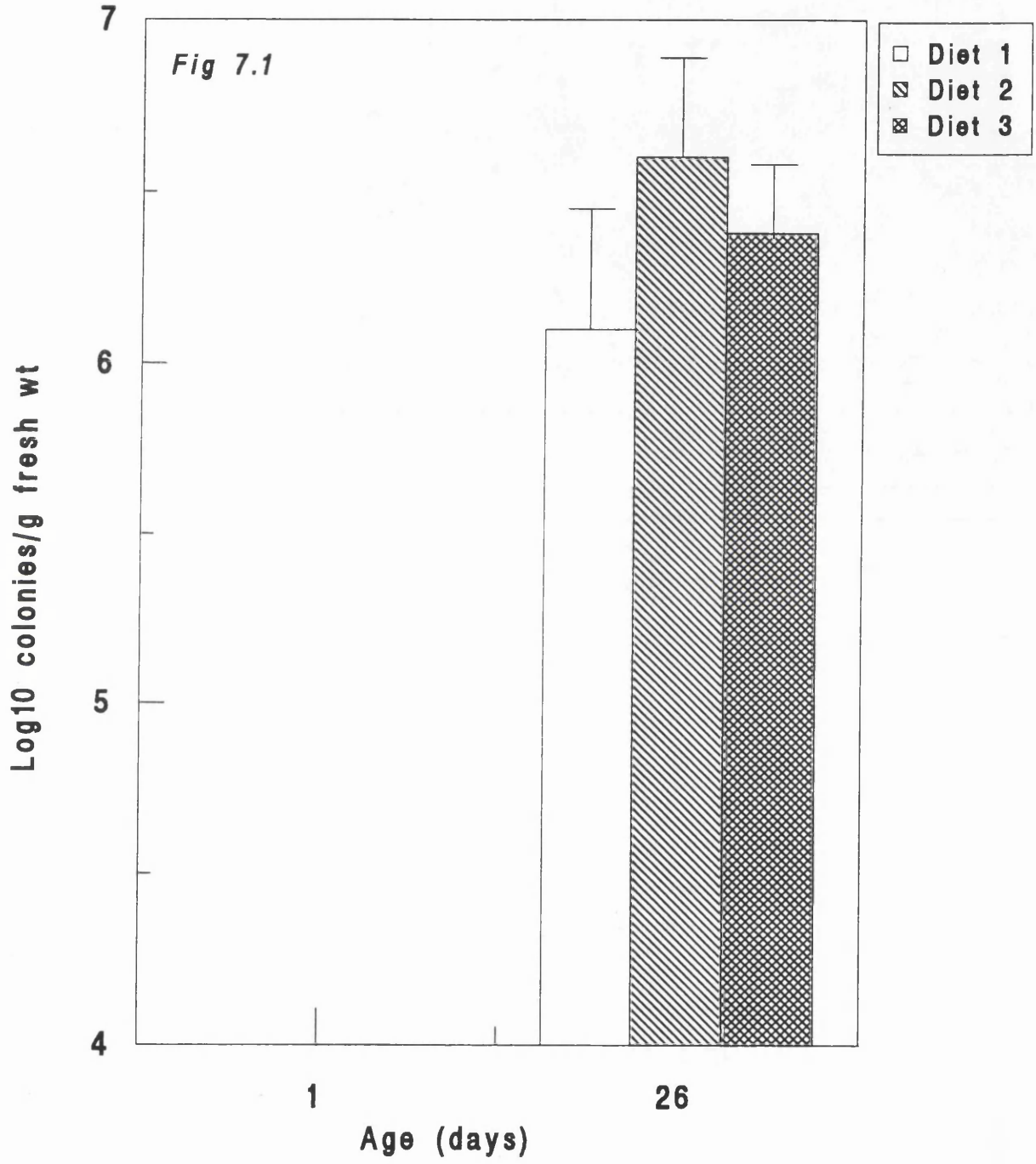
Fig 7.1
Diet 1
Diet 2
Diet 3
Log10 colonies/g fresh wt
7
6
5
4
1
26
Age (days)

changes were observed in the other diets. The numbers of Clostridia species were highest in chicks receiving the tallow oil based diet and lowest in chicks receiving the soyabean oil based diet apart from day 14 post-hatch. The changes with post-hatch age in the level of Clostridia spp. observed in chicks receiving either the tallow or the soyabean oil based diets did not show any consistency.

Lactobacilli species

The Lactobacilli were not detected in the ileum on day 1 post-hatch (see (Figure 7.2(i)). At day 7 post-hatch the population of Lactobacilli was highest in chicks receiving the Dalgety fat based diet and lowest in chicks receiving the tallow oil based diet. However, on day 14 and 26 post-hatch the numbers of Lactobacilli were highest in chicks receiving the tallow oil based diet and lowest in chicks receiving the soyabean oil based diet. Variations in the numbers of Lactobacilli observed between days 7 and 26 post-hatch in chicks receiving the tallow oil based diet did not show any discernable trend.

Streptococcal species

The ileum (see Figure 7.2(ii)) contained substantial numbers of Streptococci (10^{10} colonies/ g fresh weight) on day 1 post-hatch. thereafter decreases in Streptococci numbers were observed in all dietary treatments, but with varying rates between the treatments. Between day 1 and 7 post-hatch substantial reductions; from 10^{10} to 10^{7} colonies/ g fresh weight and from 10^{10} to 10^{6} colonies/ g fresh weight were observed in chicks receiving the Dalgety fat and the soyabean oil based diets, respectively whereas, minimal changes (from 10^{10} to 10^{9} colonies/ g fresh weight) occurred in chicks receiving the tallow oil based diet. However, between days 7 and 14 post-hatch the numbers of Streptococci were reduced to approximately 10^{7}

Figure 7.2 (i-iii): The distribution of Lactobacilli and Streptococci spp. and the Coliforms in the ileal contents

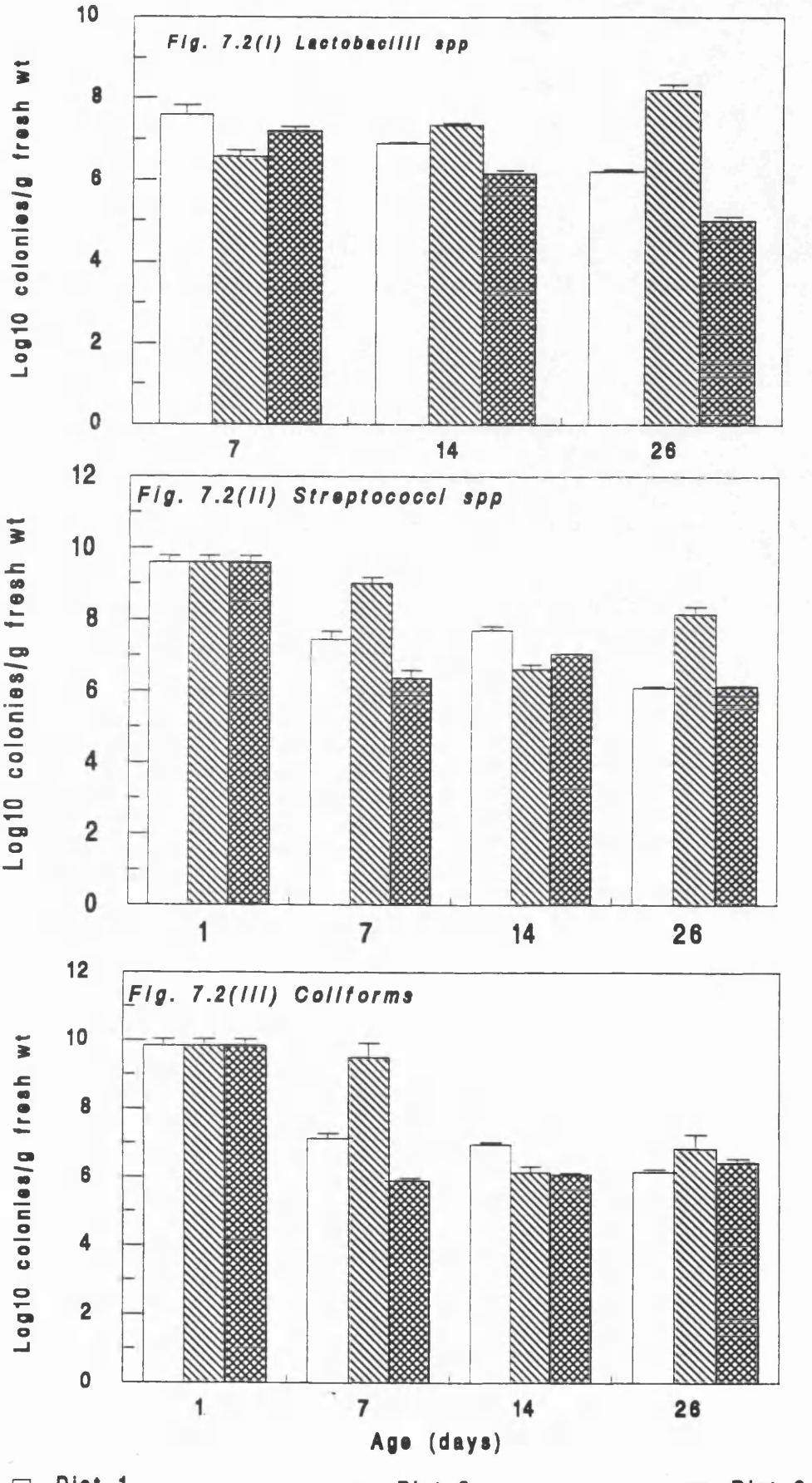
Fig. 7.2(I) Lactobacilli spp
Log10 colonies/g fresh wt
10
8
6
4
2
0
7
14
26
Fig. 7.2(II) Streptococci spp
Log10 colonies/g fresh wt
12
10
8
6
4
2
0
1
7
14
26
Fig. 7.2(III) Coliforms
Log10 colonies/g fresh wt
12
10
8
6
4
2
0
1
7
14
26
Age (days)
Diet 1
Diet 2
Diet 3

colonies/ g fresh weight in chicks receiving the tallow oil based and only small increases occurred in chicks receiving either the Dalgety fat or the soyabean oil based diets. In contrast between days 14 and 26 post-hatch a substantial increase in numbers occurred in chicks receiving the tallow oil based diet and decreases were observed in chicks receiving either the Dalgety fat or the soyabean oil based diets. A gradual decline in Streptococci levels with post-hatching age was observed in all dietary treatments.

Coliform species

The ileum also contained about 10^{10} colonies/ g fresh weight of Coliforms on day 1 post-hatch. The number of Coliforms were highest in chicks receiving the tallow oil based diet and lowest in chicks receiving the soyabean oil based diet on day 7 post-hatch. Between days 1 and 7 post-hatch substantial decline in the numbers of Coliforms (from 10^{10} to 10^{7} and 10^{10} to 10^{6} colonies/ g fresh weight were observed in chicks receiving the Dalgety fat the soyabean oil based diets, respectively, whereas, decreases (from 10^{10} to 10^{6} colonies/ g fresh weight) in chicks receiving the tallow oil based diet occurred between days 7 and 14 post-hatch. On day 26 post-hatch the differences in the population of Coliforms between the dietary treatments were relatively small.

(C) Caecum

Clostridial species

The numbers of Clostridia were more than 10^{9} colonies/g fresh weight on day 1 post-hatch. Decreases in the levels of Clostridia were observed in all dietary treatments between days 1 and 7 post-hatch reaching less than 10^{6} in chicks receiving the Dalgety fat based diet and 10^{8} in chicks receiving the tallow and

soyabean oil based diets. After day 7 post-hatch further decreases in the population of Clostridia were only observed in chicks receiving either the tallow oil or the soyabean oil based diets.

Lactobacilli species

Lactobacilli were not detected on day 1 post-hatch. However, on day 7 post-hatch the caeca contained substantial populations of Lactobacilli in the range of 10^7 to 10^8 colonies/ g fresh weight being highest in chicks receiving the Dalgety fat based diet and lowest in chicks receiving the soyabean oil based diet. The numbers of Lactobacilli remained relatively unchanged in chicks receiving the Dalgety fat and the tallow oil based diets, whilst a sharp decline (from 10^7 colonies/ g fresh weight to negligible numbers) was observed in chicks receiving the soyabean oil based diet between days 14 and 26 post-hatch.

Streptococcal species

A substantial population of Streptococci (approx. 10^7 colonies/ g fresh weight) was present in the caeca on day 1 post-hatch. Increases in the levels of Streptococci species during the first week post-hatch were observed for all dietary treatments. After day 7 post-hatch further increases were confined only to chicks receiving the tallow oil based diet whereas, decreases occurred in chicks receiving the other diets. A downward trend in the population of Streptococci was observed in chicks receiving the Dalgety fat and the soyabean oil based diets after day 7 and in chicks receiving the tallow oil based diet after day 14 post-hatch.

Figure 7.3(i-iii): The distribution of Lactobacilli and Streptococci species and the Coliform in the caecal contents

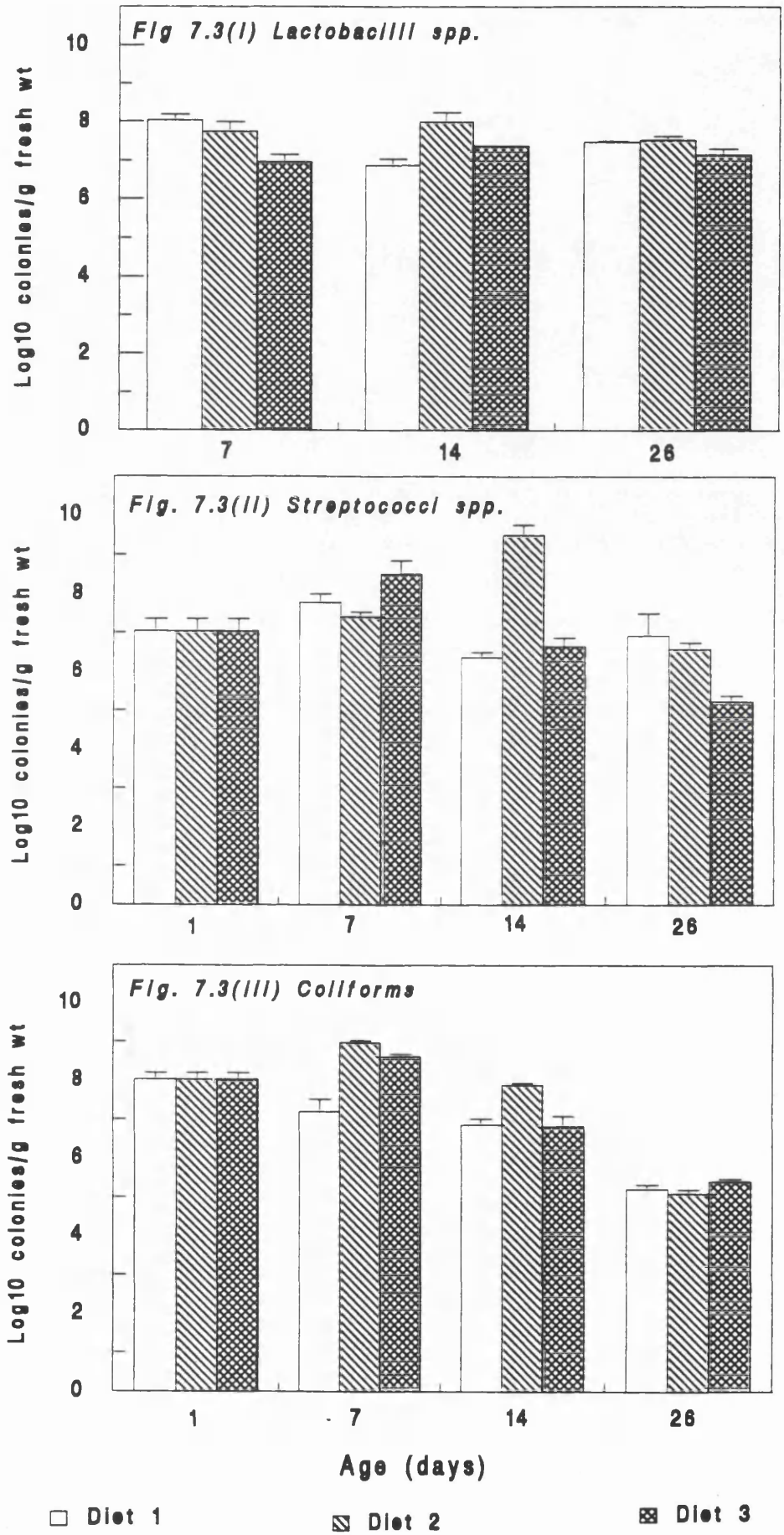
Fig 7.3(I) Lactobacilli spp.
Log10 colonies/g fresh wt
10
8
6
4
2
0
7
14
26
Fig. 7.3(II) Streptococci spp.
Log10 colonies/g fresh wt
10
8
6
4
2
0
1
7
14
26
Fig. 7.3(III) Coliforms
Log10 colonies/g fresh wt
10
8
6
4
2
0
1
7
14
26
Age (days)
Diet 1
Diet 2
Diet 3

Coliform species

The caeca contained about 10^8 Coliforms on day 1 day post-hatch. Between days 1 and 7 a gradual increase in the level of Coliforms were observed in chicks receiving the tallow and the soyabean oil based diet. Decreases occurred in all dietary treatments thereafter to a level of about 10^5 by day 26 post-hatch.

7.4 DISCUSSION

The effect of age and dietary fat sources on the establishment, types and numbers of the GIT micro-organisms in the young chick are discussed in relation to the results obtained in the present experiment. The low microbial population obtained on day 1 post-hatch in the present study particularly in the duodenum and ileum accord to the findings reported in other studies (Shapiro and Sarles, 1949; Lev and Briggs, 1956; Lev *et al.*, 1957; Salanitro *et al.*, 1978). At day 7 post-hatch substantial numbers of Clostridia and Streptococci species were present in the duodenum whilst neither, Lactobacilli nor Coliform species were detected. This is in agreement with the earlier work reported by Barnes *et al.* (1978) and Salanitro *et al.* (1978). However, the absence of Lactobacilli species observed in the present study after the second week post-hatch is in contrast with findings reported by Barnes *et al.* (1978) which showed that the duodenum at this stage was dominated by Lactobacilli species this stage. Reasons attributing to these contradicting findings are unclear. The effects of dietary fat sources on the microbial population in the duodenum, in particular the Streptococci species were relatively small. This might have been due to the minimal influence which the dietary fat sources have on duodenal lipid composition since most of the lipid in this section is of biliary origin (Freeman, 1976).

Unlike the duodenum, the ileal microbial population was composed of substantial numbers of Clostridia, Streptococci, and Coliform species and slightly lower numbers of the Lactobacilli species. This is in agreement with findings reported by Shapiro and Sarles (1949) which showed that the ileal microbial population was higher than that of the duodenum. Nonetheless, the results obtained in the present study with respect to Lactobacilli differ markedly from other studies which showed that the ileal flora, was mainly composed of Lactobacilli species after few days post-hatch (Shapiro and Sarles, 1949; Willingale and Briggs, 1955; Barnes *et al.*, 1972; Salanitro *et al.*, 1978; Kimura *et al.*, 1986). The difference in the population of Lactobacilli was further observed from day 7 to 26 post-hatch. The reasons underlying these differences are unclear, although dietary differences could have a played major part. The microbial population changes in the ileum, during the present study, were affected by the dietary treatments. Significant differences were noted between chicks receiving the tallow oil based diet than those receiving either the Dalgety fat or the soyabean oil based diets. This is in agreement with other reported work, showing that the establishment of the various types of micro-organisms in the GIT of the chick was affected by many factors including diet (Barnes *et al.*, 1978; Salanitro *et al.*, 1978; Barnes *et al.*, 1980). The ileal contents from chicks receiving the tallow oil based diet contained, with a few exceptions, higher levels of Lactobacilli, Streptococci and Coliforms from day 7 to 26 post-hatch compared to levels obtained in the other diets. From the present study it would appear that the growth of most micro-organisms was enhanced by diets containing high levels of the saturated fat. These findings are in agreement with work reported by Nath *et al.* (1948). The high numbers of Lactobacilli at day 7 post-hatch in chicks receiving either the Dalgety fat or soyabean oil based diets accord with suggestions made by Nath *et al.* (1948), that higher levels of oleic acid

in the fat had a growth stimulating effect on Lactobacilli. However the cause of reductions in the numbers of Lactobacilli after day 7 post-hatch in chicks receiving either the Dalgety fat or the soyabean oil based diets and concomitant increases in chicks receiving the tallow oil based diet is equivocal. In the present study the population of Streptococci and Coliforms were generally higher in chicks receiving the tallow oil based diet. These findings contradict the work of Smith (1965) which showed that Lactobacilli predominated over Coliforms and Streptococci species. In the present study, reductions in most of the bacterial species identified studied occurred after day 14 post-hatch in all treatments. This feature agrees with the normal pattern of microbial establishment in the GIT which is thought to be completed between days 9-13 post-hatch (Shapiro and Sarles, 1949; Salanitro *et al.*, 1978; Furuse *et al.*, 1991).

The absence of Lactobacilli in the caeca on day 1 post-hatch is in close agreement with the findings of Barnes *et al.* (1978), which showed that the initial flora of the caecum was mainly composed of Streptococci and *Coli aerogenes* whilst, the establishment of Lactobacilli was slow and occurred mainly after the commencement of feeding. Variation in the caecal flora between chicks receiving the different dietary treatments observed in this study were in contrast to findings reported by Nath *et al.* (1948) which showed that the caecal flora was not influenced by dietary fat. The numbers of Lactobacilli, Streptococci and Coliforms in the caecal flora of chicks receiving either the Dalgety or the soyabean oil based diets, at day 7 post-hatch, were in most cases higher than those present in the ileum. These findings to some extent agree with (Barnes *et al.*, 1978; Baba *et al.*, 1991) who showed that the caecum contain the highest number of micro-organisms in comparison to the other parts of the GIT. The changes in the numbers of micro-organisms with age particularly up to day 14 post-hatch observed in this study accord with the findings

reported in other studies. Humbert *et al.* (1989) showed that the morphological and quantitative changes of the caecal microflora were completed when the chick was almost 15 days old.

The lower body weight gain was observed in chicks receiving the tallow oil based diet when compared to those receiving the other diets. Since the numbers of most bacterial species were higher in chicks receiving the tallow oil based diet it is possible that there may have been a competition for nutrients between the micro-organisms and the host (Furuse and Yokota, 1985b). It has also been shown that high numbers of micro-organisms in the GIT result in lower energy retention by the chick (Furuse and Yokota, 1985b). This is accompanied by increases in the the weight and thickness of the intestinal wall. These physical changes to the GIT reduces the absorption of nutrients (Coates *et al.*, 1981). The variations in the GIT flora observed in this study with post-hatching age were generally in accordance with findings reported elsewhere. Differences in the GIT flora between chicks receiving different dietary treatments indicate that fat composition like other nutrients affects the establishment and growth of the micro-organisms.

CHAPTER 8

GENERAL DISCUSSION

Modern broiler production requires very rapid growth rates immediately following chick emergence. To achieve this end the newly hatched chick is supplied with an energy rich diet containing high levels of fat. Under these circumstances the chick is forced to deal with enormous amounts of endogenous and exogenous sources of lipid. However, the ability of the chick to satisfy both the natural metabolic changes associated with emergence and the commercial pressures demanded by the producer is limited. A further complicating feature arising from commercial interests is the overriding tendency to consider the lipid components of the diet as energy sources with scant respect being paid to the aspects of quality. Whereas, in other domesticated animal species dietary lipid quality is of major importance. Although such a situation might be less detrimental during the later stages of the broiler bird's life, marked detriments are observed in the early neonatal period where the establishment of metabolic features associated with free existence are of prime significance. Therefore it is important that consideration ought to be given during diet formulation to factors which may influence the balance between the establishment of the physiological and metabolic features and the need to obtain maximum growth as early as possible.

The present series of experiments aimed at delineating three major features of lipid metabolism in the newly hatched chick:

(i) the role played by residual yolk material during the immediate period following hatching and associated lipid changes in comparison to those existing during embryonic development

(ii) Lipid changes in the major sections of the GIT following hatching in particular with respect to post-hatch age and different dietary fat sources

(iii) Changes in the microbiological populations of the GIT with respect to post-hatch age and different dietary fat sources

The major role of lipids in regard to the development and maturation of the chick embryo is well documented (Noble and Cocchi, 1990). Their metabolism is now known to be associated with a range of unique features which aid the movement of lipid from the yolk into the embryo and tissue composition (Noble, 1987). In spite of the extreme intensity of yolk lipid metabolism and extensive absorption during the latter part of embryonic development, a large amount of unabsorbed lipid remains unabsorbed at the time of hatching. Since it has been shown that many chicks begin to eat 24 to 48 hours after hatching, the presence of yolk lipid is a critical factor in the early performance of the chick and may increase chick survival by sparing protein during stress-induced tissue catabolism. This lipid is also freely accessible to the newly hatched chick as an immediate source of essential nutrients and energy.

The proportion of the residual yolk material relative to the chick's body weight of 6-11 percent obtained in the present study accord with previous observations (Romanoff, 1960; Noble and Moore, 1964b; Noble and Ogunyemi, 1989). It was clear from results obtained in this study that the rapid removal of residual yolk lipid is associated with extensive and fast changes in tissue lipid composition. Many of the embryonic features are lost within few days after hatching to be replaced by features expected of the adult bird and indeed animal tissues in general. However, certain embryonic tissue lipid features are retained for some considerable time following hatching, whilst, others seem to bear little resemblance to the lipids available either from the yolk sac or the diet. Several suggestions have been made as to the mode and sequence of events involved in the assimilation of the remnant yolk lipid. On one hand it has been postulated that much of the lipid is absorbed via the yolk sac membrane by a process involving endodermal release directly into the the circulation of the chick in a similar way to that which operates

during embryo development (Freeman and Vince, 1974; Lambson, 1974). The presence after hatching of high activities within the yolk sac walls of enzymes associated with absorption has also been taken as an indication of the maintenance of endodermal involvement well after hatching (Kusuhara and Ishida, 1974). By contrast other evidences have been obtained showing that the remnant yolk material may be expelled through the yolk stalk into the GIT resulting in a rapid regression of the yolk sac wall and its function (Esteban *et al.*, 1991). Additionally Svanberg (1971) showed that the residual yolk lipids were deposited into the liver before being distributed to other parts of the body.

The present observations on the lipid and fatty acid compositions of the residual yolk and the liver are in favour of the preferential removal of lipid from the yolk contents via the endoderm and the involvement of the liver in the subsequent distribution process. Thus, in contrast to the composition of the yolk sac membrane lipids during embryonic development, there is high accumulation of cholesterol ester together with C20 and C22 polyunsaturated fatty acids during the immediate period after hatching (Noble and Shand, 1985; Noble and Ogunyemi, 1989). The continuation and enhancement of the compositional changes that were previously associated with the yolk sac membrane and their contrast with the lipid and fatty acid compositions changes of the GIT, indicate a segregation of fat utilization by the yolk complex from that of the GIT. The extremely high cholesterol ester levels within the liver of the post-hatch chick and the maintenance of high oleic acid levels within the cholesterol ester fraction, indicates to some extent, that the role of the liver in the distribution of the residual yolk lipid in post-hatched chicks is similar to that which occurs within the embryo. The liver possibly continues to function as a deposit for remnant lipoprotein material arising from the yolk sac membrane metabolism. However, this unique liver function persisted only for few days after

hatching. The changes observed thereafter were marked by the rapid replacement of the high cholesterol ester levels with triglyceride and phospholipid fractions. Changes in lipid fractions were also accompanied by alterations in the fatty acid composition differing markedly from that associated with embryo and the residual yolk sac materials (Noble and Moore, 1967; Noble, 1987). These changes were indicative of a rapid alteration in the role of the liver with respect to lipid metabolism. As already noted during embryonic development the liver in particular and other tissues may serve as depository organs for preformed components derived from yolk resorption. However, following hatching the liver of the chick clearly acquires an extensive capacity of its own for synthesis of fat for both structural and storage purposes (Leveille et al., 1975; Annison, 1971; Hill, 1983; Noble and Ogunyemi, 1989). This new role quickly outweighs the substantial contribution of fat accumulation coming from the residual yolk sac materials.

The overall weight changes of the yolk material during the early post-hatch period were to some extent affected by dietary fat sources; whilst effects on lipid and fatty acid compositions were not observed. The slow disappearance of the residual yolk material in chicks receiving the soyabean oil based diet was an indication that there might have been an interaction in the supply of nutrients between the exogenous and the endogenous sources. On the other hand Daly and Peterson (1990) suggested that the rapid decline of the yolk contents during the early post-hatch days was an indication of the depletion of the yolk materials. This feature might have occurred in this study in chicks receiving either the Dalgety chick starter or the tallow oil based diets. However, the advantages associated with supplying readily available energy from exogenous sources very early in the chicks life are still questionable. As pointed out by Noble and Ogunyemi (1989) the presence of the residual yolk material provides a continuation of nutrients during the critical change

from embryonic to free living development. The presence of a substantial amount of unabsorbed yolk material may also continue to affect tissue lipid patterns and metabolism during the neonatal period. Similar suggestions were also made by Daly and Peterson (1990) who showed that more efficient mobilization of nutrients from the yolk facilitate the transition to external use of energy sources mainly fats and carbohydrates, self sufficiency and encourages continued tissue development. The results obtained in the present study showed slightly reduced growth rates were observed in chicks receiving the soyabean oil based diet. This was probably due to the fact that although the soyabean oil based diet was able to contribute towards the nutrient requirements of the chick during this period, its ability was to some extent limited. Although the diet provided was of high quality its ability to contribute to the energy requirements of the chick was limited. The probable cause for this observation could be the immaturity of most of key digestive elements particularly those involving lipid metabolism (Krogdahl, 1985; Akiba *et al.*, 1988) and an interference with normal developmental processes during the early post-hatch period required to accommodate the exogenous materials. Additionally the lack of differences in the body weight changes during the early post-hatch days between chicks receiving diets differing in their fat sources were a reflection of the insignificance of dietary fat to the very young chick. This is in agreement with other studies (Fedde *et al.*, 1960). From these findings it could be suggested that introducing high fat diets early in the chick's life is unessential.

The changes in the lipid composition of the liver were mainly affected by post-hatch age and to some extent dietary fat sources. The liver lipids from chicks receiving the soyabean oil based diet contained higher and lower levels of triglyceride and phospholipid fractions, respectively, compared with the other diets. The lower levels of triglyceride and high phospholipid in the liver lipids from chicks

receiving the soyabean oil based diet conform to the suggestions (Abraham, 1970; Giordani *et al.*, 1988) that, although hepatic lipogenesis is limited by feeding diets containing high fat levels, the effects were more enhanced by increased proportions of unsaturated fatty acids in the diet. Dietary effects were also observed in the distribution of fatty acids within the different fractions of the liver. Sim *et al.* (1973) pointed out that the fatty acid compositions of the liver lipid fractions during the early post-hatch period were influenced more by dietary fat sources when compared to overall lipid changes. This was assumed to arise from the fatty acid specificity of the enzyme systems during hepatic lipogenesis processes. Most of the changes in the fatty acid compositions of the various lipid fractions occurred during the first 6 days post-hatch. This is a further indication that the major metabolic changes associated with lipid digestion and absorption occur during the first few days following hatching and that feeding various types of dietary fat within this period has a distinctive effect on the development of these processes.

A series of experiments to investigate the effects of dietary fat sources differing in lipid and fatty acid compositions and age on the performance of broiler chicks were also undertaken. The lipid and fatty acid compositions of the fat sources and their respective compounded diets obtained in the present studies were generally within the expected range. The high triglyceride levels observed in the tallow and soyabean oils conformed to previous observations which showed that triglyceride was the major lipid fraction in fat sources of both plant and animal origin (Enser, 1984; Gurr, 1984; Noble, 1987; McDonald *et al.*, 1988). However, the high free fatty acid levels in the Dalgety fat were unexpected. Similar high free fatty acid levels with respect to commercial fat sources were reported by Akinyemi (1989). A major cause for this may include the wide-spread use of fat and oil by products in poultry feed formulation which in some cases are of doubtful quality. Another cause

of the free fatty acids accumulation might be the deterioration of the fat sources during storage. However this can be minimized by improving storage conditions and suitable additions of to prevent hydrolysis. In contrast, to the fat sources, the compounded diets contained substantial levels of free fatty acid regardless of the fat source. The additional levels of free fatty acids in the compounded diets were most likely to have arisen from the hydrolytic processes during feed compounding (Noble, 1984; Christie, 1989; Wiseman *et al.*, 1990). There is no reason to suggest that the compounded diets used in the present study had peculiarities arising either from methodologies used or the components added. Extensive evidence exists from previous investigations that the presence of high free fatty acids levels within poultry diets may be the norm rather than the exception. It is also a convention of modern poultry feeding of broiler and laying stock, that the lipids have a high level of linoleic acid.

The present study has clearly delineated major lipid changes that occur along the GIT of the newly hatched chick and modification of these changes as a result of both post-hatching age and the nature of the dietary lipids. The sharp contrast in lipid composition between the diets and the GIT, was an indication that the diet, once ingested undergoes a series of physical and probably chemical processes before reaching the GIT (Senior, 1964). However, the lipid changes observed within the different sections of the GIT reflected the normal physiological features of the chicken GIT and the occurrence of the digestion and absorption processes within these sections. The prominent feature observed in relation to this was the high levels of phospholipid within the duodenum throughout the experimental period. Similar observations have been reported elsewhere (Freeman, 1976; Sklan, 1979; Griminger, 1986). The high phospholipid levels within the duodenal contents are a reflection of bile secretion. The secretion of bile is to some extent influenced by post-hatching

age and the nature of the dietary nutrients (Sklan *et al.*, 1973; Polin *et al.*, 1980; Krogdahl, 1985). The significant differences in the lipid composition between the duodenum and the jejunum, notably the decrease in phospholipid levels indicated the intensity of the digestive activities between these sections. On the other hand, it was also an indication of the importance of bile secretion in lipid digestion and absorption (Senior, 1964; Hurwitz *et al.*, 1973; Freeman, 1976).

Sklan *et al.* (1975) showed that over 95 percent of the dietary triglyceride was broken down in the duodenum mostly into free fatty acids with some mono- and di-glycerides. The products were then absorbed slowly during their passage along the GIT. However, these findings differed markedly from other reports which indicated that the major lipid digestion processes occurred beyond the duodenum (Freeman, 1976). The present observations conformed to the latter suggestion since high proportion of free fatty acids in the GIT first appeared in the upper ileum and remained high thereafter. The proportion of free fatty acids was particularly high in chicks receiving the tallow oil based diet. The presence of such high levels of free fatty acid within the GIT was bound to have an effect on the establishment and diversity of the microflora (Ferket, 1991).

Immediate after hatching, feeding systems which involve exposure of chicks to diets with high levels of unsaturated fatty acids in non esterified form, may impair the normal development of the gut microflora. As a result a host of factors would be affected, in particular those associated with the GIT function such as dietary absorption and excretion. This sometimes leads to ailments such as enteric disorders. The present study provides some evidence of a lipid dietary effect on the establishment of the GIT microflora population. The groups of micro-organisms identified were generally higher in the chicks which received the tallow oil based diet in comparison with the other diets. In view of nutrient availabilities associated

with gut microflora, it is most likely that this diet was beneficial to the development of the chick. On the other hand, it is generally accepted that high levels of dietary saturated fatty acids impair lipid digestion and absorption (Fedde *et al.*, 1960; Renner and Hill, 1961; Krogdahl, 1985; Sell *et al.*, 1991). Furthermore, an enhanced microflora proliferation is known to result in thickening of the intestinal wall which in turn diminishes lipid uptake. This situation might be exacerbated by the fact that large numbers of GIT micro-organisms possibly increases competition for nutrients between the microflora and the chick itself.

The differences in dietary fatty acid composition were also reflected in fatty acid digestibility. As expected digestibilities of the fatty acids in the present experiment diminished significantly with increased saturation irrespective of the diet. The increased utilization of satuated fatty acids in the presence of unsaturated fatty acids, observed in this study were in agreement with observations reported by Ketels and De Groote (1987). The decrease in the total lipid content within the GIT and amount of excreted fat and also increases in the digestibilities of total lipid and the individual fatty acids with post-hatching age were indicative of the improvements in fat utilization with age. The factors which may have contributed to these improvements include increased bile secretion and enzymic activities and probably improved adaptation to the external environmental conditions. Various reports have shown that the utilization of fat is limited during the early post-hatch period and the nutrient requirements of the chick during this period are low. Hence when formulating diets the inclusion of high levels of dietary lipids initially may be questionable due to large amount of lipid from the remnant yolk sac. The GIT of the newly hatched chick needs time to adapt to feeds from external sources and therefore it is unable to adequately deal with high lipid intakes during this period. Also lipid exposure of the GIT microflora with a range of consequential effects on

other nutrient availabilities and morphological changes. Furthermore, in husbandry and production, feeding chicks with diets containing high lipid content leads to wasteful excretion of undigested lipids especially if such lipids are non-esterified and contain high levels of unsaturated fatty acids (Noble, 1993 personal communication). This can result in inability to maintain ideal litter conditions (Patisson, 1989) with consequential effects to both the animal and the operator. Therefore effects of exogenous lipid supply with respect to the developmental processes, both physiologically and anatomically should be given due consideration during feed formulation. The observations obtained in the present studies clearly showed that fat utilization and subsequent performance of the chick during the early post-hatch period was influenced by controllable factors with the exception of residual yolk material.

In conclusion the main features observed in this study were:

(i) the disappearance of the residual yolk sac materials during the first five days days post-hatch

(ii) lipid changes in the residual yolk sac materials, liver, GIT and faeces during the first week post-hatch

(iii) the influence of dieatry fat sources on (i) and (ii)

(iv) improved digestibilities of total lipid and the major long chain fatty acids with post-hatching age

(v) influence of dietary fat sources on (iv), feed intake, feed efficiency and the establishment and numbers of GIT micro-organisms

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APPENDIX TABLES

1: Statisical analyses on the effect of dietary fat sources on the weights of the chick, residual yolk sac material and liver

		age (days)				
		1	3	6	9	12
body weight	diets					
	1 vs 2	N.S	*	**	**	*
	1 vs 3	N.S	*	**	*	N.S
	2 vs 3	N.S	N.S	N.S	N.S	*
residual yolk sac						
	1 vs 2	**	N.S	N.S	-	-
	1 vs 3	**	N.S	*	-	-
	2 vs 3	N.S	N.S	**	N.S	N.S
liver weight						
	1 vs 2	N.S	N.S	N.S	**	**
	1 vs 3	N.S	N.S	N.S	**	**
	2 vs 3	N.S	N.S	N.S	N.S	N.S

2: Statistical analyses on the effects of dietary fat sources and age on the lipid composition of the residual yolk sac materials

		age(days)			diets		
		3	6		1	2	3
lipid group	diets						
cholesterol ester	1 vs 2	**	**	1 vs 3	***	***	***
	1 vs 3	***	***	3 vs 6	**	*	N.S
	2 vs 3	*	**	1 vs 3	***	***	***
triglyceride	1 vs 2	N.S	***	1 vs 3	***	***	**
	1 vs 3	**	***	3 vs 6	**	*	**
	2 vs 3	N.S	**	1 vs 6	***	***	***
free fatty	1 vs 2	*	*	1 vs 3	*	*	*
acid	1 vs 3	N.S	**	1 vs 6	*	*	*
	2 vs 3	*	*	3 vs 6	*	*	*
phospholipid	1 vs 2	*	N.S	1 vs 3	*	**	N.S
	1 vs 3	***	**	3 vs 6	*	N.S	**
	2 vs 3	***	**	1 vs 6	*	**	*

Diet 1= commercial diet; Diet 2= tallow oil based diet; Diet 3= soyabean oil based diet

3: Statistical analyses on the effect of dietary fat sources on fatty acid distributions in cholesterol ester and triglyceride lipid fractions within the residual yolk sac materials

lipid fraction		CE			TG		
age (days)		1	3	6	1	3	6
	diets						
16:0	1 vs 2	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	2 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
16:1	1 vs 2	N.S	*	N.S	N.S	N.S	N.S
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	2 vs 3	N.S	*	N.S	N.S	N.S	N.S
18:0	1 vs 2	N.S	*	*	N.S	N.S	*
	1 vs 3	N.S	*	N.S	N.S	N.S	*
	2 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
18:1	1 vs 2	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	2 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
18:2	1 vs 2	N.S	N.S	N.S	N.S	*	N.S
	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	2 vs 3	N.S	N.S	N.S	N.S	*	N.S

4: Statistical analyses on the effect of age on fatty acid composition of cholesterol ester and triglyceride fractions in the residual yolk sac materials

lipid fraction		CE			TG		
diet		1	2	3	1	2	3
Fatty acid	age						
16:0*	1 vs 3	**	*	*	N.S	N.S	N.S
	3 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 6	N.S	*	*	N.S	N.S	N.S
16:1	1 vs 3	N.S	*	N.S	N.S	N.S	N.S
	3 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
18:0	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	3 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 6	N.S	N.S	N.S	*	*	N.S
18:1	1 vs 3	N.S	*	*	N.S	N.S	N.S
	3 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 6	N.S	*	*	N.S	N.S	N.S
18:2	1 vs 3	N.S	N.S	N.S	N.S	N.S	N.S
	3 vs 6	N.S	N.S	N.S	N.S	N.S	N.S
	1 vs 6	*	N.S	N.S	*	N.S	N.S

5: Statistical analyses on the effects of dietary fat sources and age on liver lipid composition

		3	6	9	12	diet	age a	b	c	d
cholesterol	1 vs 2	**	**	*	N.S	1	***	**	***	N.S
ester	1 vs 3	**	**	N.S	N.S	2	**	***	***	*
	2 vs 3	N.S	**	*	N.S	3	**	***	***	N.S
triglyceride	1 vs 2	***	***	**	***	1	***	**	N.S	N.S
	1 vs 3	***	***	**	***	2	*	***	***	**
	2 vs 3	N.S	*	***	***	3	*	***	N.S	N.S
free fatty	1 vs 2	*	N.S	**	***	1	-	*	***	N.S
acid	1 vs 3	*	*	**	*	2	N.S	N.S	N.S	N.S
	2 vs 3	*	*	*	**	3	*	**	N.S	N.S
phospholipid	1 vs 2	*	N.S	**	*	1	N.S	**	N.S	**
	1 vs 3	N.S	N.S	***	**	2	*	**	N.S	N.S
	2 vs 3	*	N.S	**	**	3	*	**	**	N.S
free	1 vs 2	N.S	*	N.S	N.S	1	N.S	N.S	N.S	N.S
cholesterol	1 vs 3	N.S	*	**	**	2	*	N.S	N.S	*
	2 vs 3	*	*	*	**	3	*	*	*	N.S
partial	1 vs 2	N.S	N.S	N.S	*	1	-	*	N.S	*
glyceride	1 vs 3	*	N.S	N.S	N.S	2	N.S	N.S	N.S	N.S
	2 vs 3	*	N.S	N.S	N.S	3	N.S	*	N.S	N.S

Age comparisons: a= 1 vs 3; b= 3 vs 6; c= 6 vs 9; d= 9 vs 12

6: Statistical analyses on the effects of dietary fat sources and age on fatty acid composition of the phospholipid fraction in the liver

		age (days)								
		3	6	9	12	diet	a*	b	c	d
fatty acid	diets									
16:0*	1 vs 2	*	N.S	*	*	1	**	N.S	N.S	N.S
	1 vs 3	*	**	**		2	N.S	N.S	N.S	N.S
	2 vs 3	N.S	*	*	*	3	N.S	N.S	*	N.S
16:1	1 vs 2	*	N.S	*	N.S	1	***	N.S	N.S	N.S
	1 vs 3	**	*	**	**	2	-	*	*	*
	2 vs 3	N.S	N.S	**	***	3	N.S	*	*	N.S
18:0	1 vs 2	*	N.S	N.S	N.S	1	N.S	N.S	N.S	N.S
	1 vs 3	**	N.S	N.S	N.S	2	*	*	N.S	N.S
	2 vs 3	*	N.S	N.S	N.S	3	N.S	*	*	N.S
18:1	1 vs 2	N.S	*	**	**	1	**	*	N.S	N.S
	1 vs 3	*	N.S	*	*	2	*	*	*	N.S
	2 vs 3	*	*	**	**	3	N.S	**	N.S	N.S
18:2	1 vs 2	N.S	*	N.S	*	1	*	*	*	*
	1 vs 3	*	*	*	*	2	*	N.S	N.S	N.S
	2 vs 3	*	*	*	**	3	***	N.S	N.S	N.S
20:4	1 vs 2	*	N.S	*	*	1	***	*	N.S	**
	1 vs 3	**	N.S	*	**	2	**	**	*	N.S
	2 vs 3	*	N.S	*	**	3	*	**	N.S	N.S
22:6	1 vs 2	N.S	*	*	*	1	**	*	*	N.S
	1 vs 3	*	*	*	N.S	2	*	*	N.S	*
	2 vs 3	*	N.S	N.S	N.S	3	***	*	*	*

* age comparison as per description in No 5

7: Statistical analyses on the effects of dietary fat sources and age on fatty acid composition of the free fatty acid fraction in the liver

fatty acid	diets	age 3	6	9	12	diet	a*	b	c	d
16:0	1 vs 2	*	**	**	**	1	-	N.S	N.S	N.S
	1 vs 3	**	***	***	***	2	*	*	N.S	N.S
	2 vs 3	*	*	*	*	3	N.S	N.S	N.S	N.S
16:1	1 vs 2	**	*	N.S	N.S	1	-	*	*	N.S
	1 vs 3	**	N.S	*	*	2	**	N.S	N.S	N.S
	2 vs 3	N.S	*	N.S	*	3	*	*	*	N.S
18:0	1 vs 2	*	*	N.S	*	1	-	N.S	*	N.S
	1 vs 3	*	N.S	*	*	2	*	N.S	N.S	N.S
	2 vs 3	*	*	N.S	N.S	3	N.S	*	N.S	N.S
18:1	1 vs 2	*	*	**	***	1	-	N.S	N.S	*
	1 vs 3	***	*	*	**	2	*	**	**	N.S
	2 vs 3	*	N.S	**	*	3	N.S	**	N.S	*
18:2	1 vs 2	N.S	*	*	*	1	-	*	*	N.S
	1 vs 3	**	**	***	***	2	N.S	N.S	*	N.S
	2 vs 3	**	*	**	***	3	**	N.S	N.S	N.S
20:4	1 vs 2	**	**	*	N.S	1	-	*	N.S	N.S
	1 vs 3	**	N.S	*	N.S	2	*	*	*	N.S
	2 vs 3	*	**	*	*	3	*	***	N.S	N.S
22:6	1 vs 2	*	*	N.S	N.S	1	-	N.S	N.S	N.S
	1 vs 3	*	N.S	N.S	N.S	2	**	*	*	N.S
	2 vs 3	*	*	N.S	N.S	3	**	N.S	N.S	N.S

* age comparison as per description in No 5

8: Statistical analyses on the effects of age and dietary fat sources on the acid composition the triglyceride fraction of the liver

fatty acid	diets	age 3	6	9	12	diet	a*	b	c	d
16:0*	1 vs 2	*	N.S	N.S	*	1	**	*	*	N.S
	1 vs 3	N.S	N.S	*	*	2	**	**	N.S	N.S
	2 vs 3	*	N.S	N.S	N.S	3	**	N.S	N.S	N.S
18:0	1 vs 2	*	N.S	N.S	N.S	1	*	N.S	N.S	N.S
	1 vs 3	*	*	*	*	2	**	**	*	N.S
	2 vs 3	*	*	*	**	3	**	N.S	N.S	*
18:1	1 vs 2	*	*	**	**	1				
	1 vs 3	N.S	*	*	**	2				
	2 vs 3	*	N.S	*	*	3				
18:2	1 vs 2	N.S	N.S	N.S	*	1	**	*	*	N.S
	1 vs 3	**	*	**	**	2	**	*	N.S	N.S
	2 vs 3	*	*	*	**	3	N.S	*	N.S	N.S

9: Statistical analyses on the effect of dietary fat sources and age on fatty acid composition in liver cholesterol ester

fatty acid	diets	age 3	6	9	12	diet	a*	b	c	d
16:0	1 vs 2	N.S	*	-	*	1	*	*	***	**
	1 vs 3	*	*	*	*	2	*	N.S	-	-
	2 vs 3	*	N.S	-	*	3	*	N.S	**	N.S
16:1	1 vs 2	N.S	N.S	-	-	1	N.S	N.S	*	*
	1 vs 3	N.S	*	-	-	2	N.S	N.S	-	-
	2 vs 3	N.S	**	-	-	3	N.S	**	-	-
18:0	1 vs 2	**	**	***	**	1	**	*	***	**
	1 vs 3	*	**	-	***	2	*	**	*	***
	2 vs 3	N.S	N.S	-	*	3	**	**	-	-
18:1	1 vs 2	N.S	**	***	***	1	**	N.S	**	**
	1 vs 3	N.S	**	**	**	2	**	***	N.S	***
	2 vs 3	N.S	N.S	**	*	3	***	**	***	**
18:2	1 vs 2	*	*	**	*	1	*	**	**	N.S
	1 vs 3	*	*	*	**	2	*	**	N.S	**
	2 vs 3	N.S	N.S	***	**	3	**	**	***	**

* age comparison as per descrption in No 5

10: Statistical analyses on the effects of dietary fat sources and age on gall bladder bile lipid composition

lipid group	diets	age 3	6	9	12	diet	a*	b	c	d
cholesterol ester	1 vs 2	***	-	*	N.S	1	***	**	*	N.S
	1 vs 3	***	-	N.S	-	2	***	-	-	*
	2 vs 3	***	-	*	-	3	*	-	-	-
triglyceride	1 vs 2	***	***	***	***	1	**	**	***	**
	1 vs 3	***	-	***	***	2	N.S	*	**	*
	2 vs 3	**	-	**	N.S	3	**	-	-	*
free fatty acid	1 vs 2	N.S	N.S	*	N.S	1	*	*	*	-
	1 vs 3	N.S	*	*	N.S	2	*	*		*
	2 vs 3	N.S	*	N.S	N.S	3	*	*	N.S	**
phospholipid	1 vs 2	**	***	**	***	1	***	**	**	N.S
	1 vs 3	***	*	*	***	2	***	***	**	**
	2 vs 3	***	***	***	*	3	**	***	*	**
free cholesterol	1 vs 2	***	**	*	**	1	*	N.S	**	N.S
	1 vs 3	**	***	**	*	2	***	**	*	N.S
	2 vs 3	**	***	***	**	3	*	***	**	***

11: Statistical analyses on the effects of dieatry fat sources and age on fatty acid composition of phospholipid fraction in the gall bladder bile

fatty acid	diets	age 3	6	9	12	diet	a*	b	c	d
16:0*	1 vs 2	N.S	*	**	N.S	1	N.S	N.S	*	**
	1 vs 3	N.S	N.S	N.S	N.S	2	N.S	*	*	*
	2 vs 3	N.S	*	**	N.S	3	*	*	*	*
18:0	1 vs 2	**	N.S	N.S	N.S	1	*	*	N.S	N.S
	1 vs 3	N.S	N.S	N.S	N.S	2	*	*	N.S	N.S
	2 vs 3	N.S	*	**	N.S	3	N.S	N.S	N.S	N.S
18:1	1 vs 2	***	N.S	*	**	1	**	**	*	*
	1 vs 3	N.S	N.S	**	**	2	***	*	*	*
	2 vs 3	***	N.S	**	**	3	*	**	N.S	N.S
18:2	1 vs 2	*	N.S	*	N.S	1	*	N.S	N.S	N.S
	1 vs 3	**	**	**	**	2	N.S	N.S	N.S	N.S
	2 vs 3	**	**	***	**	3	**	*	*	*

* age comparison as per description in No 5

12: Statistical analyses on the effects of dietary fat sources and age on the performance of chicks in Experiment 3

		age (days)					
		3	6	9	12	15	21
	diets						
body weight	1 vs 2	N.S	N.S	**	**	***	**
	1 vs 3	N.S	*	**	**	**	N.S
	2 vs 3	N.S	N.S	N.S	N.S	**	**
feed intake	1 vs 2	N.S	N.S	N.S	*	**	N.S
	1 vs 3	N.S	N.S	*	*	*	N.S
	2 vs 3	N.S	N.S	*	N.S	*	N.S
fat intake	1 vs 2	N.S	*	*	**	N.S	**
	1 vs 3	N.S	**	*	***	**	**
	2 vs 3	N.S	**	N.S	**	**	N.S