EFFECT OF DIETARY LIPIDS ON POULTRY BIRD, *GALLUS DOMESTICUS*

BY
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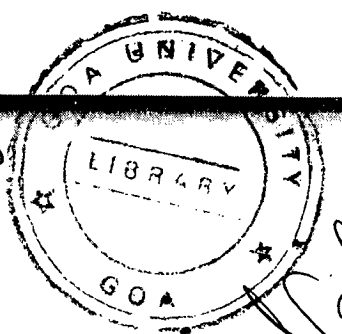
THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (ZOOLOGY)
GOA UNIVERSITY
GOA (INDIA)
2004

Dedicated to ....

My Father

Whose Love and Silent Support
enriches my Life ...



All the corrections are incorporated in the Thesis

28/4/05
(Research Guide)

28/4/05
(Prof. N.V. Nandakumar)
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-- *Certificate* --

This is to certify that **Ms. Suphala Pujari** has worked on the thesis entitled "**Effect of dietary lipids on poultry bird, *Gallus domesticus***" under my supervision and guidance.

This thesis, being submitted to Goa University, Goa, for the award of the degree of Doctor of Philosophy in Zoology, is an original record of the candidate herself and no part of the work has been submitted to any other University or Institute for the award of any degree or diploma. The thesis presented is worthy of consideration for the award of the Ph. D. degree.

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is my original contribution and that the same has not been submitted on any previous occasion for any other degree or diploma of this university or any other university /institute.

To the best of my knowledge, the present study is the first comprehensive study of its kind from the area mentioned.

The literature related to the problem investigated has been cited.
Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Suphala Pujari

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Acknowledgement

I express my deepest sense of gratitude to my guide Dr. R. Roy, for giving me this excellent opportunity to work on this Sunrise subject. I am highly indebted to him for his valuable guidance, constant encouragement and for extending all the help to me; not forgetting the immense amount of patience to drive dissertation to a success. It was in fact a great experience to discuss and learn various dimensions of the subject from him.

I extend my sincere thanks to Prof. P. V. Desai, Head, Department of Zoology, Goa University for providing the necessary laboratory facilities.

I sincerely appreciate the critical suggestions given by Dr. C. T. Achuthankutty, subject expert and VC's nominee, throughout my research work.

My sincere thanks to Dr. Saroj Bhosle, for her valuable suggestions and help to carry out the microbiological analysis. I wish to thank Prof. S. Mavinkurve, the then Head of Department of Microbiology for allowing me to work in the Micobiology laboratories. Thanks are also due to Dr. Mahender K., Department of Earth Science for his help in carrying out the statistical analysis of data.

The financial support towards the conduct of the project from Department of Ocean Development (DOD), Govt. of India, Ocean Science and Technology Cell (Marine Micriobiology) is greatly acknowledged.

I thank Dr. S.K. Shyama, Dr. A. B. Shanbhag and Dr. I. K. Pai for their encouragement and valuable suggestions during the period of research work.

I wish to express my sincere thanks to Prof. V. Kallapur, Dharwad University, for his help and suggestions during my stay in the University Campus. With deep sense of

gratitude I acknowledge the help and assistance afforded to me by Dr. Khub Singh, Director, NDRI, Bangalore in my quest for knowledge.

I thank Mr. Shekhar, Mandovi Hatcheries, for providing sufficient number of experimental animals in time and Dr. V. S. Bhave for his help and valuable suggestions

I am profoundly greatful to Dr. Sundaram, Dr. B. K. Swain, ICAR, Ela – Goa for allowing me to work in their laboratories. I also thank Dr. Avinash Nirmale, ICAR, Ela – Goa for providing the necessary literature relarted to my work.

Thanks are due to Mr. Parmar M/s., Hexa Analytical Laboratories, Mumbai, for his help in Gas chromatographic Analysis. Thanks are also due to Mr. Vishant Gaonkar, Health care Clinical Laboratory, Sanquelim for his help to carry out the pathological investigations.

And not forgetting the valuable efforts given by Mr. Ujjwal Chandawar, who gave me a friendly atmosphere and surrendered all help.

I extend my feelings of utmost garatitude towards Dr. A. L. Paropkari and Dr. Govind Ranade Scientist 'F' and Mr. Sanjeev Afzalpurkar, scientist E2, NIO, Dona Paula, Dr. K. S. Muralidhar, Head Dept. of Zoology, Govt. college Sanquelim and Ms. Preethi Thomas, lecturer, Govt. college Sanquelim for their unconditional support and timely help extended towards me in this task.

Can forget the valuable efforts given by Late Ms. Pushan Bhosle. Her kindheartedness, endearing nature which I reveal from my heart. My sincere gratitude will surely be heard by her.

I remain greatly indebted to Lekha, Minal, Nandini, Sonali, Shanti, Shashikumar, Brahma, Naveen, Upal, Galileo, Shivdas, Raghu, Neelam, Sarita, Chanda, Rasika, Deepa, Trupti, Vikrant for their ready help and assistance in times of need.

I gratefully acknowledge the help rendered to me by Librarian and Staff of Goa University library.

I remain ever grateful to Mr. Suresh and Mr. Ravi Gogia for the kind help extended to make this thesis a success.

I am greatly indebted to Kakodkar family and Bhosle family for their love, concern and moral support extended during the course of my research work. I am indeed grateful to Mr. Jayant Hariawala, for his valuable guidance and encouragement.

Thanks are due to Shailesh, Dasharath, Rama, Tanu, Sudesh, Sangeeta, Gulabi (non teaching staff) for kind help rendered to me while carrying out this work.

At this juncture, I wish to place on record my sincere thanks to Albertina Almeida, Angela, Afroz and Pushpa for their timely assistance. The friendly assistance from Praveena, Deepti, Kavita, Niranjan, Julius, Girish, Ramdas, Sameer, Yashpal, Mukul is greatly acknowledged.

I would be failing in my duty if I do not express my sense of gratitude to all my well wishers who have either directly helped me in various capacities in the successful completion of my research thesis.

I thank my parents for enough freedom and indispensable support bestowed upon me during the tenure of my Ph.D. and my brothers for being an unseen source of my confidence and inspiration.

I thank Mr. Milind Raikar for his help in styling the thesis.

Finally I thank the Almighty for his blessings.

Suphala

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INTRODUCTION

ABBREVIATIONS

TCA	Tricarboxylic acid
CoA	Coenzyme A
NADPH	Nucleotide diphosphate sugar (reduced form)
ACC	Acyl CoA carboxylase
ACP	Acyl carrier protein
$FADH^+$	Flavin adenine diphosphate(reduced form)
NAD^+	Nicotinamide adeninedinucleotide
CDP	Cytidine diphosphate
PAF	Platelet activating factor
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein
LCAT	Lecithine cholesterol acyl transferase
EFA	Essential fatty acid
PUFA	Polyunsaturated fatty acid
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid

A constant supply of energy is necessary for all animals to sustain life. Energy for all animal systems comes from the feed eaten or in times of feed deprivation from body stores. The food that we eat is digested and assimilated in the body and used for its maintenance and growth. It furnishes us the energy that is required for normal growth and metabolism. Food chiefly consists of complex organic substances such as lipids, proteins, carbohydrates, vitamins and also the inorganic components like minerals calcium, water etc.

1.1 NUTRIENTS FOR LIFE

Nutrients play an important role in the protection of the host against invading pathogens. Nutrient deficiencies can affect immune function, usually in a negative manner. Certain nutrients are capable of modulating the function of the immune system through a variety of mechanism.

1.1.1 Carbohydrates

Carbohydrates form the most abundant of all the classes of natural organic compounds. They are mostly compounds of carbon, hydrogen and oxygen. Some carbohydrates contain other elements such as nitrogen and sulfur. They are the main source of energy. Carbohydrate molecules range from simple sugars, which are readily digestible by all species, to the complex carbohydrates like cellulose and lignin, which can be digested only by bacteria. Carbohydrate is the least expensive source of energy. The commonly occurring carbohydrates in food are starch, glucose, fructose and lactose. They are the cheapest source of energy. Glucose derived from the digestion of carbohydrates is used as the main source of energy in the body. Hence, the diet should

contain adequate amount of carbohydrates in order to meet a greater part of the energy needs of an individual (Swaminathan, 1986).

Several pathways involving sugar metabolism exist in cells. One, the pentose phosphate pathway, known also as the hexose monophosphate shunt or the 6-phosphogluconate pathway, is particularly important in animal cells. Its functions side by side with glycolysis and the tricarboxylic acid cycle for production of reducing power in the form of NADPH and pentose intermediates. Another important function is to convert hexoses into pentoses, particularly ribose 5 – phosphate through pentose phosphate pathway (Devlin, 1997). This C5 sugar or its derivatives are components of ATP, CoA, NAD, FAD, RNA, and DNA.

Normal diet contain adequate amount of carbohydrate in order to meet a greater part of the energy needs of a carbohydrate is calculated by using average caloric conversion factors of 4.10 K calories / gm of carbohydrate (Henken *et al.*, 1986).

Benson *et al* (1993) reported that at an equal dietary energy corn starch decreased the growth suppressive effects of lipopolysaccharide injection of chick relative to diets containing corn oil.

1.1.2 Proteins

Proteins are required for the growth and maintenance of body weight. Proteins also provide energy to a small extent. They constitute about 20 percent of the body

weight. Body proteins are derived from dietary proteins. The body loses continuously some quantity of proteins and this loss has to be compensated by dietary proteins. Proteins are made up of simpler chemical substances known as amino acids. The amino acid contents of proteins have been found to differ from one protein to another (Halver, 1989).

The nutritional value of proteins depends on their amino acid contents. A large amount of information is available on the nutritive value of dietary proteins and the protein requirement in farm animals (Halver, 1989).

The important functions of dietary proteins are:

1. To replace the daily loss of body proteins
2. To provide amino acids for the formation of tissue proteins during growth
3. To provide amino acids necessary for the formation of enzymes, blood proteins and certain hormones of protein nature, and
4. To provide amino acids for growth of the fetus and also for the production of milk proteins.

The studies conducted by Osborne and Mendel (1913) showed that rats fed on a synthetic diet containing 'zein' (a protein contained in Maize) as the only source of protein failed to grow. Chemical analysis of zein showed that it did not contain tryptophan or lysine. When these two amino acids were added to zein, animals grew normally.

Experiments conducted on chicks have shown that amino acids like methionine acid are very essential in the growth of chicks and turkeys. Methionine was able to replace part of the glycine in the diet (Takashahi *et al*, 1994).

Diet supplemented with lysine and methionine gives better growth performance with better protein retention and efficacy ratio in fish (El-Danhar and El-Shazly, 1993).

Bhargava *et al* (1970) found that methionine deficiency resulted in increased antibody level. While Tsiagbe (1987) suggested that the requirement for methionine for maximum antibody titres was greater than that for growth.

The protein or the amino acids are channelized for the energy yielding purpose. The amino acids through various transaminases and glutamate dehydrogenase enzyme convert into keto sugars, which enter in the TCA cycle for yielding energy. Besides, ammonia is been produced as a nitrogenous waste in the body which may directly be excreted out or converted into less toxic substances like urea and uric acid and then excreted out from the body (Lehninger, 1984). The energy content of the food is calculated by using the average caloric factor 5.65 K calories per gram for protein (Henken *et al*, 1986).

The important factors affecting the utilization of dietary proteins are:

- Calorie intake
- Digestibility coefficient of proteins and
- Biological and nutritive value of proteins.

For the maximum utilization of dietary proteins, the calorie intake should be adequate. If the calorie intake is inadequate, a part of the dietary protein will be wasted in meeting the energy requirements and the protein need will not be satisfied. In the utilization of dietary protein, a part of the protein is lost in digestion and metabolism. Thus, protein should be supplemented in the diet from different sources like oil seeds, cereals, pulses, nuts etc.

1.1.3 Lipids

The lipids are a group of organic substances of fatty nature, which are insoluble in water but soluble in organic solvents like ether, alcohol, chloroform and benzene. In general, lipids are the esters of fatty acids with glycerol or with other organic compound.

Lipids rarely exist in an organism in the free state but are usually associated with proteins or combined with polysaccharides. They are important dietary constituents providing energy, vitamins, essential fatty acids and often give flavor and palatability to food. Lipids act as lubricants and insulators and the fat stores in the adipose tissue of the body are rich source of energy. Combination of the lipids and proteins are of particular cellular importance especially in membrane structures and also as a means of transporting lipids in the blood. The steroid hormones are derived from cholesterol and very small amounts of these exert potent physiological effects.

Apart from these, fats also help in forming the structural materials for cells and tissues such as cell membrane and other organal components. The fatty acids and glycerol react with alkali present in the small intestine to form their salt. These salts

being water soluble are readily absorbed by the blood and carried to the body cells (Gurr and James, 1976). The energy content of the food is calculated by using the average caloric factor 9.45 K calories per gram of lipid (Henken *et al*, 1986).

1.1.4 Vitamins

Vitamins are ordinarily defined as substances that act in trace amounts. Hence, the definition includes only substances with catalytic functions. Vitamins are classified as either fat soluble or water soluble. They are essential food components just as the essential amino acids are. Vitamins are indispensable for the growth and maintenance of the organism and occur both in animals and plants. A deficiency of certain vitamins is reflected in characteristic disturbances (deficiency diseases). Such diseases are the consequences of unbalanced nutrition. Pantothenic acid, one of the vitamins of vitamin B2 complex, can prevent or cure a specific type of dermatitis (chick pellagra) in chicks (Swaminathan, 1986). Vitamin E deficiency in rats results mainly in atrophy of the testes and dystrophy of muscles (Halver, 1989). In animal experiments, vitamin A deficiency is manifested first by cessation of growth and in man the deficiency leads to night blindness (Karlson, 1970). In animal experiments, a deficiency of riboflavin impairs normal growth and causes symptoms of skin impairment and in man the principal symptoms are dermatitis ("pellagra sine pellagra") and inflammations around the mouth (Karlson, 1970).

1.1.1 Minerals

The body contains about 24 minerals, all of which are derived from diet. The important minerals are, the *anions* like phosphate, chloride, iodide, fluoride and the *cations* viz., calcium, potassium, sodium, magnesium, iron, zinc, copper, manganese, cobalt and possibly others. Among the heavy metals, iron and zinc occupy the top slots. The minerals are essential for various body functions, as indicated below.

i. Sodium, potassium and chloride are essential for maintaining water balance in the body.

ii. Iron and copper are required for the formation of hemoglobin and

iii. Iodine is needed for the normal functioning of thyroid glands (Halver, 1989)

These substances are also metabolized. They are taken up in food and eliminated again in urine, feces and sweat. The metabolism of inorganic ions or simply "mineral metabolism" differs from the metabolism of other substances. In contrast to proteins, carbohydrates or fats, minerals are neither produced nor consumed in the organism. Their intake from food can be regulated only very roughly, if at all. Most animal species, nevertheless, in the course of evolution have developed the ability to keep the concentration of ions constant in the body fluids, thus providing a constant "*milieu interne*". Several ions have special depots, which can be mobilized in periods of insufficient intake (Karlson, 1970).

Copper deficiency can decrease antibody response, mitogen induced blastogenesis and mixed – lymphocytes reaction in mice and addition of copper to poultry diets increased primary antibody response. Zinc deficiency also has been demonstrated to suppress immune function in mammals and poultry (Cook, 1991).

1.2 CHEMISTRY OF LIPIDS

Lipids are structurally diverse. They contain much lower proportion of oxygen atom than do carbohydrates and broadly classified into fats, oil and wax. The function of lipids are to maintain structural integrity of membrane, to supply energy for physiological activities, transport of various metabolites in and out of the cells and regulation of physiological functions through secondary metabolites of lipid (Lehninger, 1984). The major dietary lipid components are namely, triglycerol, phospholipid, cholesterol, glycolipid and fatty acid.

1.2.1 Fatty Acids

Fatty acids are building block components of most lipids. They are long chain organic acids having from 4 – 24 carbon atoms; they have a single carboxyl group and a long, non popular hydrocarbon "tail" which gives most lipids their water insoluble and oily or greasy nature. Many different kinds of fatty acids have been isolated from the lipids of various species. They differ from each other in chain length and in the presence, number and position of their double bonds; some fatty acids also have methyl group branches. Fatty acids that contain no carbon carbon double bonds are termed **saturated fatty acids**; those that contain double bonds are **unsaturated fatty acids**. Unsaturated

fatty acids are further classified as **monounsaturated fatty acids** (e.g. Palmitic acid, Stearic acid) **and polyunsaturated fatty acids** (dienoic, trienoic, tetraenoic).

Polyunsaturated fatty acids are also classified into two major groups, **n3 or ω3** and **n6 or ω6** series depending upon the position of last double bond from methyl end.

The fatty acid biosynthesis occurs in the cytoplasm through a multistep reaction catalyzed by a set of enzymes commonly termed as *fatty acid synthase system.* The *fatty acid synthase system* catalyses the following overall reaction, in which one molecule of acetyl-CoA and seven molecules of the 3-carbon malonic acid, in the form of its CoA thioester, malonyl-CoA are assembled in succession to make a molecule of the 16-carbon palmitic acid, with release of seven molecules of CO_2.

$$\textbf{Acetyl-CoA + 7 malonyl-S-CoA + 14 NADPH+20H}^{+} \longrightarrow \textbf{(CH}_3\textbf{(CH}_2\textbf{)}_{14}\textbf{COO +}$$

$$\textbf{7CO}_2\textbf{+8CoA- SH + 14NADP + 6H}_2\textbf{O}$$

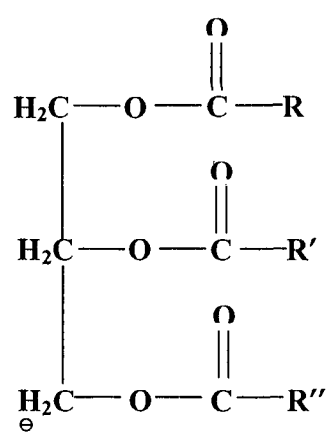
The reducing power required to make the singly bonded hydrocarbon backbone of fatty acids is furnished by NADPH.

The fatty acids produced by fatty acid synthase system do not contain any double bond between carbon – carbon atoms. The desaturation of the fatty acid occurs in the endoplasmic reticulum and catalyzed by a group of enzymes known as desaturation system. In mammals desaturation system comprises of 3 proteins, viz., cytochrome b5, cytochrome b5 reductase and fatty acyl CoA desaturases (Lehninger, 1984).

The fatty acids of the body fat are also channelized for yielding energy through β oxidation pathways, which occurs in the mitochondrial matrix. The oxidation of fat involves a reduction of $FADH^+$ and NAD^+. Through this pathway fatty acids are oxidized by successive loss of two carbon fragments. The fatty acid components of lipids furnish a large fraction of the oxidative energy in animals. Free fatty acids are first activated by esterification with CoA to form acyl-CoA esters. Four reaction steps are required to remove each acetyl-CoA residue from the carboxyl end of saturated fatty acyl-CoAs.

1.2.2 Acyl Glycerols

The most wide spread acyl glycerol is triacylglycerol, also called triglyceride or neutral lipid. Triacylglycerides are composed of a glycerol backbone to which 3 fatty acids are esterified.



[R, R' & R" are acyl chains esterified to glycerol backbone]

Fatty acids are stored for future use as triacylglycerols in all cells, but primarily in adipocytes of adipose tissue. Diacyl glycerols and monoacyl glycerols do not occur in appreciable amounts in nature but are important intermediates in a number of biosynthetic reactions.

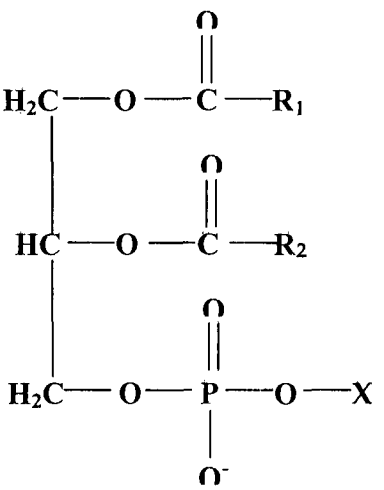
The glycerol backbone of triacylglycerols is activated by phosphorylation at the C-3 position by ***glycerol kinase***. The fatty acids incorporated into triacylglycerols are activated to acyl-CoAs through the action of ***acyl-CoA synthetases***. Two molecules of acyl-CoA are esterified to glycerol-3-phosphate to yield 1,2-diacylglycerol phosphate (commonly identified as **phosphatidic acid**). The phosphate is then removed, by ***phosphatidic acid phosphatase***, to yield 1,2-diacylglycerol, the substrate for addition of the third fatty acid. Intestinal monoacylglycerols, derived from the hydrolysis of dietary fats, can also serve as substrates for the synthesis of 1,2-diacylglycerols (Lehninger, 1984).

Generally triacylglycerol biosynthesis and oxidation occur simultaneously in a steady state, so that the amount of body fat stays relatively constant over long periods although there may be minor shortterm changes as the caloric intake fluctuates. However, if carbohydrate, fat or protein is consumed in excess above normal energy needs, the excess calories are stored in the form of triacylglycerols. Both carbohydrates and carbon chains of amino acids can give rise to acetyl CoA required for the net biosynthesis of fatty acids and triacylglycerols.

The rate of triacylglycerol biosynthesis is profoundly altered by the action of several hormones like insulin, pituitary growth hormone, adrernal cortical hormones and glucagon.

1.2.3 Phospholipids

The basic structure of phospholipid is very similar to that of the triacylglycerides except that the position of the glycerol backbone is esterified with phosphoric acid.



[Backbone of Phospholipid; X – Head alcohol group]

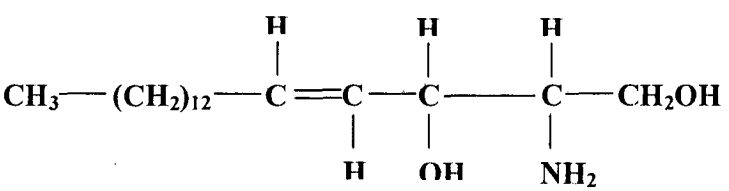
The building block of the phospholipid is phosphatidic acid, which results when the X substitution in the basic structure shown in the Figure above is a hydrogen atom (Gurr and James, 1976). Substitutions include ethanolamine (phosphatidyl ethanolamine), choline (phosphatidyl choline, also called lecithins), serine (phosphatidyl serine), glycerol (phosphatidyl glycerol), *myo*-inositol (phosphatidyl inositol, these compounds can have a

variety in the numbers of inositol alcohols that are phosphorylated generating polyphosphatidyl inositols), and phosphatidylglycerol (diphosphatidylglycerol more commonly known as cardiolipins).

Phospholipids are the basic lipid components of all membranes. The biosynthetic enzymes are associated with the endoplasmic reticulum (eukaryotic cells) or plasma membrane (prokaryotic cells). Phospholipids can be synthesized by two mechanisms. One utilizes a CDP-activated polar head group for attachment to the phosphate of phosphatidic acid. The other utilizes CDP-activated 1,2-diacylglycerol and an inactivated polar head group.

1.2.4 Sphingolipids

Sphingolipids are composed of a backbone of sphingosine which is derived itself from glycerol. Sphingosine is N-acetylated by a variety of fatty acids generating a family of molecules referred to as ceramides (Lehninger, 1984).



Sphingosine

Sphingolipids predominate in the myelin sheath of nerve fibers. Sphingomyelin is an abundant sphingolipid generated by transfer of the phosphocholine moiety of

phosphatidylcholine to a ceramide, thus sphingomyelin is a unique form of a phospholipid.

The other major class of sphingolipids (besides the sphingomyelins) are the glycosphingolipids generated by substitution of carbohydrates to the *sn*1 carbon of the glycerol backbone of a ceramide. There are 4 major classes of glycosphingolipids:

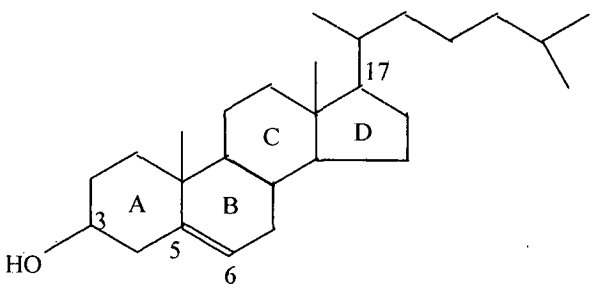
- **Cerebrosides:** contain a single moiety, principally galactose.
- **Sulfatides:** sulfuric acid esters of galactocerebrosides.
- **Globosides:** contain 2 or more sugars.
- **Gangliosides:** similar to globosides except also contain sialic acid.

1.2.5 Glyceryl Ethers (Plasmalogens)

Plasmalogens are glycerol ether phospholipids. They are of two types, alkyl ether and alkenyl ether. Dihydroxyacetone phosphate serves as the glycerol precursor for the synthesis of glycerol ether phospholipids. Three major classes of plasmalogens have been identified: **choline, ethanolamine** and **serine plasmalogens**. Ethanolamine plasmalogen is prevalent in myelin. Choline plasmalogen is abundant in cardiac tissue. One particular choline plasmalogen (1-alkyl, 2-acetyl phosphatidylcholine) has been identified as an extremely powerful biological mediator. This molecule is called **platelet activating factor, PAF** (Gurr and James, 1976). These are found in varying portions in marine organisms and other animal species.

1.2.6 Cholesterol

Cholesterol is complex and fat soluble having four fused isoprene rings. It has a polar head group, hydroxyl group at position 3. The rest of the molecule is a relatively rigid non-polar structure.



Cholesterol

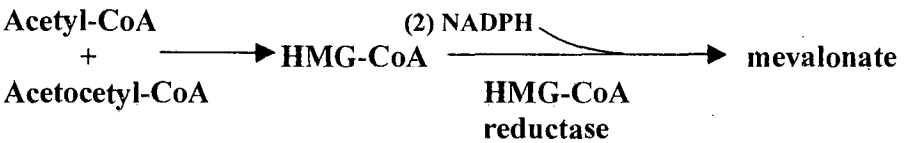
Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the **steroid hormones** and bile acids. Both dietary cholesterol and that synthesized *de novo* are transported through the circulation in **lipoprotein particles**. The same is true of cholesteryl esters, the form in which cholesterol is stored in cells.

The synthesis and utilization of cholesterol is tightly regulated in healthy animals in order to prevent over-accumulation and abnormal deposition within the body. The abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries is of particular importance clinically. Such deposition, eventually leading to atherosclerosis, is the leading contributory factor in diseases of the coronary arteries.

Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA.

The process has five major steps:

1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
2. HMG-CoA is converted to mevalonate
3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2
4. IPP is converted to squalene
5. Squalene is converted into lanosterol and then to cholesterol.



The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms (Lehninger, 1984):

1. Regulation of HMGR activity and levels
2. Regulation of excess intracellular free cholesterol through the activity of *acyl-CoA: cholesterol acyltransferase*, ACAT
3. Regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport.

Regulation of HMGR activity is the primary means for controlling the level of cholesterol biosynthesis. The enzyme is controlled by four distinct mechanisms: feedback inhibition, control of gene expression, rate of enzyme degradation and phosphorylation-dephosphorylation.

The first three control mechanisms are exerted by cholesterol itself. Cholesterol acts as a feedback inhibitor of pre-existing HMGR as well as inducing rapid degradation of the enzyme. The latter is the result of cholesterol-induced polyubiquitination of HMGR and its degradation in the proteosome.

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesized by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDLs. The liver synthesizes VLDLs and these are converted to LDLs through the action of endothelial cell-associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL-associated enzyme LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (apo-D), which is associated with HDLs. **Reverse cholesterol transport** allows peripheral cholesterol to be returned to the liver in LDLs (Gurr and James, 1976).

1.3 DIETARY ROLE OF LIPID

Lipid metabolism in animals can no longer be considered to be simply a matter of dietary fatty acids. The dynamics of membrane structure and function depend upon the complex role of lipid and help the organism to adapt a new environment (Hazel, 1984; Cossins, 1983; Dey *et al.*, 1993; Farkas *et al.*,, 1994; Roy *et al.*, 1997). The interrelationship between the dietary fatty acid, membrane fluidity and membrane integrity and metabolic pathways in animal are evident and the state of the lipids in the animal is in a constant flux.

The main function of fats in the body is to provide a steady energy. Fats provide twice as much energy as that provided by the same amount of carbohydrate, because fats molecule contain higher percentage of carbon and hydrogen, but less percentage of oxygen than that of carbohydrates. Fats are the richest source of energy to the body but they are more expensive than carbohydrates.

Fats fulfill important functions in our diet. It is a source of essential fatty acids and carries lipid soluble vitamins. The amount and type of fat consumed is the focus of much interest on maintaining the good health. Fat is important for slowing the digestive process so one does not feel hungry an hour after eating a meal. One role of fat in the diet is to aid in the absorption of fat soluble vitamins, which include vitamins A, D, E and K.

Polyunsaturated fatty acids cannot be synthesized *de novo* by animals (Henderson and Tocher, 1987) as the animals lack the enzymes (Δ_{12} and Δ_{15} desaturases) required for the addition of extra double bond to monoenoic or oleic acid towards the methyl end to synthesize linoleic (9,12, Octadecaenoic acid) and linolenic (9,12, 15 Octadecatrienoic acid) acids respectively.

Because these two fatty acids are essential to body function they must be obtained through diet. Therefore they called ***essential fatty acids*** (EFA). Animals do possess the machinery to synthesize other long chain polyunsaturated fatty acids, once these two essential fatty acids are supplied to the body. These EFAs are used as parts of cell membranes and in the synthesis of hormone like substances.

The natural distribution of these two fatty acids is not in a same order. Synthesis of linoleic acid from oleic acid is very common among the terrestrial plants where as the linolenic acid, which is further a desaturated product of linoleic acid by Δ_{15} desaturase enzyme is very restricted in terrestrial ecosystem.

These two fatty acids are very important for animal beings in terms of producing other ω3 and ω6 series of PUFAs and are involved in temperature adaptation and production of secondary metabolites (Lands, 1987).

Fats with a high content of saturated fatty acids (SFA) include tropical oils like coconut oil, palm kernel oil, cocoa, butter, palm oil etc. SFA can increase blood cholesterol levels. Higher levels of blood cholesterol increase the risk of heart disease.

Foods high in PUFA include corn oil, safflower oil, soybean oil, sunflower oil, cotton seed oil, walnuts, and seafood. Foods like olives oil, canola oil, peanuts, almonds, hazelnuts, cashew nuts are rich in monounsaturated fatty acid (MUFA). MUFA and PUFA both have a blood cholesterol lowering effect and can lower the risk of heart disease.

PUFAs are very essential for the growth and development and maintenance of cellular functions in animals. EPA and DHA are important for normal growth and development in children and are also active in brain and eye development; these fatty acids may also be important in the prevention and treatment of heart disease, hypertension, arthritis and cancer, especially in adults (Simopoulus, 1991; Salem *et al.*, 1996).

Fish oil and fish meal, rich sources of ω3 fatty acids are the most effective means of correcting the dietary balance of ω6: ω3 fatty acids in terms of the improvements seen in health and fertility (Allen and Dandorth, 1988).

The ω6 and ω3 series of fatty acids play a significant role to maintain physiological homeostasis in animals. The secondary metabolites viz., prostaglandin and thromboxane produced from PUFA regulate the various metabolic and physiological functions in animals (Lands, 1987).

Each PUFA synthesize different prostaglandin and thromboxane. Dietary linoleate is converted to arachidonate, which is further converted to prostaglandin required for biosynthesis of steroid. On the other hand, the eicosanoid produced from n3 PUFA act as anti-inflammatory mediators. All eicosanoid use same biosynthetic pathway (i.e. cycloxygenase or lipoxygenase pathway) but they work in antagonistic fashion. Eicosanoid of one group work as competitive inhibitor for the production of other eicosanoid. It is important to recognize that linoleic acid will not substitute alpha linolenic acid in providing n3 PUFA to various tissues (Mantzioris *et al*, 1995).

1.4 LITERATURE SURVEYED:

Investigations to determine the essential fatty acid requirement of poultry bird began in 1950 and in 1960 linoleic acid requirement was established for chickens. Presently a dietary level of 1% linoleic acid is recommended for adequate growth of Chickens, Turkeys and Quails (National Research Council, 1984). Even though essential fatty acid requirement is known, little information is made available to the practical nutritionist regarding the metabolic and physiological importance of essential fatty acid in poultry. Essential fatty acid deficiency symptom in chicks include retarded growth,

increased water consumption, reduced resistance to disease, enlarged liver with increased lipid content and alteration of tissue fatty acid composition (Balnava, 1971). Linoleic acid requirement for growing quail as determined by maximum growth rate and minimum liver size, was estimated to be about 1% of the diet but the requirement of the same for egg production was estimated to be about 0.70% of the diet (Murai *et al*, 1994). Although linoleic acid is accepted as essential fatty acid for the poultry birds, linolenic acid is also essential for the normal development and growth of poultry birds as in other animals. Linoleic acid cannot be synthesized *de novo by* animals (Henderson and Tocher, 1987), it is to be consumed along with the food. Hence, it is also known as essential fatty acid in animals including the poultry birds. It is important to recognize that linoleic acid will not substitute linolenic acid in providing n3 PUFA to various tissues. The long chain n3 PUFA, derived from linolenic acid, is present in the retina and nervous tissue (Rezenka, 1998). The recognized deficiency symptoms of n3 fatty acid in mammals include defective vision (Neuringer *et al.*, 1998) and impaired learning ability (Yamamoto *et al.*, 1987, Bourre *et al.*, 1989). Although dietary linoleic acid may protect chicks from nutritional encephalomalacia induced by vitamin E deficiency (Budowski and Crawford, 1986), an absolute requirement for a linolenic acid in poultry has not been demonstrated. Both linoleic and linolenic acid are readily absorbed through the intestinal wall where resynthesis of triacylglycerol and the packaging of lipid into proto microns occur for transport to the liver (Krogdahl, 1985). Metabolic machinery of birds altered during post hatching development depending upon the type and proportion of metabolite consumed along with the diet (Asnani and Pilo, 1991). Halle (1999) reported that the

fertility, hatchability and the growth of progeny of poultry birds depend upon the dietary fatty acids.

Broadhurst *et al* (2002) proved that polyunsaturated fatty acid is dietary essential for brain intellectual growth and development. Watts and Browse (2002) proved that PUFA are important membrane component and precursor of signaling molecules. The long chain PUFA during infancy has been related to neonatal growth and development (Patrix and Gerard, 2002). Polyunsaturated fatty acid derived from essential fatty acid plays an important role in prenatal visual and neural development. It was found that in malnourished infants a nutrient formula enriched with long chain fatty acid of n6 and n3 series could be helpful to achieve erythrocyte fatty acid pattern and a visual function similar to that obtained in a breast fed infant (Marin *et al.*, 2000). Bell *et al.*, (1994) showed that diet rich in PUFA influences the growth as well as the production of secondary metabolites like prostaglandin and thromboxane in juvenile trouts.

The metabolic utilization of dietary fat affects the carcass composition and meat quality in farmed animals (Doreau *et al.*, 1997) including the poultry birds (Hartfiel, 1995; Mieczkowska *et al*, 1999). Klinger *et al* (1996) suggested that dietary lipid effect several hematological factors of culture channel cat fish. Fish fed with fish oil diet had significantly lower hematocrits, higher thrombocyte count and higher serum iron concentration. Dietary lipid affects the fatty acid composition of blood leucocytes and plasma eicosanoid concentration in European Sea Bass (Farndale *et al.*, 1999). It was proved that deficiency of EFA in chicks lead to cessation of growth and loss of feathers

on the body (Swaminathan, 1986). Shafey and Cham (1994) showed that by manipulating hen's diet the cholesterol and fatty acid content of the eggs can be altered for better human consumption. The dietary fatty acid influence the fatty acid composition of egg yolk fatty acid and the utilization of essential fatty acid by developing chick embryo (Lin *et al.*, 1991). Rinogi *et al* (2000) showed that, the daily heat production of cocks fed the diet with fish oil rich in long chain PUFA was found to be higher than that in those fed with control diet containing soya bean oil and linseed oil. This result suggests that also in poultry long chain PUFA induces thermogenesis, particularly during daylight.

Dietary fat induced changes in tissue lipid parameters have already been reported in various animals including mammals and non-mammals. The undigested fraction of soya bean protein supplemented with 10% safflower oil induces the changes in leukotrienes and prostaglandin E production in the spleen of hamsters (Gatchalian *et al.*, 1994). However, when the hamster was fed on safflower oil with soybean protein diet leukotriene B4 production was not influenced (Gatchalian *et al.*, 1995). 1% conjugated linoleic acid supplemented diet significantly increases the body mass gain along with the increased ratio of HDL – Cholesterol and total cholesterol ratio in rat (Szymczyk *et al.*, 2000). Similarly, conjugated linoleic acid did not modify the performance parameter (weight gain and feed conversion ratio), but altered the fat and protein content of the tissues in chicks (Simon *et al.*, 2000). Work done by An Byong *et al* (1997) showed that dietary fat, the degree of unsaturated fat, plays a very important role in the growth of chicks, when the four weeks old chicks were fed with fat varying in

saturated and unsaturated index from different sources and the metabolism in the growing chicks was significantly affected. Lopez *et al* (2001) reported that high fish oil concentration decreases the saturated and monoenoic fatty acid content in the thymus sample. Production of platelet thromboxane A2 and aortic prostacyclin decreased in rat with higher intake of n3 fatty acid (Yamada, 1996). DHA and EPA prevent atherosclerosis development by reducing hypercholesterolemia and modifying the platelet function in rat (Ikeda *et al.*, 1996,1998; Adan *et al.*, 1999). Castillo *et al* (2000) showed that fish oil produced a significant reversion of the hyper cholesterolemia previously induced by coconut oil feeding. Fish oil also produces a clear decrease in plasma triacylglycerine level. PUFA reduces the incident of NEC (nectorizing enterocolitis) by modulating PAF (platelet activating factor) metabolism and endotoxin trans location.

Watkins (1995) reviewed the dietary and hormonal control of PUFA formation and the role of PUFA in eicosonoid production during cell differentiation, oviposition and bone cell metabolism of poultry bird. Dietary administration of γ - linolenic acid increased in vitro production of prostaglandin E1 derived from dehomo γ - linolenic acid but did not significantly influenced the production of prostaglandin - E2 derived from Arachidonic acid in rat (Quoc *et al.*, 1996). Although the capability of fat digestion increased with dietary supplementation of lipase, the feed intake in terms of crude protein remain unaltered in broiler chicks (Ala-Marazooqi and Leeson, 1999). Cholesterol metabolism of the growing birds varies with age of the growing birds (Innariea *et al.*, 1992).

Marine bacteria are known to produce wide range of compounds, which have potential applications as bioactive compounds, probiotics and nutritional supplements. These organisms are now being screened for the production of polyunsaturated fatty acids as well as specific fatty acids (Watanabe *et al.*, 1996 & 1997; Yazawa, 1996). The concept of using microorganisms in feed or enriching the feed with some specific microorganisms in fish is well established in Asian countries. The use of living microbial supplementation in diet as an additional ingredient for enhancing growth of animal has been the thrust area of nutritionist in the recent past. The probiotics have multiple effects on intestinal microflora and act as health promoting microorganisms (Yano *et al.*, 1994).

1.5 OBJECTIVES

So far, the nutritional studies carried out with respect to fish nutrition, poultry nutrition, cattle nutrition or human nutrition is focused on the requirement of dietary protein and how best this could be supplemented. Only very recently the emphasis is given on requirement of EFA in the nutritional biochemistry work. From the literature review it has been observed that both the linoleic and linolenic fatty acids are required in the diet for better growth of animal. It is been suggested that only 5% of lipid should be supplemented in the diet either in the form of linoleic or linolenic acid or both. There is absolute dearth of knowledge about what is the exact quantity of the linoleic and linolenic acid should be there in the diet in order to maintain animal in a **well being condition**.

Poultry products like chicken meat, eggs are in great demand as it provides protein rich comparatively low priced non-vegetarian diet, which is unique and tasty. It becomes an essential substitute of protein foe human consumption. Although the poultry science in India and other countries is well established with regard to improvement of the meat and production of eggs through dietary manipulation, feed formulation of the poultry has not been aimed to improve the health of the consumer (human beings) as well as poultry bird itself.

The work that has been carried out so far on importance of dietary lipids (as reviewed in section 1.4) depicted that fatty acid profiles of tissues depend on dietary lipids. But how the dietary lipids are influencing PUFA composition has not been studied. The effect of dietary cholesterol on serum and tissue lipid composition on the different organism has been worked out but how dietary cholesterol modulates lipid composition is not studied. Mostly scientists have worked on poultry birds of 14 days age and above and there is no information about the requirement of dietary lipid in neonatal period. Although much work is done to increase sustainable level of n3 PUFAs, in fish, poultry birds and other animals, the requirement of particular fatty acids as growth promoter are not known.

The concept of enriching the feed with some specific microorganisms in fish and other animals is well established in Asiah countries. These act as additional ingredient for enhancing growth of animal and have multiple effects on intestinal microflora and acts as health promoting factors.

It has been observed that most of the available commercial poultry diets contain 35-40% crude protein and 5-10% of crude fat, out of which 60-70% is linoleic acid and 2-3% is linolenic acid. Since, varying the dietary levels of essential fatty acids will modify the composition of long chain PUFA of both ω6 and ω3 series in the tissues of poultry birds, enriching poultry meat with specified PUFA can be done to meet the consumer demand. Furthermore, the changes in the types of PUFA in the tissues of bird may offer potential benefits to the birds by modulating eicosanoid production which would help the birds to be in a **"well being state"** and so also the human beings.

In the present research work, poultry bird, *Gallus domesticus* is used as an experimental model. The emphases were laid on the quality and quantity of lipid in a diet. The main aim of the study was to increase the sustainable level of n3 and 0r n6 PUFA so that the consumer of these animals get benefit by synthesizing sufficient amount of metabolically active compounds required for being in a **"well being state."**

Efforts were taken to see the effect of dietary lipids on poultry bird during post hatching development from 1st day to 35th day, to see change in pattern of lipid metabolism during this period and also to find out the amount of EFA required for them to be in healthy state during this period.

The assumed Ph.D. work is based on the following broad objectives:

1. **To study the lipid profiles in the growing birds during post hatching development.**

2. **Role of some dietary lipids (some oils) in the tissues of poultry bird during growth and development (post hatching)**

3. **To find out whether some marine bacteria can be used as an alternative source of ∝- linolenic acid (n-3 fatty acid).**

Materials and Methods

ABBREVIATIONS

RBC	Red blood cell
WBC	White blood cell
FCR	Feed conversion ratio
Gw	Daily instantaneous growth rate
PCA	Perchloric acid
BSA	Bovine serum albumin
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
NaCl	Sodium chloride
BHT	Butylated hydroxy toluene
ATP	Adenosine triphosphate
TG	Triglycerol
CH	Cholesterol
NAD	Nicotinamide adenine dinucleotide
ALP	Alkaline phosphatase
GPT	Glutamate pyruvate transaminase
GOT	Glutamate oxoloacetate transaminase
LDH	Lactate dehydrogenase
$NADPH^+$	Nucleotide diphosphate sugar (reduced form)
DNPH	Dinitrophenyl hydrazine

The present work was carried out at Department of Zoology, Goa University, Goa. Goa is the smallest state of India lying on west coast. It is located between latitudes $15^0$48′33″N and $14^0$53′54″N, longitudes $74^0$20′13″E and $73^0$40′33″E.

2.1 COLLECTION OF MATERIALS

2.1.1 Chemicals

The chemicals like organic solvents (chloroform, methanol, benzene, ether etc.) and acids (sulphuric acid, phosphoric acid, acetic acid etc.) were procured from M/s., Sds-fine Chemicals. Other chemicals, namely, the various salts (sodium chloride, potassium chloride, copper sulphate, sodium sulphate, sodium hydroxide, potassium hydroxide, ferric chloride, ammonium molybdate, magnesium nitrate, ascorbic acid, EDTA, phosphotungstic acid, etc.) were obtained from M/s., Sds-fine Chemicals and or M/s., Hi-media. Standard chemicals like cholesterol, tripalmitin, lipid standard, fatty acid standard as well as the chemicals required for the enzymatic analysis (p-nitrophenyl, di - nitro phenyl hydrazine, aspartic acid, α - ketoglutaric acid, alanine, cysteamine, NADPH, HMG –CoA, acetyl CoA, acetoacetyl Co-A, glycocholate, colipase, etc.) were imported from M/s., Sigma Chemical Co., USA. Most chemicals used for this research work were either in AR or general grade.

2.1.2 Glasswares And Labwares

The required glass wares like beakers, pipettes, test tubes, funnels, conical flask, burettes, etc. were procured from M/s., Borosil and other lab wares like plastic storage bottles, micro vials, centrifuge tubes, pipette stands etc. were obtained from

M/s., Tarsons. All the glassware and lab wares were cleaned properly with teepol detergent and chromic acid and were dried before use.

2.1.3 Experimental Animals

After obtaining the approval from the local animal ethics committee, Goa University, *Gallus domesticus* was used as the experimental animal. Day old chicks (*Gallus domesticus*) broiler strain were obtained from M/s., Mondovi Hatcheries (Ponda, Goa).

2.1.4 Feed For The Experimental Animals

Commercial feed (Shankh Brand) was used to feed the experimental animals (broiler chicks). The same was procured from M/s., Vinod Traders, Ponda - Goa. The oils used in the experiment viz., sunflower oil (Sundrop brand of M/s., Hindustan Lever Ltd.) and coconut oil (Cococare brand of M/s., Raj Mills Pvt. Ltd.) were procured from the local market. The fish (Sardine) oil was obtained from M/s., Sigma Chemical Co. USA.

2.1.5 Marine Bacteria

In our laboratory we cultured bacteria collected from coastal sediments, which were having high efficacy for *de novo* synthesis of α - linolenic acid when grown in sodium acetate medium. (Pujari *et al.*, 2004). These bacterial isolates were grown on large scale and used as a supplement of α - linolenic acid.

2.2 MAINTENANCE OF EXPERIMENTAL ANIMALS

The chicks were maintained in the aviary room of the animal house attached to the Department of Zoology, Goa University. The day old chicks were kept in a cage (size 51cm x 51 cm x 51 cm) inside the quarantine room for six days. The cages were cleaned regularly. Six birds were kept in each cage. During this tenure they were subjected to brooding temperature by switching on 25W bulb. They were also given 1% glucose s and 1% tetracycline solutions. On 7th day healthy birds were picked and vaccinated (with Lasota 100) as per recommendation of veterinary doctor attached to Mandovi Hatchery and were shifted to the aviary room. The room was cleaned and disinfected properly.

2.3 EXPERIMENTAL SET UP

The birds were maintained till 35 days in the aviary room and utmost care was taken with respect to their cleanliness, food and water. The water was given *ad libitum*. The ration given per week per bird is shown in the chart below (as per the recommendation of veterinary doctor attached to the Mandovi hatchery).

Age of the bird	**Feed given**
1st week	10 gm per bird per day
2nd week	20 gm per bird per day
3rd week	40 gm per bird per day
4th week	60 gm per bird per day
5th week	80 gm per bird per day
6th week	100 gm per bird per day

During the first week the chicks were given water and feed in the saucers. Later the same was replaced by the feeders (size 32 cm x 9 cm x 6 cm) and waterers (size 15 cm x 15 cm x 6 cm). Six birds at a time were sacrificed from the day of hatching till the marketable size with a gap of seven days.

In the second phase, 7 days old chicks were divided in four groups. Group 1 was maintained with the commercial diet (which served as control). Remaining three groups were supplemented with different doses (2.5%, 5%, 10%) of coconut oil selected for the study, along with the commercial diet. The birds were sacrificed on 16th day (21 day old bird) and 31st day (35 days old birds) of feeding. After 15 days of feeding 3 chicks from each cage were sacrificed and remaining 3 chicks were maintained for another 15 days and then sacrificed. The same experiment was repeated once again. All the birds were given feed as per the recommended ration (mentioned as earlier).

Similarly, in the third and fourth phases the feeding experiments were conducted with different doses of sunflower oil and fish oil respectively. The said experiments were also repeated once again.

The day of start of feeding experiment with different oils is indicated as '0 day' in tables and figures. D1, D2 and D3 refers to the different doses of oils (2.5%, 5% and 10% respectively).

In the fifth phase, another 4 groups were maintained in the laboratory. Group 1 served as control and the remaining 3 groups were supplemented with different strains

(*Pseudomonas sp.*, *Streptococcus sp.*, *Staphylococcus sp.*) of marine bacteria (0.25g wet bacterial cells per bird per day) along with the commercial diet. The birds were sacrificed after 15 days and 30 days of feeding. The selected bacterial strains were grown in mineral salt medium (MSM) containing 5% sodium acetate and were incubated on rotary shaker (180 rpm) at room temperature for 48 hrs. Cells were then harvested by centrifuging at 10,000 *x g* for 10 min at 16° C (Remi centrifuge, model C-24) and washed with 0.85% saline. The bacterial cells obtained were then killed by heat treatment and were mixed with the commercial diet to feed the chicks. The said feeding experiment was repeated once again.

The proximate composition of commercial feed and experimental feed were analysed in the ICAR laboratory, Old Goa, Goa (ref. Table 1). The fatty acid composition of feed, oils and bacteria were analysed in the laboratory (ref. section 2.8.2.4).

2.4 COLLECTION OF TISSUES

For haematological analysis, blood was collected by pricking the vein going to the wing. For the analysis of serum, the blood was collected from the dorsal aorta with the help of syringe without any anticoagulant and transferred into a centrifuge tube (anticoagulant free) and incubated for 15 minutes at room temperature for complete coagulation of the blood. The clean serum was obtained by centrifuging the tube at 600 *x g* (Remi Centrifuge, model C23) for 10 mins. The serum thus obtained was stored in the vials and refrigerated immediately for various estimations and analysis.

For the collection of other tissues namely, liver, muscle (*Pectoralis major*), large intestine and pancreas, the general viscera was exposed. The tissues were collected and kept in ice immediately for various biochemical estimations and enzymatic analysis.

2.5 GROWTH

Growth of chicks was monitored over a specific time period. The weight of the individual chick was recorded every week by using monoplane balance. Each chick was tagged individually with different numbers using cello tape and labels. Number was written on the label and it was tagged around the leg of each chick. Daily instantaneous growth rate (G_w) and percentage increase in weight per day (%W/day) was tabulated following the formula given by Hardy (1989).

Daily instantaneous growth rate (G_w) = ln W_1 –ln W_2 / T

where, W_1= weight of the bird at start of study

W_2 = weight of the bird at end of study

T = time intervals in days

Feed conversion ratio (FCR) = Feed intake / Weight gain

2.6 ROUTINE HAEMATOLOGY (Sood, 1996)

2.6.1 Total count of the blood cells

Total Blood cells count was done as per the method of Sood (1996). The Neubauer chamber was used for WBC and RBC counting.

2.6.1.1 Erythrocyte count

3.98 ml of RBC diluting fluid was taken into the test tube and to this 0.02ml of blood (obtained by pricking the vein going to the wing) was added. Dilution of the blood was two hundred times. A small drop of diluted blood was taken on the counting chamber. The numbers of red cells seen on 5 small squares (each 0.04 sq.mm) were counted.

2.6.1.2 Leucocyte count

0.38 ml of WBC diluting fluid was taken into the test tube and to this 0.02 ml of blood (which was obtain by pricking the vein going to the wing) was added. Dilution of blood was twenty times. A small drop of diluted blood was taken on the counting chamber. The number of leucocyte seen in the four corners (WBC square each one sq. mm) were counted.

Calculation

Total number of cells /c. mm

= Average number of cells counted per square x F (Factor)

F = Dilution Factor / Area of each square counted *x* volume of fluid

2.6.2 Haemoglobin

Haemoglobin estimation was done by using Sahli's hemoglobino meter. Haemoglobin tube was filled till 20 mark with 0.1N HCL. To this 20 ul of the blood (obtained by pricking the vein going to the wing) was added and kept for 10 minutes. During this time the mixture of acid and blood in the tube was kept stirring. Then the distilled water was added until a match was obtained with the

brown standard (comparator) provided. The lower level of fluid meniscus on gm/100 ml of blood was read.

2.7 TISSUE PROTEIN

2.7.1 Extraction of Protein (Das and Prosser, 1967; Roy *et al.*, 1991)

0.5gm of tissue (liver/ pectoral muscle/ large intestine) was homogenated with 5ml of ice-cold water and 5ml PCA (Perchloric acid). It was centrifuged at 800 *x g* for 15 min. The residue was washed with different solvent like chloroform methanol and diethyl ether. The washed residue was dissolved in 4ml 1(N) NaOH. This was considered as crude protein.

For extraction of crude serum protein, 0.1ml of serum was dissolved in 2 ml of 1(N) NaOH.

2.7.2 Estimation of protein (Lowry *et al.*, 1951)

The protein aliquot was diluted to 0.5ml with distilled water and to this 5ml of protein reagent (Lowry's reagent) was added. The mixture was incubated for 15 min at room temperature. To this 0.5ml of diluted (12) Folin's Ciocalteu reagent was added. This mixture was again incubated for another 10 min at room temperature. The intensity of colour was measured against a suitable blank at 690nm. Finally the quantification of the protein content of the sample was calculated with the help of a prepared standard curve of BSA.

★ **Standard Solution**

250 ug /ml BSA solution was prepared in 1N NaOH

★ Protein Reagent (Lowry's reagent)

To 98 ml of 4% sodium carbonate solution, 1 ml of 2% copper sulphate solution and 1 ml of 4% potassium sodium tartarate were added to prepare protein reagent.

2.8 TISSUE LIPID PROFILE

2.8.1 Extraction of Lipid (Roy *et al.*, 1991)

0.5gm of tissue was homogenated with 5ml of ice cold water and 5ml of 10% Perchloric acid (PCA). This was centrifuged at 800 *x g* for 15 min. The supernatant was discarded and the residue was washed with chloroform-methanol (11), chloroform-methanol (21), and diethyl ether. The supernatant of each wash was collected in a same test tube. To this 5% NaCl was added and mixed thoroughly and was kept over night in the refrigerator for phase separation (Folch *et al.*, 1957). For the extraction of lipid from the serum, the serum collected after centrifugation was mixed with chloroform - methanol (21). The mixture was kept on rotary shaker for 30 mins at room temperature. To this, 5 % of NaCl solution was added and mixed thoroughly and was kept over night in the refrigerator for phase separation.

The lower organic phase that is lipid phase was collected, filtered and dried completely (under the vacuum). The dried lipid was resuspended with hexane (containing 1mM BHT, as antioxidant) and was stored at (–) 20°C for further analysis.

2.8.2 Estimation of Lipid (Kates, 1986)

2.8.2.1 Total Sterol

To the dried lipid aliquot 6ml of acetic acid and 4ml of ferric chloride reagent were added. The mixture was then kept in ice bath for 15 min. The intensity of the colour was measured against a suitable blank at 550nm. Finally the quantification of the sterol content of the sample was calculated with the help of a prepared standard curve for cholesterol.

★ **Standard Solution**

1.3 umole /ml Cholesterol solution was prepared in Acetic acid.

★ **Ferric chloride reagent**

4 ml of 2.5% ferric chloride stock solution in orthophosphoric acid was diluted to 50 ml with concentrated sulphuric acid. (prepared freshly).

2.8.2.2 Total Triglycerol

The lipid aliquot was saponified at 60°-70° C for 30 min with 0.5ml of 0.4% ethanolic sodium hydroxide solution. The mixture was then cooled and neutralized by adding 0.5ml of 0.25(N) sulphuric acid and the ethanol was completely removed on a water bath. To this 0.1 ml of 0.05M sodium per iodate, 0.05M sodium arsenate and 5ml of freshly prepared chromotropic acid reagent were added at an interval of 10 min. The mixture was kept for 30 min on a water bath to develop the colour. The tubes were then cooled and the intensity of the colour was measured against a blank at 570nm. The quantification of the triglycerol content of the sample was calculated with the help of a prepared standard curve of tripalmitin.

★ Standard Solution

4 umole/ml Tripalmitin solution was prepared in Benzene.

★ Chromatrophic acid reagent

0.448 gm of sodium salt of chromotropic acid was dissolved in 40 ml of distilled water and the mixture was kept for cooling in the refrigerator. To this an ice-cold mixture of 120 ml of concentrated sulphuric acid and 60 ml of distilled water was added.

2.8.2.3 Total Phospholipid

Phospholipid was estimated as phosphate in the sample. A lipid aliquot was taken and evaporated by keeping on a water bath. After evaporation, 0.2ml of 10% alcoholic magnesium nitrate was added and the mixture was charred at 300^0C(using Bunsen burner) till a white powder was formed. This powder was dissolved in 1.5ml of 0.5(N) HCl. To this 3.5ml of freshly prepared ammonium molybdate reagent was added and the mixture was kept on a water bath for 30 min for colour development. The intensity of the colour was then measured against a blank at 820nm. The quantification of the phospholipid content in term of phosphate was calculated with the help of a prepared standard curve of ATP.

★ Standard Solution

4 umole /ml ATP solution was used.

★ **Ammonium molybdate reagent**

0.42% of ammonium molybdate solution in 1N sulphuric acid and 10% of aqueous ascorbic acid solution were taken in the ratio of 61 in order to prepare the reagent.

2.8.2.4 Fatty Acid Profile of Lipid

For esterification, a lipid aliquot was taken in screw cap test tube and it was dried off. To this 5ml of 5% methanolic HCl and 2-3 drops of concentrated sulphuric acid were added. The tubes were placed in water bath (90^0C) for 3-4 hours. Then it was cooled and to this 0.5 ml of water and 3.5ml of hexane were added and vortexed for 15 min. Upper phase was collected as methyl ester. The same was dried under vacuum and stored in hexane (0.2ml). Fatty acid profile of the prepared methyl ester was analysed with the help of Gas Chromatogram (Chemito make model 8610) equipped with FID (Flame ionising detector) and 10% DEG (Di ethylene glycol) packed column. The oven temperature was programmed (Roy *et al.*, 1992) with the initial temperature 170° C, then raised to 180°C at the rate of 1°C per min and held for 10min and then again raised to 190°C at the rate of 2°C per min and finally held for 15min. The flow of the carrier gas was fixed at 15ml/min. The identification of the obtained peeks was done with the help of prepared standard chromatogram of the known fatty acids under the same programme.

2.8.3 Serum Lipid Profiles (Trinder, 1969)

Serum lipid profiles mainly triglycerides, total cholesterol were measured by using the commercial diagnostic kits (M/s. Crest Biosystems, Goa).

2.8.3.1 Serum Triglycerides

Lipoprotein lipase hydrolyses triglycerides to glycerol and fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate, which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample. The intensity of colour was read at 505 nm.

The test solution (marked as T) contained 1 ml of working reagent and 0.01 ml of serum sample. The standard solution (marked as S) contained 1 ml working reagent and 0.01 ml of triglyceride standard (conc. 200 ug/dl). The solutions were mixed properly and incubated at 37° C for 5 minutes. The absorbance of standard and test sample was measured against a suitable blank within 60 minutes.

Calculation

Triglycerides in μg/dl = Abs. T/Abs. S x 200

★ Working Reagent

4 parts of Enzyme reagent 1 and 1 part of enzyme reagent 2 was mixed before the estimation.

2.8.3.2 Serum Cholesterol

Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4 – aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample. The intensity of colour was read at 505 nm.

The test solution (marked as T) contained 1 ml of working reagent and 0.01 ml of serum sample. The standard solution (marked as S) contained 1 ml working reagent and 0.01 ml of cholesterol standard (conc.200 ug/dl). The solutions were mixed properly and incubated at 37° C for 5 minutes. The absorbance of standard and test sample were measured against blank within 60 minutes.

Calculation

Cholesterol in ug/dl = Abs. T/Abs. S x 200

★ Working Reagent

4 parts of Enzyme reagent 1 and 1 part of enzyme reagent 2 was mixed before the estimation.

2.8.3.3 Serum HDL Cholesterol

When the serum is reacted with the Polyethylene Glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in

the supernatant and is then assayed as a sample for cholesterol using the Cholesterol (CHOD/PAP) reagent. The absorbance is measured at 505 nm.

For precipitation of VLDL and LDL, 0.1 ml of precipitating reagent (L1) and 0.1 ml of serum was taken in a test tube. The content of the test tube was mixed well and incubated at room temperature for 5 minutes. Then it was centrifuged at 800 *x g* to obtain a clear supernatant.

For the Cholesterol assay, 1 ml of working reagent (section 2.8.3.3) and 0.05 ml of supernatant was taken in a test tube marked 'T'. The test tube marked as 'S' contained 1 ml working reagent and 0.05 ml of HDL standard (25ug /dl). The solutions were mixed properly and incubated at 37° C for 5 minutes. The absorbance of standard and test sample were measured against blank within 60 minutes.

Calculation

HDL Cholesterol in ug/dl = Abs. T/Abs.S x 25 x 2

(where 2 is the dilution factor due to the deproteinization step)

Serum LDL-cholesterol as well as VLDL - cholesterol were calculated using Freidewalds's formula as mentioned below

LDL -Cholesterol = Total Cholesterol - TG/5 – HDL- Cholesterol

VLDL-Cholesterol = TG/5

2.9 ENZYMATIC ANALYSIS

For the enzymatic assay serum and liver was taken. Serum was collected in the similar manner as mentioned before (section 2.4) and it was kept in ice bucket. 1% liver homogenate was prepared by using buffer (Glycine or Phosphate buffer

depending on the type of enzyme). Then it was centrifuged and the supernatant was collected and stored in the ice bucket for the enzymatic assays.

2.9.1 Alkaline Phosphatase [EC 3.1.3.1] (Godkar, 1994)

The reaction mixture contained 0.1M glycine buffer pH 10.5, 2mM magnesium chloride as activator, and 5mM of *p*-nitro phenyl phosphate as substrate in a total volume of 0.5 ml. To this 0.05 ml of serum or 0.25 ml of 1% liver homogenate was added. The reaction was carried out for 30 min. at 37° C and the reaction was stopped by adding 5 ml of 0.25 N NaOH. At the same time substrate blank tube was also maintained. The intensity of the productants of this reaction (*p*-nitro phenyl) was measured at 405 nm. The amount of the *p*-nitro phenyl released by the action of the alkaline phosphate was quantified by prepared *p*-nitro phenyl standard curve. Different concentrations of *p*– nitrophenol solution was taken to a total volume of 0.5 ml glycine buffer. To this 5 ml of 0.25 N NaOH was added and was incubated at room temperature for 10 minutes. The intensity of the colour was measured at 405 nm against a suitable blank. Enzyme activity was expressed in terms of μ mole of product formed per mg of enzyme protein per minute.

★ Standard Solution

5mM *p* - Nitrophenol solution was prepared.

2.9.2 Lactate Dehydrogenase [EC 1.1.1.27] (Godkar, 1994)

1 ml of the buffered substrate was incubated at 37° C for 5 minutes. To this 0.2 ml of NAD solution was added and incubated at 37° C for exactly 15 minutes. Then 1 ml of dinitrophenyl hydrazine (DNPH) reagent and 0.025 ml serum or 1%

liver homogenate was added. The contents of the tube were mixed properly and kept at room temperature for 15 minutes. Finally 10 ml of 0.4 N sodium hydroxide was added and mixed thoroughly. After 10 minutes the optical density was measured against blank at 440 nm.

For the preparation of the standard graph, different concentrations of pyruvate and NADH (both in equal volumes) were taken in the test tubes and the volume was adjusted to 1 ml with buffered substrate. Then 0.2 ml of nicotinamide adenine dinucleotide (NAD), 0.1 ml of distilled water and 1 ml of DNPH were added to all the tubes.. The contents of the tubes were mixed thoroughly and kept at room temperature for 15 minutes. Finally 10 ml of 0.4 N sodium hydroxide was added and kept at room temperature for exactly 10 minutes. The optical density was measured against blank at 440 nm.

Enzyme activity was expressed in terms of μ mole of product formed per mg of enzyme protein per minute.

★ Buffered substrate

125 ml of 0.1 M glycine buffer was mixed with 75 ml of 0.1 N sodium hydroxide. 4 gm of lithium lactate was added to this, mixed and adjusted to pH 10.

★ DNPH Reagent

It contained 200mg of dinitrophenyl hydrazine and 85 ml of conc. Hydrochloric acid in distilled water, final volume should be adjusted to one litre by using distilled water.

★ Standard Solution

0.22 mg/ml of sodium pyruvate was used as standard solution..

2.9.3 Glutamate Oxaloacetate Transaminase [EC2.6.1.1] (Godkar, 1994)

The reaction mixture contained 0.25 ml of buffered substrate specific for glutamate oxaloacetate transaminase (GOT) and to this 0.1 ml of serum or 0.5 ml of 1% liver homogenate was added. The reaction was carried out for 30 min. at 37°C. Further 0.5 ml di nitro phenyl hydrazine reagent was added and this mixture was incubated for 20 min at room temperature. To this 5 ml of 0.4N sodium hydroxide was added and incubated for 10 minutes at room temperature. The intensity was measured at 540 nm.

For the preparation of standard graph, different concentrations of pyruvate were taken in test tubes and the volume was adjusted to o.5 ml by addition of GOT substrate. Then 0.1 ml of distilled water and 0.5 ml of DNPH were added to all the tubes and kept at room temperature for 20 minutes. The reaction was terminated by addition of 5 ml of 0.4 N sodium hydroxide solutions. The tubes were incubated at room temperature for 10 minutes and the intensities were read at 540 nm.

Enzyme activity was expressed in terms of μ mole of product formed per mg of enzyme protein per minute.

★ Substrate For GOT.

2.66 gms of aspartic acid was taken and to this 30 mg of α Ketoglutaric acid was added and this was dissolved in 20ml of 1 N sodium hydroxide and the final volume was made to 100 ml by adding 0.05M phosphate buffer (pH 7.45).

★ Standard Solution

0.22mg/ml of sodium pyruvate.

2.9.4 Glutamate Pyruvate Transaminase [EC 2.6.1.2] (Godkar, 1994)

The reaction mixture contained 0.25 ml of buffered substrate specific for glutamate pyruvate transaminase (GPT) and to this 0.1 ml of serum or 0.5 ml of 1% liver homogenate was added. The reaction was carried out for 30 min. at 37°C. Further 0.5 ml di nitro phenyl hydrazine reagent was added and this mixture was incubated for 20 min at room temperature. To this 5 ml of 0.4N sodium hydroxide was added and incubated for 10 minutes at room temperature. The intensity was measured at 540 nm.

Enzyme activity was expressed in terms of μ mole of product formed per mg of enzyme protein per minute.

★ Substrate For GPT

1.78 gms of alanine was taken and to this 30mg of α Ketoglutaric acid and 0.5 ml of 1 N NaOH was added and the volume was made to 100ml by adding phosphate buffer.

2.9.5 3-Hydroxy-3-Methylglutaryl-Coa Reductase [EC1.1.1.34] (Siedel, 1983)

The enzyme activity was measured with UV – visible spectrophotometer (model TCC-240A) capable of exact measurement at 339 nm with a thermostat cuvette holder. The reaction was developed in a cuvette. The reaction mixture contained 0.9 ml reagent mixture, 0.02ml serum sample or 0.05 ml 1% liver homogenate in a final volume of 1ml adjusted with distilled water. The reaction mixture was stirred properly and incubated for 5 mins at 37^0 in a thermostat cuvette holder to establish a constant base line rate at 339nm (Δ A1). Then 0.02 ml of HMG-CoA was added. The decrease in absorbance was monitored over a period of 5 mins after addition of HMG-CoA (Δ A2).

The catalytic concentration of the enzyme in the sample (µ mole of HMG-CoA reduced per minute and litre) is calculated from the decrease in absorbance according to calculation formula

Enzyme catalytic unit $U/l = 79.4 / v \times \Delta A/\Delta t$

$\Delta A = \Delta A1 - \Delta A2$ where, v is sample volume in ml and t is the time of reaction in min.

The final result was expressed in U/l/mg of protein (U - Units)

★ Reagent Mixture

1 ml water was mixed with 14ml Phosphate buffer (0.1 mol/l; pH 6.5), 0.4ml EDTA solution (0.1 mol/l), 0.4 ml Cysteamine solution (0.1mol/l) 1.2 ml 1%serum albumin and 1 ml NADPH solution (5mmol/l).

2.9.7 3-Hydroxy-3-Methylglutaryl-Coa Synthase [E.C 2.3.3.10] (Miziorko, 1985)

The reaction was developed in a cuvette and the enzyme activity was measured with UV – visible spectrophotometer capable of exact measurement at 300 nm with a thermostat cuvette holder. The standard assay mixture contained 0.5 ml of 0.2 *M* Tris –HCL (pH 8.2) containing 0.2 m*M* EDTA, 0.02 ml of 10 m*M* acetyl CoA (pH 4.5), 0.05 ml of 1 m*M* acetoacetyl CoA (pH 4.5),, and 0.03 ml serum or 0.08 ml 1% liver homogenate. The final volume is adjusted to 1 ml with distilled water. The enzyme is incubated for 5 mins at 37^0C in cuvette containing all components of assay mixture except for acetyl CoA. Thus the base line absorbance at 300 nm is established prior to starting the reaction by addition of acetyl CoA. The decrease in absorbance was monitored over a period of 5 mins after addition of acetyl CoA. The final volume of the sample was 1 ml. The catalytic activity of the enzyme was calculated by the following formula

$$= \Delta A1 - \Delta A2 / \Delta t \times \text{protein}$$

2.10 STATISTICAL CALCULATION (Bailey-1994)

All the recorded observation was expressed in the form of arithmetic mean of six samples and the standard error by using the following formulae

Standard error = Standard deviation / $\sqrt{n}$

Standard deviation = $\sqrt{\sum(X - X_i)^2 / (n-1)}$

where X = Arithmetic mean

X_i = Individual observation

n = No. of observation

2.10.1. Student "t" Test

Comparison test of the obtained data for each sample groups (control, treated and between the different treatment days) were calculated by using common student 't' test. The formula for 't'

$$t = X1 - X2 / \sqrt{(SE1)^2 + (SE2)^2}$$

where, X1, X2 = mean values of the data

SE1, SE2 = standard error of respective mean values

Degree of freedom = n1 + n2-2

The calculated 't' value was tallied from the tabulated 't' values at the probability level 0.05 (at respective degree of freedom, in our case degree of freedom was 10). If the calculated value was higher then the tabulated value at the probability level 0.05 then the difference was accepted as significant difference. However, if the calculated value lies between the value of the probability level 0.1 and 0.05 then the difference were accepted as equivocal (means the difference may or may not be statistically significant). The lesser value of calculated 't' then the tabulated value at probability level 0.1, were considered as non-significant difference.

2.10.1 ANOVA (Analysis of Varience) Test

The comparison of the different parameter between control and all the treated groups were also statistically verified by calculating 'F' value of Analysis of Variance. This was calculated by calculating the variation between the columns and their sum of square on one hand and on another hand by calculating variation within the column and their sum of squares and finally 'F' value was calculated by calculating the values of both the variance that means (the ratio of the variance

between the column and the variance within the column) at their respective degree of freedom. Then calculated 'F' value was tallied from the statistical table to find whether the variation was significant or non-significant (probability table). The data of the various columns and the data within the group was computed in a PC and by using Jandel Sigmastat statistical software and the value of 'F' as well as level of significance were recorded.

Results

ABBREVIATIONS

FCR	Feed conversion ratio
Gw	Daily instantaneous growth rate
HMG CoA	Hydroxy methyl glutaryl Coenzyme A
RBC	Red blood cell
WBC	White blood cell
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein
CH	Cholesterol
TG	Triglycerol
ALP	Alkaline phosphatase
GPT	Glutamate pyruvate transaminase
GOT	Glutamate oxaloacetate transaminase
LDH	Lactate dehydrogenase
μ mole	Micromole
ηmole	Nanomole
mg	Milligrams
dl	Deciliter
mm	Millimeter
ANVOA	Analysis of variance
I	Initial
C	Control
D1	2.5 ml oil/100 gm of feed
D2	5 ml oil/100 gm of feed
D3	10 ml oil/100 gm of feed
B1 strain of bacteria	Staphylococcus *sp.*
B2 strain of bacteria	Streptococcus *sp.*
B3 strain of bacteria	Pseudomonas *sp.*

3.1 Changes during the post hatching developmental period:

3.1.1 Growth

The growth curve of the poultry bird *Gallus domesticus* (in terms of net weight gain), during post hatching development (from 1 day to 35 days) showed almost an exponential nature (figure I). The linear increase in the growth of the bird was observed till 28 days of post hatching development, which was accompanied by a steady decrease in feed conversion ratio (FCR) and a static level of daily instantaneous growth rate (G_w). It was interesting to note a sharp increase in FCR (from 0.075 to 0.341) and a sharp decrease in daily growth rate (G_w) from 0.10 to 0.034 during the post hatching developmental period from 28 days to 35 days (Table 1).

3.1.2 Hematological parameters

From Table 2 and figure II, it is evident that there were significant variations in hemoglobin concentration ($F = 11.52$, $p < 0.001$) and the total count of erythrocytes ($F = 26.57$, $p < 0.001$) and leucocytes ($F = 70.31$, $p < 0.001$) during post hatching development. Up to 7 days of post hatching development the hemoglobin concentration remained unaltered. However, about 25% decrease in the concentration of hemoglobin was noticed on 21 days of development, compared to the concentration of hemoglobin of 1 day or 7 days old chicks (Table 2a), after which it remained static till the 35 days of development. The total erythrocyte count showed a gradual increase up to 21 days of development and after which it became more or less stable (Table 2 and 2a). A decreasing trend, in the counts of total leucocytes was observed during the post hatching

developmental period of *Gallus domesticus* till 28 days of development and after that it became more or less constant (Table 2a and figure II).

3.1.3 Tissue biochemical composition

Tables 3-6 and figures III-VI represent the variation of tissue level total protein and tissue level lipid profiles mainly total triglycerol, cholesterol and phospholipid in different tissues like liver, pectoral muscle, large intestine and the total blood of *Gallus domesticus* during post hatching development (1 day to 35 days). Table 7 represents the fatty acid profile of different tissues (liver, pectoral muscle, large intestine and total blood) of *Gallus domesticus* during post hatching development (1 day to 35 days).

3.1.3.1 Protein

The dynamics of the tissue level total protein concentration is represented in Table 3 and figure III. Table 3a represents the statistical comparison of data pertaining to Table 3. The variation in tissue level total protein concentration during the post hatching development (from 1 day to 35 days) was significant at 0.1% level in all the tissues, except in the liver where the variations may or may not be statistically significant ($F = 2.33$). In liver, there was a gradual decrease in the tissue protein concentration up to 14 days (about 15% decrease, calculated 't' value –> 2.33, $p < 0.05$). After that an increase in protein value was noticed on 21 days (17% increase, $p < 0.05$) and which remained almost static till 35 days of post hatching development (Table 3a). In muscle, the gradual accumulation of protein was observed till 35 days of development. The

observed decrease (about 11%) on the 7th day of development was not statistically significant. In intestine, the gradual increase of tissue protein was observed till 28 days of development. After that about 9 % decrease of tissue protein was observed on 35 days (calculated 't' value –> 2.54, $p < 0.025$). The blood protein level remained almost static up to 14 days of post hatching development. A sharp decrease (about 35%) was observed on 21 days ($p < 0.005$) and after that it again increased (22% increase, $p < 0.05$) as observed on 35th day over the value of 21 days.

3.1.3.2 Total Triglycerol

The data in Table 4 and figure IV demonstrates the changes in tissue level total triglycerol concentration and that in Table 4a shows its relevant statistical calculation. The variations in the total triglycerol concentration during post hatching development are significant at 0.1% level in all the tissues (F = 14.43 – 68.75). A decreasing trend was noticed in the total triglycerol concentration of liver and muscle up to 14 days of post hatching development and then the level was increased. About 30 – 40 % decrease in the triglycerol level of liver / muscle was noticed in 14 days old chicks as compared to same of 1 day old chicks. In these tissues, triglycerol level was increased by 2-3 fold ($p < 0.005$) on 35 days of post hatching development compared to the triglycerol level of 14 day old chicks. In the intestine, till 14 days of post hatching development the triglycerol level remained more or less static and after that the level was enhanced by about 75% ($p < 0.001$) on the 21st day of development. However, during the 21st to the 35th day of post hatching development, again the triglycerol level remained static. In blood, a sharp increase (3 fold, $p<0.005$) in the triglycerol level in the first week of post hatching

development was noticed. After that, till 21 days of development, the blood triglycerol level remained more or less constant and then 3-4 fold augmentation in the triglycerol concentration was noticed (figure IV).

3.1.3.3 Total Cholesterol

Table 5 and figure V illustrate the variations in total cholesterol concentration of various tissues of chicks during post hatching development. The relevant statistical calculation is represented in Table 5a. The ANOVA for the total cholesterol concentration during post hatching development are significant at 0.1% level in all the tissues (Table 5). A sharp and gradual decrease (from 20% to 60%, $p < 0.005$) in the total cholesterol level of liver was noticed during the post hatching development of the chicks from the 1st to the 28th day and after that on the 35th day of post hatching development this level was increased by 20% (calculated 't' value –> 3.45, $p < 0.005$). Relatively, static and partial decreased trend was noticed in the total cholesterol concentration of the *Gallus'* muscles during the post hatching developmental period, except for about 58% increase on 7th day ($p < 0.01$). In intestine, there was a gradual increase (3 fold, $p < 0.005$) in the cholesterol concentration up to 14 days of post hatching development which was followed by gradual decrease (50%, calculated 't' value –>3.15, $p < 0.005$) till 28 days of post hatching development; and again the cholesterol level was raised by about 2 fold ($p < 0.005$) on the 35th day of post hatching development (figure V). After a sharp increase (2.5 fold, p < 0.0.01) in the total cholesterol concentration of blood on the 7th day of development, the blood cholesterol level remained almost static

till 21 days of post hatching development (Table 5, 5a) and then the level was again raised by about 80% to 2.53 fold ($p < 0.005$).

3.1.3.4 Total Phospholipid

Table 6 and figure VI highlights the changes in the total phospholipid concentration during post hatching development. The statistical analysis of the relevant data is given in Table 6a. The variations in the total phospholipid concentration during post hatching development are significant at 0.1% level in all the tissues ($F = 4.58 - 9.49$, $p < 0.001$). In liver, the total phospholipid concentration showed an increase of about 20% on 7^{th} day of post hatching development which was followed by a sharp and gradual decrease and the decreasing trend continued till 28 days of post hatching development (Table 6). About 3 fold ($p < 0.005$) increase in the total phospholipid concentration in the liver of *Gallus* was noticed from 28 days to 35 days of development. In muscle, after an initial decrease (60%, $p < 0.005$) on 7^{th} day of post hatching development the level of the total phospholipid was gradually elevated till the 28 days of development which was followed by a sharp decrease of about 20% (calculated 't' value –> 2.38 , $p < 0.025$) on the 35^{th} day of development. In intestine, although about 20% ($p < 0.005$) decrease in the total phospholipid concentration was recorded on the 7^{th} day of post hatching development, the level of the total phospholipid remained more or less static till 28 days of development followed by 2.5 fold ($p < 0.005$) increase on 35^{th} day of development (Figure VI). In the blood, the total phospholipid concentration increased gradually till 14 days of development followed by a sharp decrease (about 80%, $p < 0.005$) on 21^{st} day of development. From 21^{st} day onwards again an increased trend in total phospholipid was detected (Table 6).

3.1.3.5 Fatty acid profiles

Table 7 highlights the changes in the fatty acid profiles of total lipid in various tissues viz., liver, muscle, intestine and blood during post hatching developmental period from the 1st day to the 35th day. The major fatty acids as observed in various tissues during post hatching development of the bird are palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6).

In liver, 50% - 60% increase in linoleic acid, 60% → 2.3 fold increase in arachidonic acid and 3.5 fold – 4 fold increase in palmitoleic acid were noticed from 21 days onwards, over the same of the one day old bird, along with the increase in stearic acid (17%), and eicosapentaenoic acid (5.5 fold – 6 fold) at the cost of 45 – 50% decrease in oleic acid from 21 days onwards.

In muscle, about 45% increase in stearic acid concentration from the 7th day along with 45- 50% increase in palmitoleic acid, 2 fold increase in linoleic acid, 70 – 80% increase in arachidonic acid, 4 fold increase in eicosapentaenoic acid and 60 – 70% increase in docosahexaenoic acid from 21st day or 28th day onwards was recorded, which was accompanied by a significant decrease in level of linoleic acid (25 %) from the 7th day and oleic acid (11% – 30%) from the 21st day onwards.

In intestine, 25% – 30 % increase in linoleic acid and 50% increase in oleic acid from the 7th day and 14th day onwards respectively was noticed along with 90% → 2.3

fold increase in palmitoleic acid and about 30% increase in arachidonic acid, at the cost of 50% decrease in stearic acid from the 14th day onwards and 60 % decrease in eicosapentaenoic acid from the 21st day onwards.

About 30% increase in linoleic acid and 46% → 2.1 fold increase in arachidonic acid concentration was noticed in the blood from the 21st day onwards. This increase was accompanied with 56% decrease in oleic acid from the 21st days onwards and 45% decrease in docosahexaenoic acid from the 7th day onwards.

3.1.4 Cholesterol metabolism

Table 8 and figure VII demonstrate the activity of 3- HMG CoA Reductase (EC1.1.1.34) and 3- HMG CoA Synthase (EC 2.3.3.10) during post hatching development. These are the two key enzymes that regulate cholesterol metabolism. The cholesterol metabolism is greatly influenced by endogenous and exogenous supply of cholesterol to the body.

The activity of 3- HMG CoA Reductase in liver and serum was decreased by 50 – 55% (p <0.005) on the 7th day of post hatching development, although the 3- HMG CoA Reductase activity in liver again showed increasing trend after the 7th day of development. The activity of the same enzyme in serum remained more or less static from 7 – 35 days of post hatching development (Table 8). It was interesting to note a sharp increase 2.2fold, p <0.005) in the activity of the said enzyme in liver from 14 – 21 days of post hatching development

The activity of 3- HMG CoA Synthase in liver decreased (34.09%, $p < 0.005$) in the first week of development and remained almost static up to 3rd week of post hatching development and then it was further decreased by about 40% ($p < 0.005$) on the 35th day of post hatching development. In serum, the activity of aforesaid enzyme was decreased by 50% ($p<0.005$) in the first week and remained constant for another week. The level of activity in serum was again increased (2.49 fold, $p < 0.005$) in the third week of development and remained more or less static till the 35th day of post hatching development (Table 8,8A).

Figure I : Growth chart of the broiler chick (*Gallus domesticus*) during post hatching development.

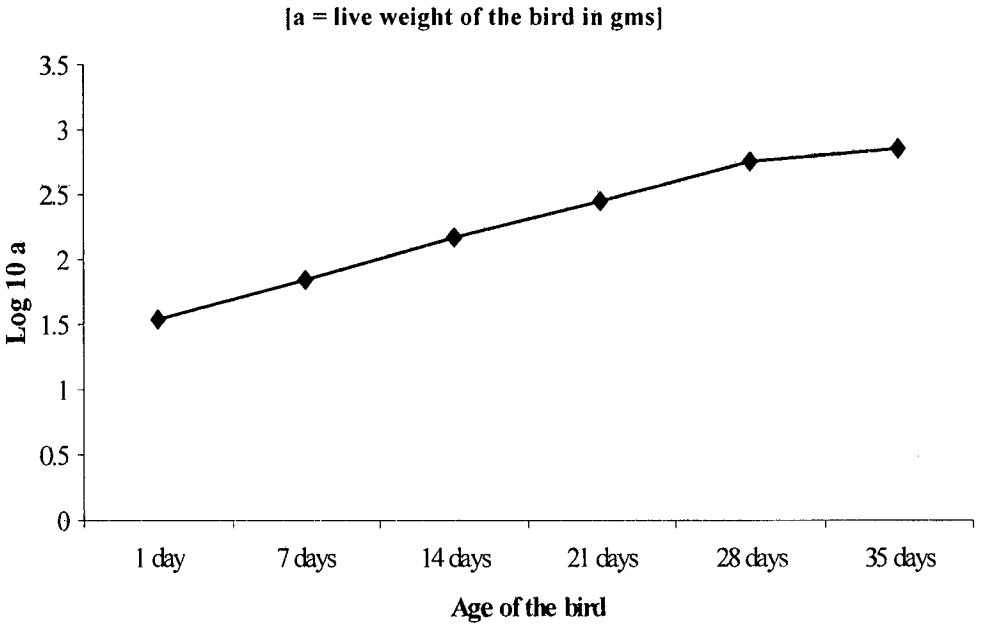


Figure II: Changes in the hematological parameters of the broiler chick (*Gallus domesticus*) during post hatching development

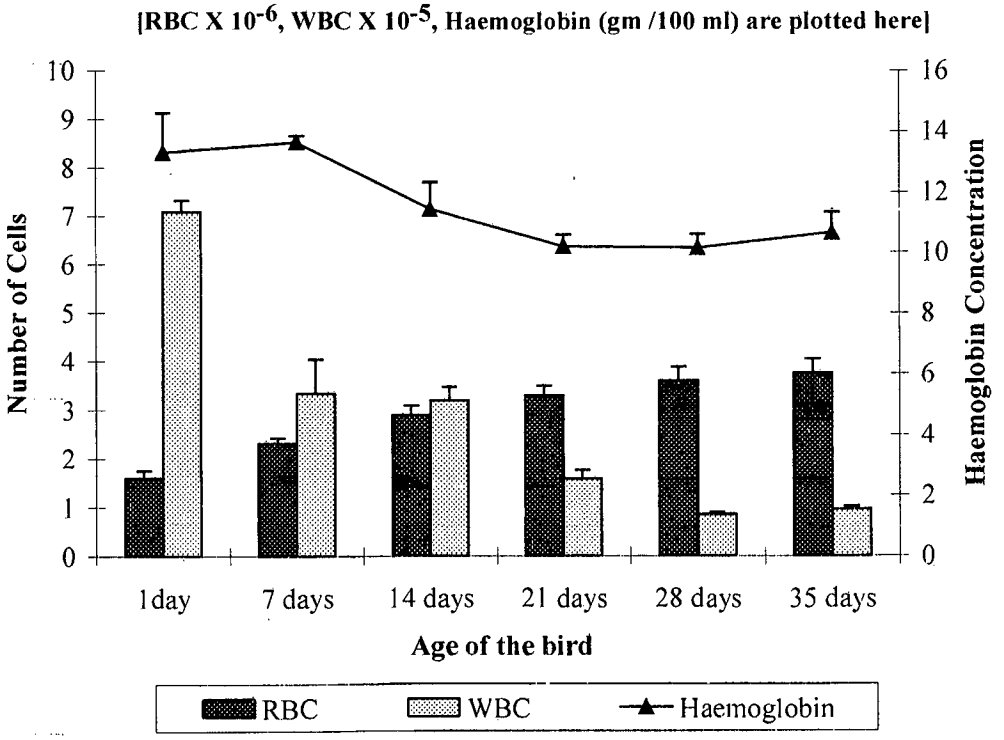


Figure III: Changes in the tissue total protein (mg/100mg) concentration of the broiler chick (*Gallus domesticus*) during post hatching development.

[Protein concentration is expressed as mg / 100 mg of tissue for liver, muscle and intestine and as mg / 100 ml for blood]

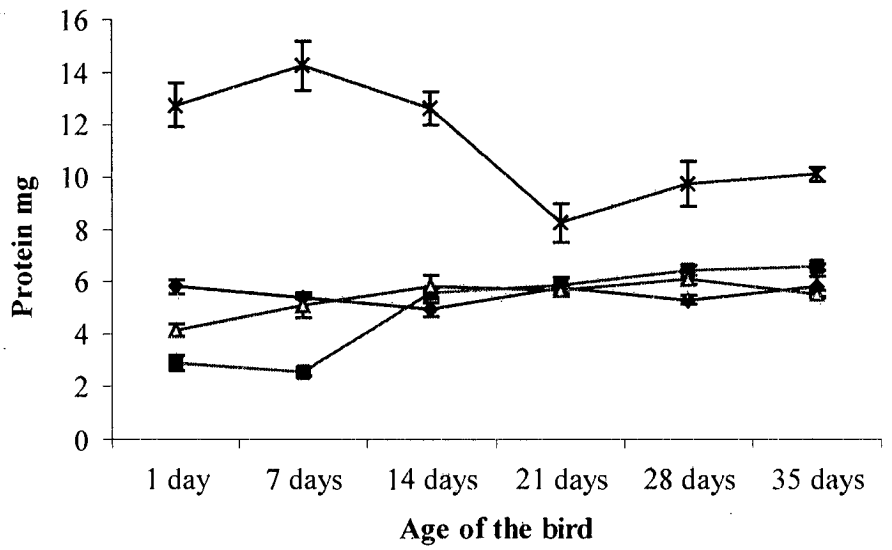


Figure IV: Changes in the tissue total triglycerol concentration of the broiler chick (*Gallus domesticus)* during post hatching development

[Triglycerol concentration is expressed as µm ole/ 100 mg of tissue for liver, muscle and intestine and as µm / 100 ml for blood]

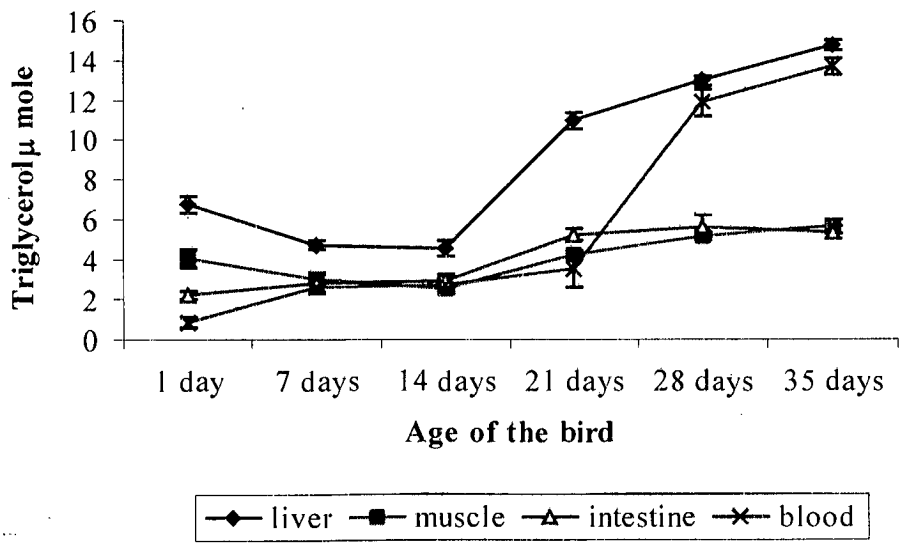


Figure V: Changes in the tissue total cholesterol concentration of the broiler chick (*Gallus domesticus)* during post hatching development

[Cholesterol concentration is expressed as μm / 100 mg of tissue for liver, muscle

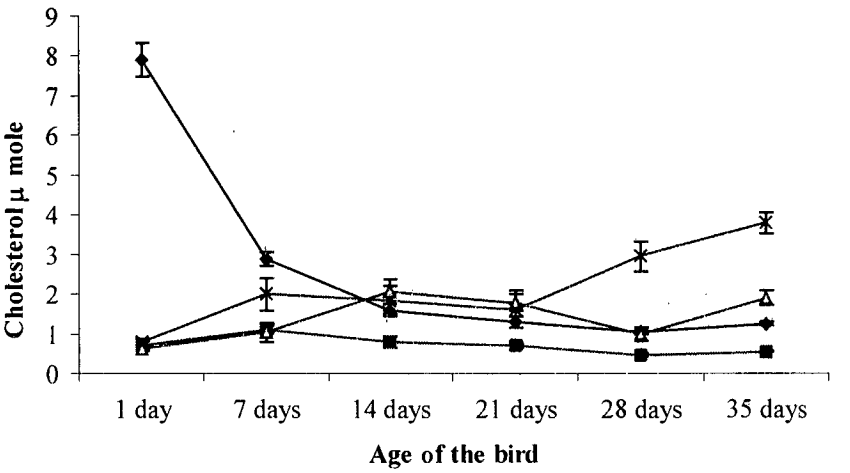


Figure VI: Changes in the tissue total Phospholipid concentration of the broiler chick (*Gallus domesticus)* during post hatching development

[Phospholipid concentration is expressed as ηmole / 100 mg of tissue for liver, muscle and intestine and as ηm/ 100 ml for blood]

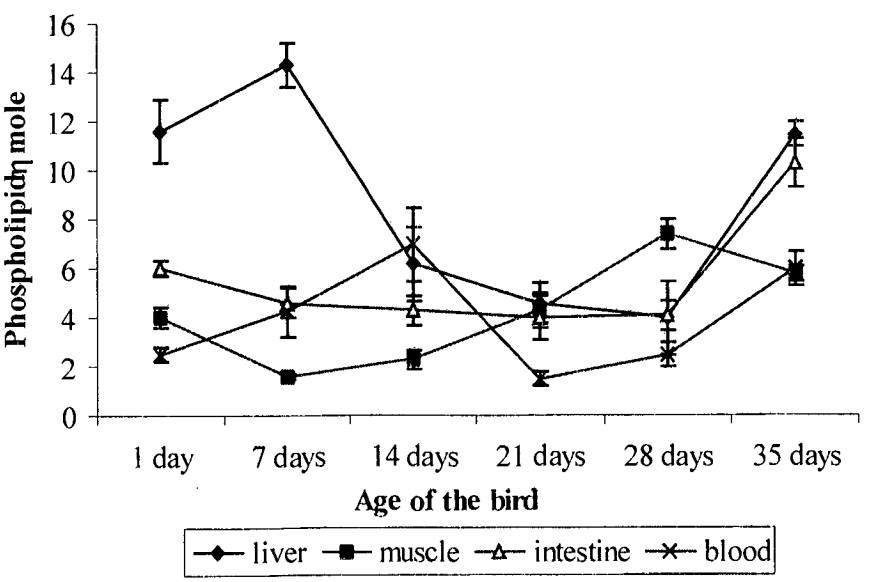
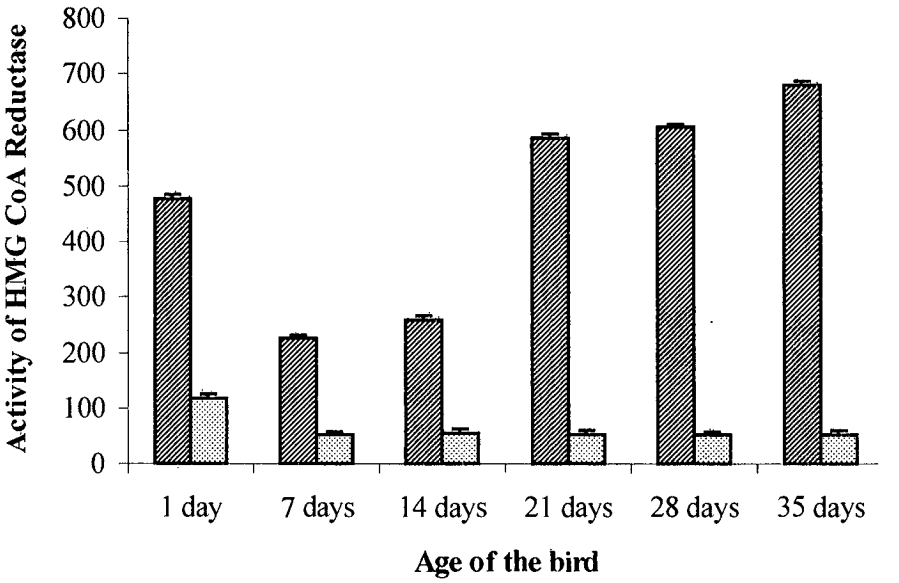


Figure VII: Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler chick, (*Gallus domesticus*) during post hatching development.

a: 3-HMG-CoA- Reductase



b: 3-HMG-CoA- Synthase

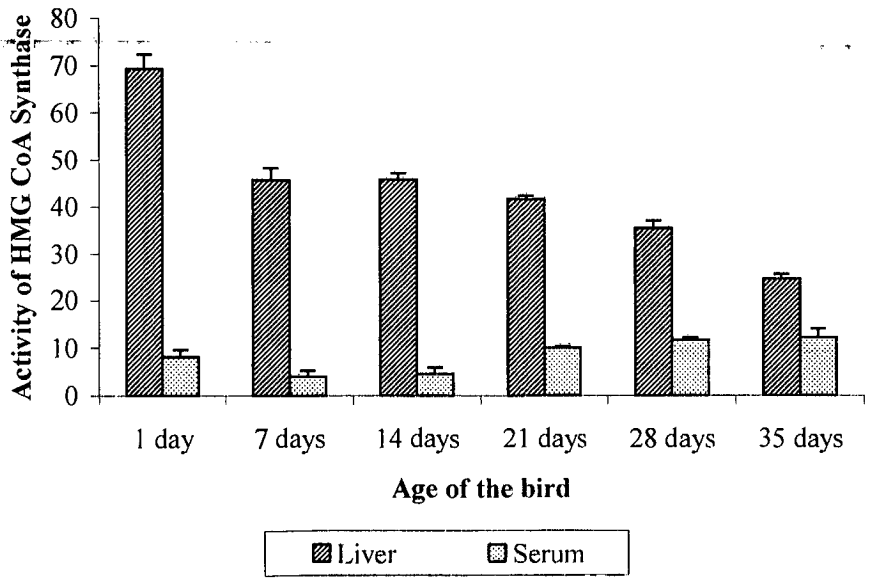


Table 1: Growth chart of broiler chick (*Gallus domesticus*) during post hatching development.

(Average weight of six birds and their standard error)

Age of the birds	Average weight of the birds and their standard error (gm)	Daily instantaneous growth rate G_w*	Feed conversion ratio (FCR) *
1day	34.33 ± 0.92	---	---
7 days	70.50 ± 2.81	0.103	0.428
14 days	148.67 ± 5.08	0.106	0.273
21 days	284.67 ± 15.88	0.093	0.193
28 days	573.17 ± 20.40	0.100	0.075
35 days	725.50 ± 27.12	0.034	0.341

* **Between two consecutive weeks. The value was calculated from the mean values of weight of birds in each week**

Table 2: Changes in the hematological parameters of broiler chick (*Gallus domesticus*)during post hatching development

(Mean values of six birds and their standard error)

Age of the bird after hatching	Hemoglobin g /dl	Total erythrocytes $x\ 10^6$ /cubic mm	Total Leucocytes x 10^5/cubic mm
1day	13.29 ± 1.30	1.60 ± 0.15	7.08 ± 0.24
7 days	13.63 ± 0.20	2.32 ±0.108	3.34 ±0.69
14 days	11.44 ± 0.87	2.9 ± 0.20	3.20 ±0.28
21 days	10.19 ± 0.39	3.30 ± 0. 20	1.59 ± 0.18
28 days	10.15 ± 0.45	3.60 ± 0.28	0.86 ± 0.04
35 days	10.65 ± 0.68	3.75± 0.30	0.96 ± 0.07
Analysis of variance *'F'* Value	11.525	26.57	70.313
Level of significance *'p'*	< 0.001	< 0.001	< 0.001

Table 2a: Statistical calculation (comparison test by using student 't' test) of Table 2.

Comparing group	Parameters	% change	't' value	*p* value
1 day v/s 7 days	**Hemoglobin gm /dl**	2.56 ↑	0.26	NS
7 days v/s 14 days		16.06↓	2.45	<0.025
7days v/s 21 days		25.24↓	7.82	<0.005
14days v/s 21 days		10.92↓	1.31	NS
21days v/s 35days		4.51 ↑	0.59	NS
1 day v/s 7 days	**Total erythrocytes $x\ 10^6$ /cubic mm**	45.00 ↑	4.626	<0.001
7 days v/s 14 days		2.17 fold↑	11.51	<0.001
7days v/s 21 days		42.24↑	9.09	<0.001
14days v/s 21 days		5.21↑	0.80	NS
21days v/s 35days		13.64 ↑	1.24	NS
1 day v/s 7 days	**Total Leucocytes x 10^5/cubic mm**	52.82 ↓	5.12	<0.005
7 days v/s 14 days		4.19↓	0.19	NS
7days v/s 21 days		52.39↓	2.45	<0.025
14days v/s 21 days		50.31	4.84	<0.001
21days v/s 35days		39.62 ↓	3.26	<0.005

Table 3: Changes in the tissue total protein concentration of broiler chick (*Gallus domesticus*) during post hatching development.

(Mean values of six birds and their standard error)

Age of the bird after hatching	Liver	Muscle	Intestine	Blood
	mg/100 mg			mg/100ml
1 day	5.80 ± 0.26	2.88 ± 0.27	4.13 ± 0.25	12.75 ± 0.85
7 days	5.38 ± 0.05	2.56 ± 0.17	5.07 ± 0.48	14.25 ± 0.92
14 days	4.94 ± 0.26	5.56 ± 0.20	5.81 ± 0.46	12.62 ± 0.62
21 days	5.78 ± 0.37	5.85 ± 0.31	5.66 ± 0.20	8.25 ± 0.75
28 days	5.30 ± 0.16	6.43 ± 0.19	6.08 ± 0.18	9.75 ± 0.85
35 days	5.83 ± 0.38	6.56 ± 0.12	5.52 ± 0.13	10.12 ± 0.25
Analysis of variance *'F'* value	2.33	65.91	6.01	6.39
Level of significance *'p'*	Equivocal	< 0.001	< 0.001	< 0.001

Table 3a: Statistical calculation (comparison test by using student 't' test) of Table 3.

Comparing group	Parameters	% change	't' value	*p* value
1 day v/s 7 days	Liver	7.24 ↓	1.62	Equivocal
7 days v/s 14 days		8.17↓	1.66	Equivocal
7 days v/s 21 days		7.43 ↑	1.08	NS
14 days v/s 21days		17.00↑	1.87	< 0.05
21days v/s 35days		0.87 ↑	0.09	NS
1 day v/s 7 days	Muscle	11.11 ↓	1.00	NS
7 days v/s 14 days		2.17 fold↑	11.51	< 0.005
7 days v/s 21 days		128.52 ↑	9.40	< 0.005
14 days v/s 21days		2.58↓	0.29	NS
21days v/s 35days		12.14 ↑	2.15	<0.05
1 day v/s 7 days	Intestine	22.76 ↑	1.74	Equivocal
7 days v/s 14 days		14.59↑	1.72	Equivocal
7 days v/s 21 days		11.64 ↑	1.13	NS
14days v/s 21days		2.58 ↑	0.29	NS
21 days v/s 35days		2.47↓	0.58	NS
1 day v/s 7 days	Blood	11.76 ↑	1.20	NS
7 days v/s 14 days		11.43 ↓	1.46	Equivocal
7 days v/s 21days		42.10 ↓	5.04	< 0.005
14days v/s 21 days		34.63↑	4.49	<0.005
21days v/s 35days		22.67 ↑	2.37	< 0.05

Table 4: Changes in the total triglycerol concentration of broiler chick (*Gallus domesticus*) during post hatching development.

(Mean values of 6 samples and their standard error)

Age of the bird after hatching	Liver	Muscle	Intestine	Blood
	μ mole /100 mg			μ mole /100ml
1 day	6.74 ± 0.43	4.06 ± 0.46	2.23 ± 0.19	0.85 ± 0.25
7 days	4.70 ± 0.24	2.98 ± 0.25	2.78 ± 0.52	2.57 ± 0.22
14 days	4.56 ± 0.40	2.51 ± 0.12	2.95 ± 0.27	2.75 ± 0.45
21 days	10.96 ± 0.44	4.20 ± 0.21	5.22 ± 0.28	3.52 ± 0.89
28 days	12.98 ± 0.25	5.14 ± 0.21	5.61 ± 0.55	11.89 ± 0.69
35 days	14.79 ± 0.26	5.64 ± 0.35	5.34 ± 0.32	13.70 ± 0.42
Analysis of variance 'F' value	68.75	14.43	14.75	15.40
Level of significance '*p*'	< 0.001	< 0.001	< 0.001	< 0.001

Table 4a : Statistical calculation (comparison test by using student 't' test) of Table 4.

Comparing group	Parameters	% change	't' value	*p* value
1 day v/s 7 days	Liver	30.27 ↓	4.16	< 0.005
7 days v/s 14 days		2.98↓	0.30	NS
7 days v/s 21 days		2.75 fold↑	21.31	<0.005
14 days v/s 21days		2.4 fold ↑	10.77	<0.005
21days v/s 35days		14.12 ↑	1.52	Equivocal
1 day v/s 7 days	Muscle	26.60 ↓	2.08	< 0.05
7 days v/s 14 days		15.77↓	1.69	Equivocal
7 days v/s 21 days		40.93 ↑	6.19	< 0.005
14 days v/s 21days		67.33 ↑	7.01	<0.005
21days v/s 35days		34.29 ↑	3.60	<0.005
1 day v/s 7 days	Intestine	24.66↑	1.00	NS
7 days v/s 14 days		6.11↑	0.29	NS
7 days v/s 21 days		87.77 ↑	4.14	< 0.005
14 days v/s 21days		76.95 ↑	5.85	<0.005
21days v/s 35days		2.29 ↑	0.28	NS
1 day v/s 7 days	Blood	2.02 fold ↑	5.21	< 0.005
7 days v/s 14 days		7.00↑	0.36	NS
7 days v/s 21 days		36.96 ↓	1.03	< 0.005
14 days v/s 21days		28.00 ↑	0.77	NS
21days v/s 35days		2.89 fold ↑	6.06	<0.005

Table 5: Changes in the total cholesterol concentration of broiler chick (*Gallus domesticus*)during post hatching development.

(Mean values of 6 samples and their standard error)

Age of the bird after hatching	Liver	Muscle	Intestine	Blood
	μ mole /100 mg			μ mole /100ml
1 day	7.89 ± 0.42	0.70 ± 0.11	0.63 ± 0.07	0.79 ± 0.80
7 days	2.88 ± 0.16	1.11 ± 0.09	1.03 ± 0.23	2.00 ± 0.41
14 days	1.59 ± 0.15	0.79 ± 0.15	2.06 ± 0.30	1.84 ± 0.35
21 days	1.29 ± 0.14	0.71 ± 0.06	1.79 ± 0.22	1.62 ± 0.46
28 days	1.03 ± 0.04	0.46 ± 0.04	0.99 ± 0.17	0.95 ± 0.39
35 days	1.25 ± 0.05	0.55 ± 0.02	1.90 ± 0.19	3.80 ± 0.27
Analysis of variance 'F' value	175.59	6.08	7.73	7.00
Level of significance '*p*'	< 0.001	< 0.001	< 0.001	< 0.001

Table 5a: Statistical calculation (comparison test by using student 't' test) of Table 5

Comparing group	Tissues	% change	't' value	*p* value
1 day v/s 7 days	Liver	63.50 ↓	11.13	
7 days v/s 14 days		44.79↓	5.89	< 0.005
7 days v/s 21 days		55.20 ↓	7.57	< 0.005
14 days v/s 21days		18.86↓	1.46	< 0.005
21days v/s 35days		3.10 ↓	0.27	Equivocal
				NS
1 day v/s 7 days	Muscle	58.57 ↑	2.88	
7 days v/s 14 days		45.56↑	1.74	< 0.01
7 days v/s 21 days		55.20↓	4.00	Equivocal
14 days v/s 21days		86.95↑	2.48	< 0.005
21days v/s 35days		22.54 ↓	2.67	<0.025
				< 0.025
1 day v/s 7 days	Intestine	63.49 ↑	1.66	
7 days v/s 14 days		6.52↓	0.35	Equivocal
7 days v/s 21 days		73.79 ↑	2.38	NS
14 days v/s 21days		6.97↓	0.27	< 0.025
21days v/s 35days		6.15 ↑	0.38	NS
				NS
1 day v/s 7 days	Blood	2.53 fold ↑	1.34	NS
7 days v/s 14 days		64.70↑	1.51	Equivocal
7 days v/s 21 days		19.00 ↓	0.61	NS
14 days v/s 21days		78.57↓	3.60	<0.005
21days v/s 35days		90.00 ↑	3.6	< 0.001

Table 6: Changes in the total Phospholipid concentration of broiler chick (*Gallus domesticus*)during post hatching development

(Mean values of 6 samples and their standard error)

Age of the bird after hatching	Liver	Muscle	Intestine	Blood
	ηmole/100 mg			η mole /100ml
1 day	11.6 ± 1.30	4.00 ± 0.40	6.00 ± 0.30	2.50 ± 0.29
7 days	14.30 ± 0.90	1.58 ± 0.10	4.60 ± 0.60	4.25 ± 1.03
14 days	6.20 ± 1.50	2.30 ± 0.40	4.30 ± 0.60	7.00 ± 1.50
21 days	4.60 ± 0.80	4.30 ± 0.70	4.00 ± 0.90	1.50 ± 0.29
28 days	4.00 ± 1.50	7.40 ± 0.60	4.10 ± 0.60	2.50 ± 0.50
35 days	11.50 ± 0.50	5.80 ± 0.30	10.30 ± 1.00	6.00 ± 0.70
Analysis of variance '*F*' value	9.49	4.58	7.26	7.59
Level of significance '*p*'	< 0.001	< 0.001	< 0.001	<0.001

Table 6a: Statistical calculation (comparison test by using student 't' test) of Table 6.

Comparing group	Parameters	% change	't' value	*p* value
1 day v/s 7 days	Liver	23.28 ↑	1.70	Equivocal
7 days v/s 14 days		56.64↓	4.63	< 0.005
7 days v/s 21 days		67.83 ↓	8.08	< 0.005
14 days v/s 21days		25.80↓	0.94	< 0.025
21days v/s 35days		2.5 fold ↑	7.34	<0.005
1 day v/s 7 days	Muscle	60.50 ↓	5.90	< 0.005
7 days v/s 14 days		45.56↑	1.74	Equivocal
7 days v/s 21 days		2.72 fold ↑	3.90	< 0.005
14 days v/s 21days		86.95↑	2.48	<0.025
21days v/s 35days		41.86↓	3.29	<0.005
1 day v/s 7 days	Intestine	23.33 ↓	2.09	< 0.05
7 days v/s 14 days		6.52↓	0.35	NS
7 days v/s 21 days		13.04 ↓	0.56	NS
14 days v/s 21days		6.97 ↓	0.27	NS
21days v/s 35days		2.57 fold ↑	3.64	< 0.01
1 day v/s 7 days	Blood	70.00↑	1.63	Equivocal
7 days v/s 14 days		64.70↑	1.51	Equivocal
7 days v/s 21 days		64.70↓	2.57	<0.025
14 days v/s 21days		78.57↓	3.60	<0.005
21days v/s 35days		4 fold ↑	5.94	<0.005

Table 7: Changes in the fatty acid profiles of total lipid in various tissues of chick *Gallus domesticus* during post hatching development

(Mean values of three estimates)

Tissues	Fatty acid	Relative % composition					
		1day	7 days	14 days	21 days	28 days	35 days
Liver	16:0	30.90	30.47	32.42	31.33	30.30	29.80
	16:1	1.10	1.47	1.30	4.40*	3.80*	4.20*
	18:0	15.49	16.22	14.45	14.21	18.23*	18.20*
	18:1	30.93	30.58	28.23	17.21*	16.24*	15.20*
	18:2	9.61	11.72	10.56	14.91*	15.23*	15.30*
	18:3	1.20	0.90	1.50	1.20	1.10	1.20
	20:4	4.37	4.64	4.23	7.38*	8.90*	10.20*
	20:5	0.40	0.40	0.35	0.46	2.20*	2.40*
	22:6	1.20	1.20	1.00	1.50	1.60	1.40
	Others	4.80	2.40	5.96	7.40	2.40	2.10
Muscle	16:0	28.02	29.73	27.13	28.90	26.20	28.30
	16:1	7.00	6.50	6.32	6.10	3.20*	3.60*
	18:0	9.84	13.97*	13.84*	13.90*	14.30*	14.30*
	18:1	26.77	27.95	25.38	23.90*	22.20*	19.30*
	18:2	18.51	13.56*	13.40*	15.60*	14.20*	14.00*
	18:3	1.20	0.90	1.00	0.80	2.20*	2.50*
	20:4	5.16	4.29	8.00	8.30*	8.80*	9.30*
	20:5	0.50	0.40	0.50	0.20	2.00*	2.20*
	22:6	1.60	1.50	1.50	1.20	2.50*	2.60*
	Others	1.40	1.20	2.93	1.10	4.40	3.90

Continued...

Intestine	**16:0**	28.65	28.74	28.57	28.50	29.50	27.50
	16:1	1.90	3.58*	3.86*	3.46*	3.90*	4.40*
	18:0	19.86	17.09	9.35*	9.35*	9.25*	10.20*
	18:1	19.06	20.30	28.26*	28.50*	27.50*	27.20*
	18:2	12.74	15.64*	15.74*	15.36*	15.80*	16.50*
	18:3	1.80	1.90	1.80	2.10	2.20	2.40
	20:4	5.92	5.25	5.72	7.90*	7.22*	7.50*
	20:5	3.20	2.50	3.30	1.90*	1.20*	1.50*
	22:6	1.80	1.50	1.50	1.60	1.50	1.40
	Others	5.07	3.52	1.90	1.33	1.95	1.40
Blood	**16:0**	24.96	24.79	25.70	27.27	26.92	25.72
	16:1	1.96	2.27	2.37	2.20	1.90	2.20
	18:0	25.85	24.50	27.49	26.88	24.50	24.29
	18:1	17.54	17.30	18.10	7.60*	7.00*	7.70*
	18:2	21.21	20.24	20.15	27.46*	28.42*	28.32*
	18:3	0.30	0.20	0.30	0.30	0.20	0.20
	20:4	4.28	4.10	4.30	6.29*	7.38*	9.36*
	20:5	0.30	0.30	0.29	0.30	0.35	0.30
	22:6	0.90	0.50*	0.50*	0.50*	0.51*	0.51*
	Others	2.70	5.80	0.80	1.20	2.82	1.40

*** These values are significant at $p < 0.05$ over the values of 1 day data.**

Table 8 : Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler chick, *Gallus domesticus* during post hatching development.

(Mean values of 6 samples and their standard error)

Age of the bird after hatching	3-HMG-CoA- Reductase (u/l/mg protein)		3-HMG-CoA- Synthase (u/l/mg protein)	
	Liver	Serum	Liver	Serum
1 day	477.48 ± 8.03	116.45 ± 8.95	69.27 ± 3.02	8.09 ± 1.47
7 days	227.20 ± 4.14	52.16 ± 4.30	45.65 ± 2.62	4.01 ± 1.24
14 days	258.22 ± 7.54	55.58 ± 6.00	45.86 ± 1.39	4.59 ± 1.32
21 days	585.97 ± 6.89	52.47 ± 5.92	41.68 ± 0.75	9.99 ± 0.33
28 days	606.23 ± 5.98	52.47 ± 3.98	35.39 ± 1.68	11.65 ± 0.52
35 days	681.21 ± 8.17	51.61 ± 7.92	24.60 ± 1.03	12.18 ± 1.85

Table 8a: Statistical calculation (comparison test by using student 't' test) of Table 8.

Comparing group	Tissues	3-HMG-CoA- Reductase			3-HMG-CoA- Synthase		
		% change	't' value	*p* value	% change	't' Value	*p* value
1day vs 7days	Liver	52.41↓	27.70	<0.005	34.09↓	5.90	<0.005
7days vs14days		13.65↑	3.60	<0.005	0.46↑	0.07	NS
7days vs 21days		2.57fold↑	44.63	<0.005	8.69↓	1.45	Equivocal
14days vs 21days		2.26fold↑	32.08	<0.005	9.11↓	2.64	<0.025
21days vs 35days		16.25↑	8.91	<0.005	40.97↓	13.40	<0.005
1day vs 7days	serum	55.20↓	6.47	<0.005	50.43↓	2.12	<0.05
7days vs14days		6.55↑	0.46	NS	14.46↑	0.32	NS
7days vs 21days		0.59↑	0.04	NS	2.49fold↑	4.66	<0.005
14days vs 21days		5.59↓	0.36	NS	2.17fold↑	3.96	<0.005
21days vs 35days		1.63↓	0.08	NS	21.92↑	1.16	NS

3.2 Effect of dietary lipid on the growth and development:

Based on the fatty acid composition, three commercial oils viz., coconut oil, sunflower oil and fish oil were selected to study the effect of dietary lipid on broiler chicks during post hatching development. The coconut oil is having around 26% of monoenoic acid and 61% saturated fatty acid. Sunflower oil contains 58% of linoleic acid, while the fish oil contains about 20% monoenoic fatty acids, 9% linoleic acid and 26% of long chain polyunsaturated fatty acids (Table 9a).

Commercial feed used in these experiments contained 36% crude protein and 6.5% total crude fat. We selected 3 different doses of the lipid sources viz., 2.5%, 5% and 10 % in the present study. From Table 9b it was very clear that with the addition of exogenous lipid, the proximate composition of the commercial feed as well as experimental diet remains unaltered except for the crude fat content only.

Table 9c represents the relative composition of fatty acid profiles of feed supplemented with different lipid sources used in the experiment. It was noticed that with the addition of coconut oil there was increase in the saturated fatty acids as well as monoenoic acids of the commercial feed, whereas the linoleic acid content had decreased. However, with the supplementation of sunflower oil, the fatty acid profiles of the feed remained unaltered. With the addition of fish oil to the commercial feed, the linoleic acid content was decreased with an increase in linolenic acid, monoenoic fatty acids and long chain polyunsaturated fatty acids.

3.2.1 Growth:

Tables 10a, 10b and 10c figure A demonstrate the growth of the chicks supplemented with different doses of coconut oil, sunflower oil and fish oil respectively. Table 10ai, 10bi and 10ci give the relevant statistical analysis of the Tables 10a – 10c.

About 19-46% increase (statistically equivocal to $p<0.005$) in the net weight of the chicks was observed over that of control group when the chicks were supplemented with different doses of coconut oil (viz., 2.5%, 5%, 10%) for 15 days . This increase in the weight gain was reflected in the daily instantaneous growth rate (G_w) and in feed conversion ratio (Table 10a). It is interesting to observe that these dose dependent changes were noticed with last two doses of coconut oil supplementation (statistically equivocal) only (Table 10ai). With prolonged feeding for another 15 days, a marginal increase (about 9%, statistically equivocal) in the average weight of the bird was observed in chicks supplemented with 10% coconut oil only (figure A).

With the supplementation of the sunflower oil for 15 days about 20-70% increase ($p < 0.025 - p< 0.005$) in the average weight of the chicks was observed (figure A). The dose dependent change in the average weight of the chicks was observed with the last two doses only. About 72% increase in the net weight of the birds, as observed in the chicks fed with the diet supplemented with 10% sunflower oil compared to the net weight of the control birds, increaased the value of daily instantaneous growth rate by about 40% and brought down the value of feed conversion ratio (FCR) by about 10% (Table 10b). With the continuation of sunflower oil for another 15 days, a significant increase in the net weight of the chicks was observed with the supplementation of with 10% sunflower

oil only. About 10% increase ($p<0.05$) in weight was recorded over the weight of control chicks. However, no much change was noticed in the daily instantaneous growth (G_w) as well as FCR with the supplementation of 10% sunflower oil for 30 days (Table 10b).

A clear dose dependent change is evident from table 10c with respect to average weight of the birds when they were given a diet supplemented with fish oil for 15 days and 30 days. When the growing chicks were supplemented with different doses of fish oil (viz. 2.5%, 5% and 10%) along with the commercial diet for 15 days about 25% to 84% ($p<0.01$ to $p < 0.001$) more growth was observed (Table 10c and 10ci). This increase in the net weight was also reflected in the increased value of daily instantaneous growth rate and decreased value of feed conversion ratio. With the continuation of the same feeding experiment for another 15 days, the maximum increase in the net weight of the chicks was observed as compared to the net weight of control chicks with the supplementation of 10% fish oil (20% increase, $p< 0.005$), but this increase was reflected by about 30% decrease in the value of feed conversion ratio (Figure A).

3.2.2 Hematology

About 10-15% increase ($P<0.025$) in the hemoglobin concentration was observed in the experimental birds over the same of the control birds with the supplementation of coconut oil (up to 5% doses) for 15 days. However, such a trend disappeared with the continuous supplementation of coconut oil for another 15 days (figure B1). No statistically significant changes were observed in the level of hemoglobin concentration after 15 and or 30 days of feeding all three doses (2.5%, 5%, 10%) of sunflower oil along

with commercial feed, (Table 11b and 11bi). However, about 22% increase ($p<0.005$) in the hemoglobin concentration was detected in the chicks fed with the diet supplemented with 10% fish oil of 15 days over the same of the control chicks. This rise in the hemoglobin concentration was decreased with the continuous supplementation of fish oil. After 30 days of supplementing 10% fish oil, the hemoglobin concentration increased by about 15% ($p<0.025$) only over the same of the control chicks (Figure B3).

It is evident from Table 11a that there was a dose dependent decrease (about 40% to 70%, p <0.05-0.005) in the count of total erythrocytes of the chicks supplemented with coconut oil for 15 and / or 30 days. With the supplementation of sunflower oil for 15 days and / or 30 days a similar trend in the count of total erythrocyte count was observed (Figure B2b). Although a sharp decrease (about 60% - 70%, $p<0.005$) in the count of total erythrocyte was observed with 2.5% sunflower oil supplementation, with increase in the doses of oil supplementations, the total erythrocyte count increased but still remained lower compared to the same of control birds (Table 11b, 11bi). No significant change in the total erythrocyte count was recorded with the supplementation of different doses of fish oil for 15 days and / or 30 days (Table 11c and 11ci).

Although 40%- 45% decrease in the total count of leukocytes was observed with the supplementation of various doses of coconut oil for 15 and / or 30 days (Table 11a, 11ai), the decrease in the count of total leukocytes was not dose dependent. It can be clearly visualized from figure B2c that the total leucocyte count of the chicks decreased by about 40% - 45 %, ($p<$ 0.01- $p<$ 0.005) when the chicks were supplemented with

different doses of sunflower oil for 15 days. However, with the continuation of the sunflower oil supplementation for another 15 days the total leukocyte count did not change significantly. With the supplementation of different doses of fish oil for 15 days, no significant change in the total leukocyte count was noticed. However, about 20-25 % increase (p<0.01-0.005) in the total count of leukocyte was observed when supplementation of fish oil was prolonged for another 15 days (Table 11c, 11ci).

3.2.3 Tissue biochemical composition

Tables 12 –15 and figures C – F represent the variation of the tissue level total protein and lipid profiles (viz., triglycerol, cholesterol, phospholipid) in different tissues of *Gallus domesticus,* supplemented with different doses of coconut oil, sunflower oil and fish oil along with the commercial feed for 15 and 30 days. Table 16 represents the fatty acid profiles of various tissues of chicks in this feeding experiment.

3.2.3.1 Total Protein

From Table 12a it is very clear that there were statistically significant changes occurred in the total protein concentration after 15 days and / or 30 days of supplementing different doses of coconut oil (p<0.005) except in the liver (after 15 days of oil supplementation). About 24% increase (p<0.005) in the total protein content of liver was detected with 10% supplementation of oil for 30 days (Table 12ai). In muscle and intestine, a dose dependent increase in the protein concentration was noticed with the supplementation of oil for 15 and / or 30 days except for the first dose (2.5% of oil)

where it remained unaltered for 15 days (in muscle and intestine) or for 30 days (in muscle) of feeding experiment (figure C1).

A statistically significant change in the tissue level total protein concentration was noticed (Table 12b) after 15 days and / or 30 days of supplementing different doses of sunflower oil ($p<0.001$) except for intestine (after 15 days of oil supplementation). A clear dose dependent increase (from about 10% to two fold), in the tissue level protein concentration was noticed (p <0.05 – 0.005) in all the tissues viz., liver, muscle and intestine with the supplementation of different doses of sunflower oil for 30 days. However this dose dependent increase was not prominent after supplementing the oil for 15 days in intestine and liver (FigureC2). In liver and intestine about 11-30 % increase (p <0.05) in the total protein concentration was noticed with 10% sunflower oil supplementation for 15 days only.

A statistically significant change in the tissue level total protein concentration was noticed (Table 12c) after 15 days and / or 30 days of supplementing different doses of fish oil ($p<0.001$). A clear dose dependent increase (about 11-80%, $p<0.005$) in the total protein concentration of liver, muscle and intestine in chicks was evident from the Tables 12c and 12ci and figure C3.

3.2.3.2 Total Triglycerol

The variations in the total triglycerol level in liver, muscle and intestine of the chicks fed with 2.5%, 5% and 10% coconut oil along with the commercial feed for 15

days and / or 30 days were statistically significant at 0.1% level (Table 13a). When the chicks were fed with a diet supplemented with 10% coconut oil for 15 and / or 30 days, a significant increase (about 43%-61%, $p<0.005$) was noticed in the liver (Table 13ai). A significant ($p<0.005$) dose dependent increase in the total triglycerol concentration of muscle and intestine was noticed with the supplementation of different doses (viz., 2.5%, 5% and 10%) of coconut oil for 15 and / or 30 days except in the muscle after 15 days of feeding (Figure D1).

The dynamics of total triglycerol concentration in liver, muscle and intestine of the chicks fed with 2.5%, 5% and 10% sunflower oil along with the commercial diet for 15 days and / or 30 days were statistically significant at 0.1% level (Table 13b) except in the intestine (after 15 days of oil supplementation). A dose dependent increase in the level of total triglycerol was observed in muscle (about 20% - 80%, $p<0.005$) with the supplementation of different doses of sunflower oil to the chicks for a period of 15 and / or 30 days (Figure D2). In liver, a dose dependent increase in the concentration of total triglycerol was recorded after 15 days of oil supplementation (figure D2). In intestine, although no change in the tissue level total triglycerol was detected after 15 days of oil supplementation, a dose dependent increase (80% - 2 fold, $p< 0.005$) was detected after 30 days of the feeding experiment (Table 13bi).

The variations in the tissue level concentration of total triglycerol of liver, muscle and intestine of the chicks supplemented with 2.5%, 5% and 10% fish oil along with the commercial feed for 15 days and / or 30 days were found to be statistically significant at

0.1% level (Table 13c). Almost a clear dose dependent increase of total triglycerol concentration in various tissues of chicks was noticed with the supplementation of different doses of fish oil after 15 days. This dose dependent increase was not seen with the continuation of fish oil supplementation for another 15 days (figure D3). A significant decrease (about 12-25%, $p<0.005$) in the tissue level triglycerol concentration was noticed in the birds supplemented with 5% and 10% fish oil compared to that of birds with 2.5% oil supplementation for a period of 30 days. However, feeding with 10% fish oil for 30 days, the total triglycerol level remained 24% to almost 3 fold higher than that of the control birds (Table 13ci).

3.2.3.3 Total Cholesterol

It is clear from Table 14a that the variations in the total cholesterol concentration in different tissues of the chicks fed with 2.5%, 5% and 10% coconut oil along with the commercial feed for 15 days and / or 30 days were statistically significant at 1% or 0.1% level. With the supplementation of different doses of coconut oil for 15 days and / or 30 days, the liver and muscle of the chicks showed a dose dependent increase (63% to 7.6 fold, $p < 0.005$) in the total cholesterol concentration (Table 14ai). Due to 10% coconut oil supplementation for a period of 30 days, the total cholesterol level was increased by 5.8, 7.6 and 2.4 fold in the liver, muscle and intestine respectively ($p<0.005$). In the intestine, significant changes in the total cholesterol concentration was detected only with 5% and 10% coconut oil supplemented chicks over the same of the control chicks (figure E1).

It is evident from Table 14b that the variations in the total cholesterol content of different tissues of the chicks supplemented with 2.5%, 5% and 10% sunflower oil along with the commercial feed for 15 days and / or 30 days were statistically significant at 0.1% except in muscle and intestine (after 15 days of the feeding experiment). With the supplementation of sunflower oil for 15 days, the total cholesterol concentration was increased by 2 - 4 fold in liver, muscle and intestine ($p < 0.005$). Whereas, by increasing the doses of oil supplementation the total cholesterol concentration in muscle and intestine remained more or less static (figure E2). The degree of increase in the tissue level cholesterol concentration in liver and muscle further increased when sunflower oil supplementation was prolonged for another 15 days. (Table 14b and 14bi).

It is evident from table 14c that the variations in the total cholesterol of different tissues of the chicks fed with 2.5%, 5% and 10% fish oil along with the commercial diet for 15 days and / or 30 days were statistically significant at 0.1%. A dose dependent increase in the total cholesterol concentration was noticed in liver and muscle up to 5% supplementation of fish oil for 15 days. But with the increase in dose from 5% to 10% these levels were decreased (by 10% - 36%, $p < 0.005$) in all the tissues (Figure E3). A similar trend was noticed after 30 days of supplementing fish oil except in the intestine where a slight increase was noticed from 5% supplementation to 10% supplementation of fish oil (Table 14c and 14ci).

3.2.3.4 Total Phospholipid

A significant variation ($p<0.05 - 0.001$) of total phospholipid concentration in various tissues was recorded due to the supplementation of various doses of coconut oil for 15 and / or 30 days (Table 15a).

About 45% - 70% ($p<0.005$) depletion in the phospholipid concentration of liver, muscle and intestine was recorded with the supplementation of 2.5% coconut oil for 15 days. After that a dose dependent increase in the level of total phospholipid was noticed in all the tissues (Table 15a). After 30 days, there was a depletion (53% - 66%, $p<0.005$) in the total phospholipid concentration of liver, muscle and intestine in the chicks fed with 2.5% coconut oil and this was followed by little increase with the supplementation of 5% and 10% coconut oil (figure F1).

The variations in the total phospholipid concentration of different tissues of the chicks fed with 2.5%, 5% and 10% sunflower oil along with the commercial feed for 15 days and or 30 days were statistically significant at 0.1% (Table 15b). With the supplementation of sunflower oil, a dose dependent increase in total phospholipid concentration of liver, muscle and intestine was noticed after 15 days. After 30 days, a similar trend was noticed except in intestine where the increase was not statistically significant with the supplementation of 2.5% sunflower oil (figure F2).

The variations in the total phospholipid of different tissues of the chicks supplemented with 2.5%, 5% and 10% fish oil along with the commercial diet for 15 days and / or 30 days were statistically significant at 0.1% (Table 15c). Supplementation

of 2.5% fish oil for 15 days did not alter the total phospholipid concentration of liver and muscle. With increase in the dose from 2.5% to 5%, an elevation (of about 2.5 fold, $p<0.005$) was recorded in all the tissues (Table 15ci). Further increase in the dose of oil from 5% to 10%, a depletion (about 22%, $p<0.005$) in the level of total phospholipid concentration was recorded in all the tissues which was higher than that of control birds (figure F3). Supplementation of 2.5% fish oil for 30 days showed decrease (about 57 %, $p<0.005$)) in the total phospholipid of liver and intestine and decrease of about16% ($p<0.01$) in that of muscle. With increase in the dose from 2.5% to 5%, the level of total phospholipid was elevated in all the tissues (Table 15ci). With further increase in dose from 5% to 10 %, the total phospholipid concentration was decreased (20-27%, $p<0.005$) in liver, muscle and intestine. With the supplementation of the highest dose of 10%, the values of total phospholipid remained lower than that of the control chicks except in the muscle where it was higher than that of the control chicks (Table 15c).

3.2.3.5 Fatty acid profiles

Tables 16a - 16d represent the relative concentration of fatty acid profiles of total lipid of liver, muscle, intestine and serum of *Gallus domesticus* given a commercial diet supplemented with 2.5% and 5% coconut oil, 5% and 10% sunflower oil and 5% and 10% fish oil for 30 days. Dose dependent changes in the relative concentration of some fatty acids were recorded in chicks supplemented with sunflower oil and fish oil only.

About 12%– 25% increase in the relative concentration of palmitic, stearic and arachidonic acid at the expense of about 35 – 45% decrease in palmitoleic acid, oleic acid

and eicosapentaenoic acid was detected in the liver of chickss supplemented with coconut oil for 30 days over that of the control group (Table 16a), 38% decrease in stearic acid, about 24 – 66% decrease in eicosapentaenoic acid and docosahexaenoic acid with 21-54% increase in linoleic acid and arachidonic acid was detected in the liver of birds given a commercial diet containing sunflower oil. Treatment with fish oil resulted in 91% to almost 6 fold increase in the relative concentration of ω3 fatty acids mainly α - linolenic acid , eicosapentaenoic acid and docosahexaenoic acid at the expense of 22-50% decrease in palmitoleic acid , linoleic acid and arachidonic acid and 10% decrease in palmitic acid along with 30% decrease in oleic acid (with 10% fish oil supplementation). All these changes are statistically significant at $p < 0.05$.

In muscle (Table 16b), 10 – 16% increase in the level of palmitic acid, stearic acid, linoleic acid and arachidonic acid was recorded along with 25 – 70% decrease in palmitoleic acid, oleic acid, linolenic acid, eicosapentaenoic acid and docosahexaenoic acid in chicks supplemented with coconut oil for 30 days. With the supplementation of sunflower oil, there was 30 – 60% increase in linoleic acid, arachidonic acid in muscle at the cost of 9 – 28% decrease in palmitic acid, stearic acid, oleic acid and docosahexaenoic acid. Supplementation of fish oil depicted 70% - 3 fold increase in α - linolenic acid, eicosapentaenoic acid and docosahexaenoic acid at the cost of 15 – 40% decrease in oleic acid and arachidonic acid and 14% decrease in stearic acid (10% Fish oil).

In the intestine (Table 16c), 11-50% increase in the concentration of palmitic, stearic and linoleic acid was noticed followed by 36 – 57% decrease in palmitoleic , oleic and docosahexaenoic acids in chicks fed with a diet supplemented with Coconut oil for 30 days. Supplementation of Sunflower oil to the diet of chicks showed about 36 – 62% increase in linoleic acid along with 20 – 31% decrease in Oleic acid and 33% decrease in linolenic acid after 30 days of feeding (with 10% oil supplementation only). When the chicks were given a diet containing fish oil, there was 91% increase increase in linoleic acid, 2 – 3 fold increase in eicosapentaenoic acid and 2 – 5 fold increase in docosahexaenoic acid accompanied by 17 – 24% decrease in palmitic, oleic acid and arachidonic acid (with 10% fish oil only).

When the chicks were supplemented with coconut oil along with the commercial feed, about 11% increase in palmitic acid level of serum was noticed along with 26% decrease in the level of stearic acid and eicosapentaenoic acid. 18 – 33% decrease in stearic acid and oleic acid was recorded in the chicks supplemented with sunflower oil along with 11 – 22% increase in linoleic acid and 19 – 40% increase in arachidonic acid. With the supplementation of fish oil to the diet, the linolenic acid eicosapentaenoic acid and docosahexaenoic acid contents of the serum increased by 3 – 8 fold, whereas, a decrease in the relative concentration of stearic acid . oleic acid, linoleic acid and arachidonic acid was noticed (Table 16d).

3.2.4 Serum lipid profiles

Table 17 and figure G represent the changes in serum lipid profiles due to supplementation of various doses of coconut oil, sunflower oil and fish oil along with the commercial feed to the growing chicks for a period of 15 days and / or 30 days.

The total cholesterol level of serum was increased by 15-22% ($p<0.005$) with the supplementation of 5% and 10 % coconut oil for 15 days. However with 2.5% supplementation of coconut oil, the total cholesterol level in serum was decreased by about 5% ($p<0.005$). Further supplementation of coconut oil for another 15, days showed a dose dependent increase (about 25-53%, $p< 0.005$) in the total cholesterol level (Table 17a). The serum total triglycerol level showed a decreasing trend with the supplementation of coconut oil for 15 days. However, with the continuation of the same the total triglycerol was raised by 8 - 24 % ($p < 0.005$). While coconut oil supplementation brought increase in the HDL cholesterol level by about 18% - 34% after 15 days of feeding, 18% rise in the same was detected only with 10% coconut oil supplementation for 30 days. LDL cholesterol cholesterol level remained more or less unaltered during the first phase of oil supplementation. With the continuation of coconut oil for another 15 days, the LDL cholesterol and VLDL cholesterol levels were increased by 2-3 fold ($p<0.005$) and by 24% ($p<0.005$) respectively (figure G1).

It is noticed from Table 17b and figure G2 that there was increase (about 12%, $p<0.005$) in the level of total cholesterol of serum after 15 days of feeding different doses of sunflower oil along with the commercial feed. The increase in the total cholesterol

level in serum was much more prominent with the continuation of sunflower oil supplementation for another 15 days. With the supplementation of 2.5% and 5% sunflower oil for 15 and / or 30 days there was a decrease (about 14% - 21%, $p<0.005$) in the level of total triglycerol. But with increase in the dose from 5% to 10% for a period of 30 days, the serum total triglycerol level increased to the normal level. About 20% - 55% ($p < 0.005$) increase in serum HDL cholesterol level was detected in the experimental birds fed with different doses of sunflower oil for 15 days and / or 30 days (figure G2). With the supplementation of sunflower oil for 15 days, the serum LDL cholesterol was decreased by about 26-34% ($p < 0.025 - p < 0.05$) while the same was increased by about 45% - 60% ($p < 0.005$ - equivocal) in the birds supplemented with 5% and 10% sunflower oil for 30 days. About 14% - 18% ($p<0.05$ - equivocal) decrease in the concentration of VLDL cholesterol in serum was noticed after 15 days of supplementation of sunflower oil and the trend remained the same even after prolonging the supplementation of sunflower oil for another 15 days except for the birds supplemented with 10% sunflower oil (Table 17b).

When the chicks were supplemented with 2.5% fish oil for 15 and 30 days, the total cholesterol concentration of serum increased by about 7% ($p<0.005$). But with the increase in dose of oil supplementation, the serum cholesterol level came back to normal level (Figure G3). A gradual decrease (about 11%-55%, $p<0.005$) in the serum total triglycerol level of serum was noticed in chicks supplemented with different doses of fish oil for 15 and / or 30 days. With the supplementation of 2.5% fish oil for 15 days the level of HDL cholesterol increased by about 33% ($p<0.005$). But with increase in the

dose, the level of HDL cholesterol decreased but it remained higher than the control value. After 30 days of feeding experimental diet containing fish oil, at initial dose the HDL cholesterol was decreased by about 14%, $p<0.005$, but, with increase in the dose of fish oil, HDL cholesterol level increased and reached either to normal level (with 5% fish oil) or significantly increased by 16% (with 10% fish oil) over the control value (Table 17c). With the supplementation of fish oil for 30 days a decrease (about 40 – 55%, $p < 0.005$) in the LDL concentration was noticed. However, the picture is not the same after the supplementation of fish oil for 15 days. About 20% decrease in the concentration of LDL cholesterol of serum was noticed with the supplementation of 2.5% fish oil for 15 days, but at a higher dose, this change was not detected. The VLDL cholesterol concentration was decreased by about 11% - 55% with the supplementation of fish oil for 15 and / or 30 days. This increase was statistically significant at 0.5% level except for 2.5% fish oil supplementation for 15 days (Table 17ci).

3.2.5 Liver and cardiac function test

Liver function test with reference to Alkaline Phosphatase (ALP, E.C. 3.1.3.1) and Glutamate Pyruvate Transaminase (GPT, EC 2.6.1.2) and cardiac function test with reference to Lactate Dehydrogenase (LDH, EC 1.1.1.27) and Glutamate Oxaloacetate Transaminase (GOT, EC2.6.1.1) in liver and serum were checked with the supplementation of 2 doses of coconut oil, sunflower oil and fish oil which is represented in tables 18, 19, 20, 21 and figures H, I, J, K.

3.2.5.1 Alkaline Phosphatase

With the supplementation of coconut oil for 15 days and 30 days, there was increase (about 22%- 2.5 fold, $p<0.005$) in the activity of alkaline phosphatase in liver and serum (Table 17ai). But the activity was lower with the supplementation of 5% coconut oil than that of 2.5% coconut oil supplementation, except for serum level after 30 days of feeding experimental diet (Table 18a and figure H).

A dose dependent decrease (18% - 63%, $p<0.005$) in the activity of alkaline phosphatase was noticed in the liver with the supplementation of 5% and 10% sunflower oil for 15 days and / or 30 days (Table18b). In serum, an increasing trend was noticed with the supplementation of 5% sunflower oil for 15 days and / or 30 days. However, in serum, the level of ALP activity was decreased with the increase in dose of oil supplementation, but the level of activity remained still higher (after 15 days of feeding) or came to the normal value (after 30 days of feeding).

Supplementation of 5% and 10% fish oil for 15 days and 30 days reduced the activity of alkaline phosphatase (about 16%- 59%, $p<0.005$) in liver and serum (Table 18ci). It can also be clearly noticed from table 18ci that the activity of alkaline phosphatase was lower with 10% fish oil after 30 days of feeding experimental diet compared to 5% supplementation of fish oil.

3.2.5.2 Glutamate Pyruvate Transaminase

It is clear from Table 19a that there were no significant changes detected in the activity of GPT in liver after 15 and 30 days of feeding coconut oil. In serum, though the activity of GPT increased (61%, equivocal) after 15 days of feeding 2.5% coconut oil, it remained unaltered with supplementation of 2.5% coconut oil for 30 days and 5% coconut oil for 15 and / or 30 days.

With the supplementation of 5% and 10% of sunflower oil (Table 19b) and fish oil (Table 19c), the GPT activity in liver as well as in serum remained unaltered after 15 days and / or 30 days of feeding experimental diet Figure I).

3.2.5.3 Glutamate Oxaloacetate Transaminase

With the supplementation of 2.5% and 5% coconut oil for 15 and / or 30 days, the GOT activity in liver was depleted (about 20%- 52%, equivocal - $p<0.05$) which was not dose dependent (Table 20a, and figure J). The serum GOT activity remained almost unaltered with 2.5% coconut oil supplementation after 15 days and / or 30 days. However, after 30 days, the serum GOT level was decreased by 12% with the supplementation of 5% coconut oil, which may or may not be statistically significant.

The liver GOT activity was decreased (about 32 –50%, $p<0.025$ – <0.005) after 15 and / or 30 days of feeding 5% and 10% sunflower oil (Table 19b). The serum GOT activity was also increased (50% - 3.6 fold) after 15 and 30 days of supplementing sunflower oil. The serum GOT activity remained lower at 10% sunflower oil

supplemented birds compared to 5% sunflower oil supplemented birds (Table 20b, figure J).

Supplementation of 5% and 10% fish oil for 15 and / or 30 days decreased (about 56% - 70%, $p < 0.01$ - 0.005) the GOT activity in liver. In serum, the GOT activity increased by about 30% ($p < 0.005$ – equivocal) with supplementation of fish oil for 15 days. With fish oil supplementation for another 15 days, the serum GOT activity was decreased by 18% - 28%, ($p < 0.025$ - 0.005).

3.2.5.4 Lactate Dehydrogenase

The liver LDH activity was increased 2 - 3 fold ($p < 0.005$) with the supplementation of 2.5% and 5% coconut oil for 15 and / or 30 days (figure K). Serum LDH activity was increased (about 55%, $p<0.005$) with the same treatment for 15 days, but with the continuation of feeding for another 15 days the activity of LDH decreased and came to normal level insignificantly (Table 21a).

Supplementation of 5% and 10% sunflower oil increased (20% - 2 fold, $p <$ 0.005) the activity of LDH in liver after 15 and / or 30 days of feeding. However, the liver LDH activity remained unaltered with increase in dose from 5% to 10% after 30days of the feeding experiment, though it was increased by about 22% ($p<0.005$) after 15 days of feeding experimental diet. The serum LDH activity remained unaltered with the supplementation of sunflower oil for 15 and / or 30 days except in the birds supplemented with 10% sunflower oil for 30 days(Tables 21b).

Table 21c clearly shows that there was increase (about 11%-19%, $p < 0.005$) in liver LDH activity after 15 days of feeding 5% and 10% fish oil. The changes were not dose dependent. However, with continuation of fish oil supplementation for another 15 days, the liver LDH activity returned to normal value (figure K). No statistically significant change was detected in the serum level activity of LDH after 15 days of fish oil supplementation. The serum activity was reduced by 16% - 23% ($p < 0.005$) with supplementation of fish oil for 30 days (Table 21c).

3.2.6 Cholesterol metabolism

The 3 - HMG CoA Reductase activity in liver was decreased (about 17%, $p<0.005$) with the supplementation of 2.5% coconut oil for 15 days. However, no change in the activity was recorded with the continuation of the same feeding experiment for another 15 days (figure L1). When the birds were supplemented with 5% coconut oil, the activity increased by 15% and 30% after 15 days and 30 days of the feeding experiment respectively ($p<0.005$). In serum, a dose dependent increase (4 – 12 fold, $p<0.005$) in the level of 3- HMG CoA Reductase was observed after 15 and / or 30 days of feeding coconut oil (Table 22 ai).

The 3 - HMG CoA Synthase activity in liver showed a dose dependent decrease (24%-63%, $p<0.025 – 0.005$) after 15 and / or 30 days (Table 22 a, figure L1). However, a dose dependent increase (62% - 2.6 fold, $p < 0.005$) in serum Synthase activity was noticed with the supplementation of coconut oil for 15 days and / or 30 days. (Tables 22a, 22ai).

Table 22 b and figure L2 clearly depict that there was a dose dependent increase in the activity of 3- HMG CoA Reductase in liver (12% - 72%, $p < 0.005$) and serum (4 – 13 fold, $p < 0.005$) after 15 and / or 30 days of supplementing the sunflower oil.

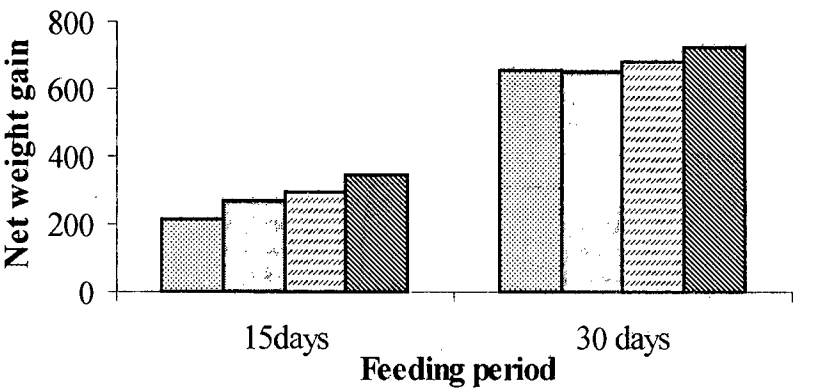
It was also evident that the 3- HMG CoA Synthase activity in liver decreased (40% - 64%, $p < 0.005$) with the supplementation of sunflower oil for 15 days and / or 30 days compared to control chicks. In serum, a dose dependent increase (3 - 12 fold, $p < 0.005$) was recorded with the supplementation of sunflower oil (Figure L2).

Table 22c and figure L3 clearly show that there was a dose dependent increase (about 25 – 55%, $p < 0.005$) in 3- HMG CoA Reductase activity of liver after 15 days of supplementing 5% and 10% fish oil. However, supplementation of fish oil for 30 days did not alter the activity of 3 - HMG CoA Reductase in except a marginal 6% increase as observed in 10% oil supplemented birds. In serum, the level of 3 – HMG CoA Reductase activity remained unaltered due to supplementation of 5% and 10% fish oil for a period of 15 days and / or 30 days (Figure L3).

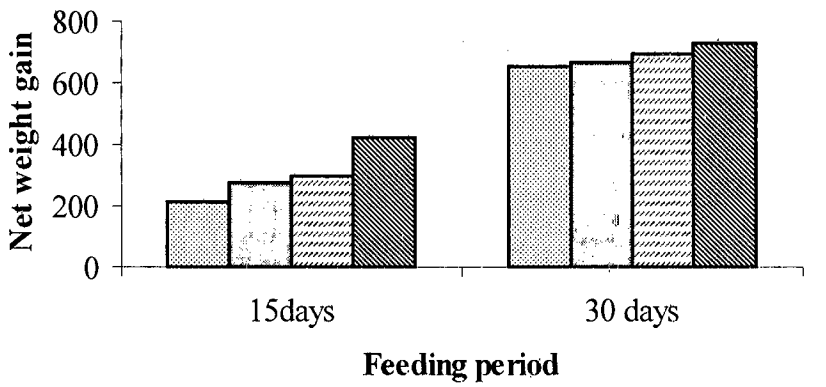
In liver a dose dependent increase (about 3-5 fold, $p<0.005$) in 3- HMG CoA Synthase activity was recorded with the supplementation of 5% and 10% fish oil for 15 and / or 30 days. In serum, the 3- HMG CoA Synthase activity was increased (2 - 4 fold, $p<0$ .005) with the supplementation of 5% and 10% fish oil for 15 and / or 30 days compared to control.

Figure A : Growth chart (net weight gain in grams) of broiler chick (*Gallus domesticus*) supplemented with various doses of different oils along with commercial feed during post hatching development.

1: Coconut oil



2:Sunflower oil



3: Fish oil

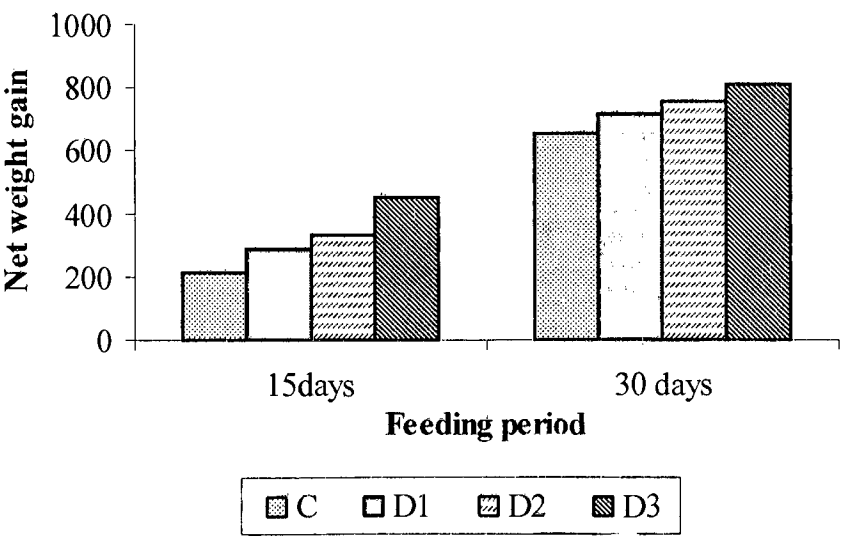
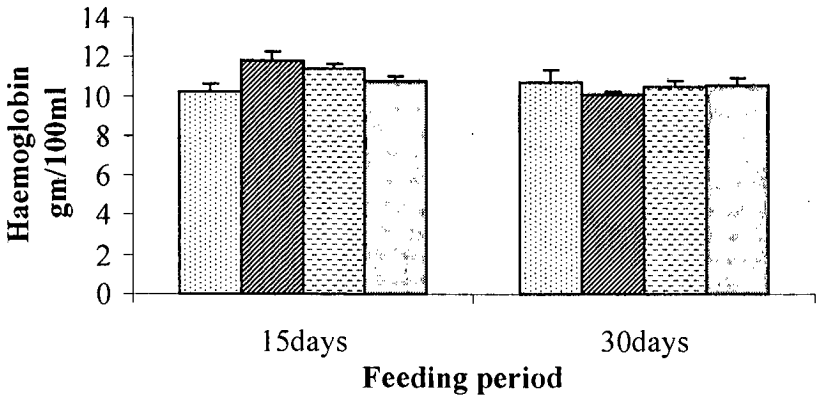
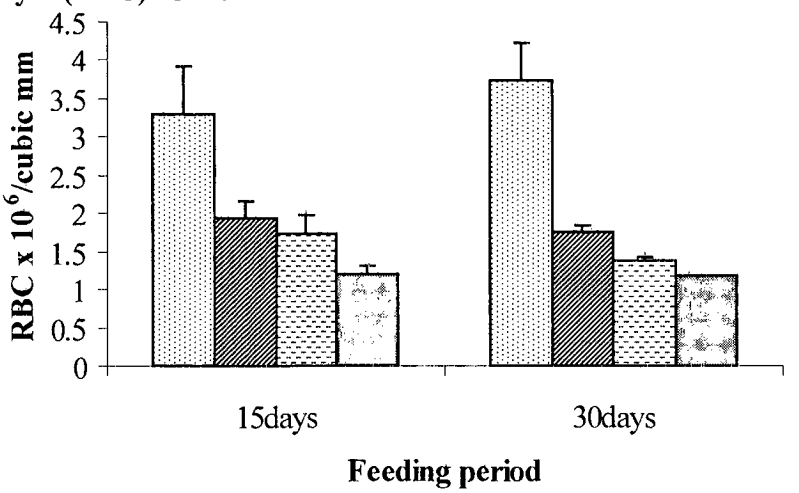


Figure B1 : Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different doses of coconut oil during along with the commercial feed during post hatching development..

a: Haemoglobin concentration



b: Erythrocyte (RBC) count



c : Leukocyte (WBC) count

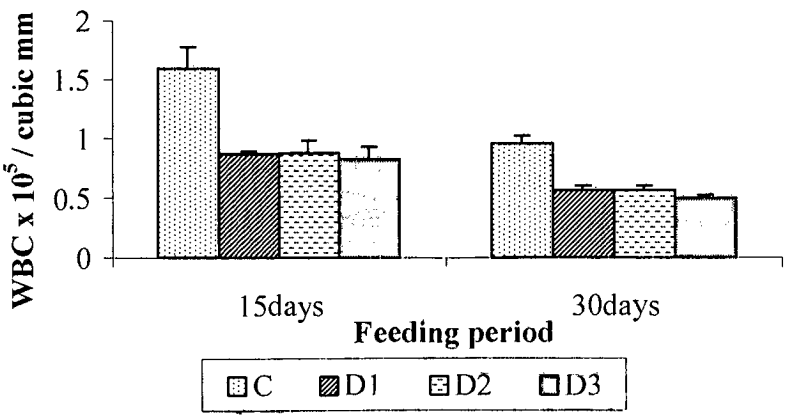
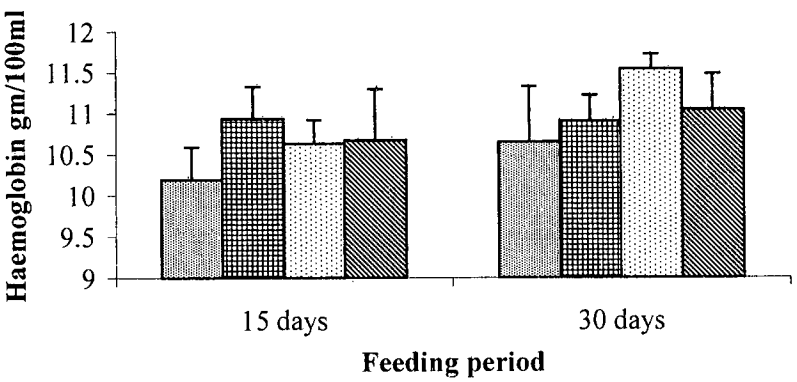
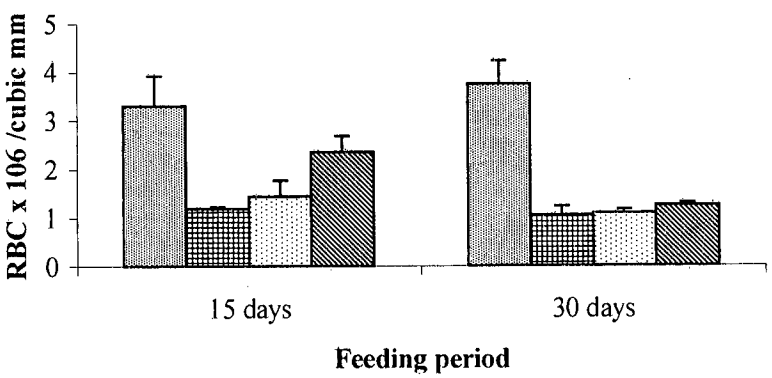


Figure B2: Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different doses of sunflower oil along with the commercial feed during post hatching development.

a: Haemoglobin concentration



b: Erythrocyte (RBC) count



c : Leucocyte (WBC) count

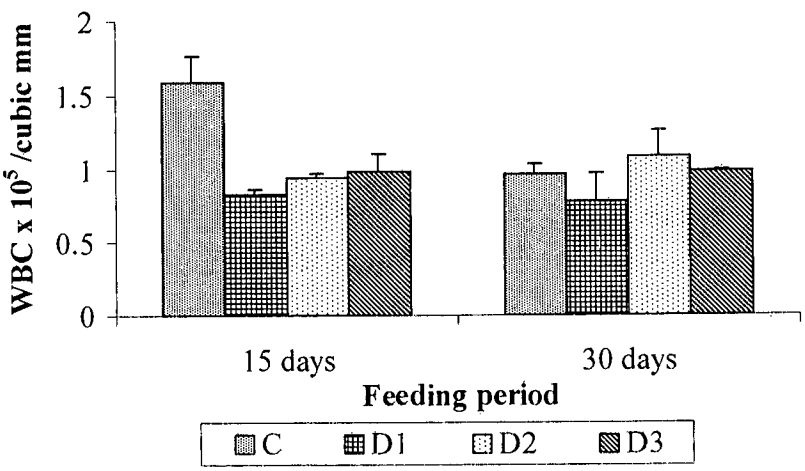
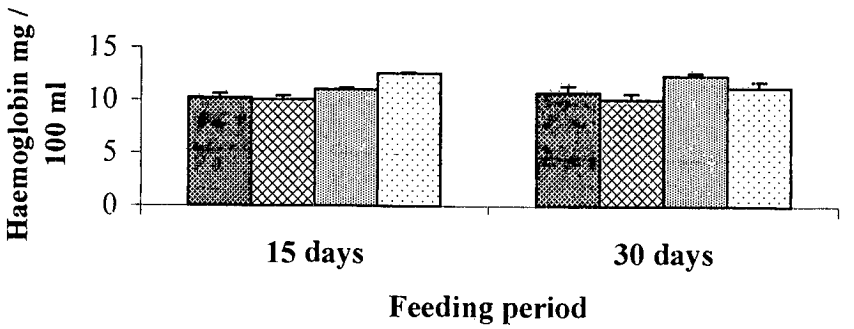
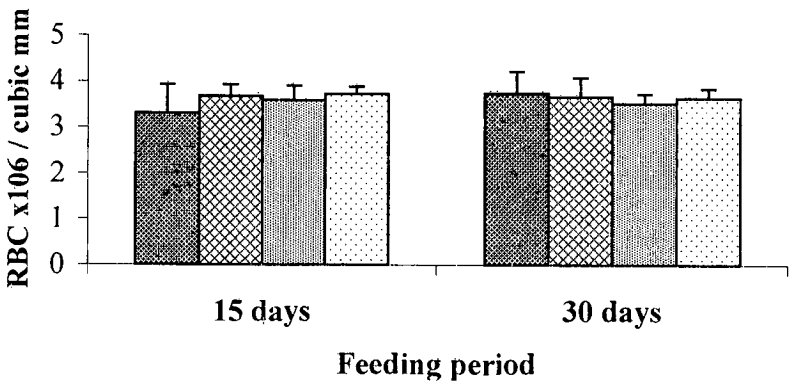


Figure B3 : Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different doses of fish oil along with the commercial feed during post hatching development.

a: Haemoglobin concentration



b: Erythrocyte (RBC) count



c : Leucocyte (WBC) count

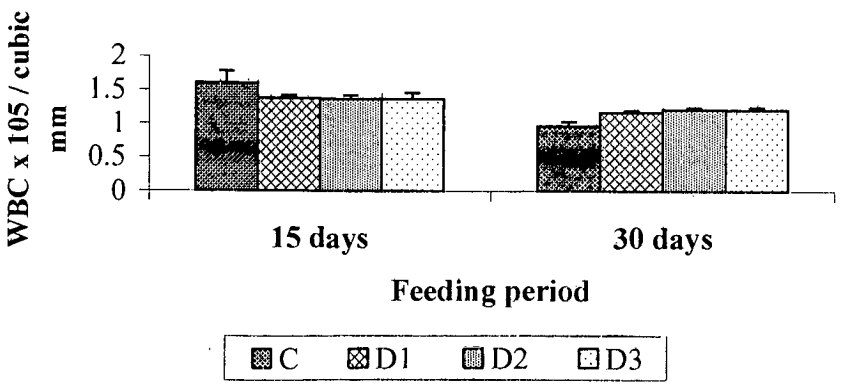
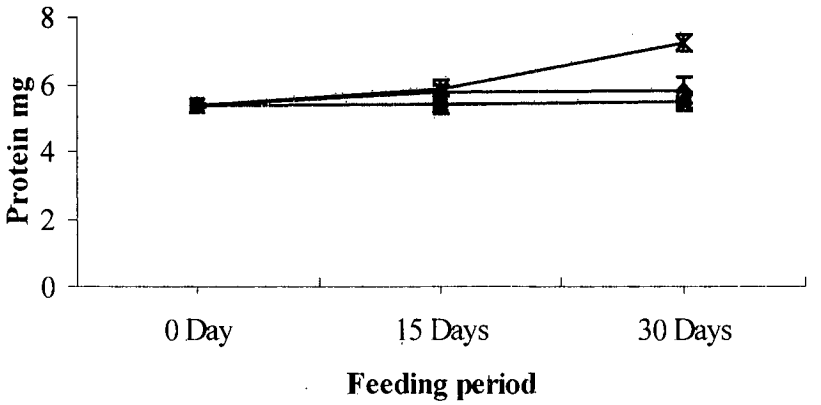
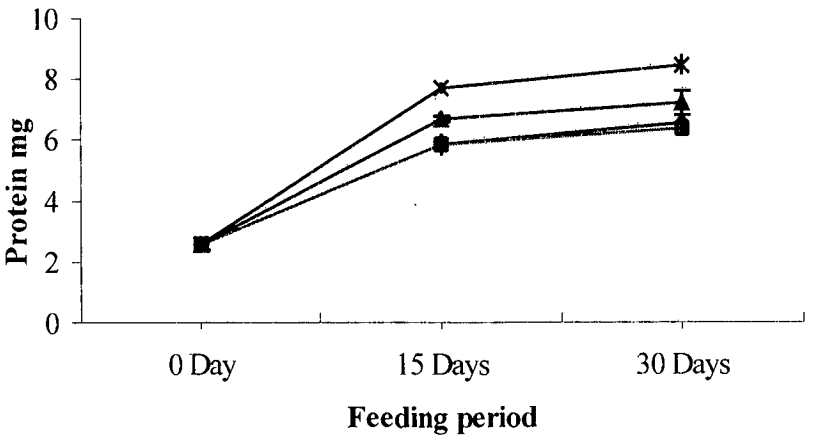


Figure C1: Changes in the total Protein (mg/100mg) concentration of the broiler chick *(Gallus domesticus)* supplemented with different doses of Coconut oil along with the commercial feed during post hatching development

a: Liver



b: Muscle



c :Intestine

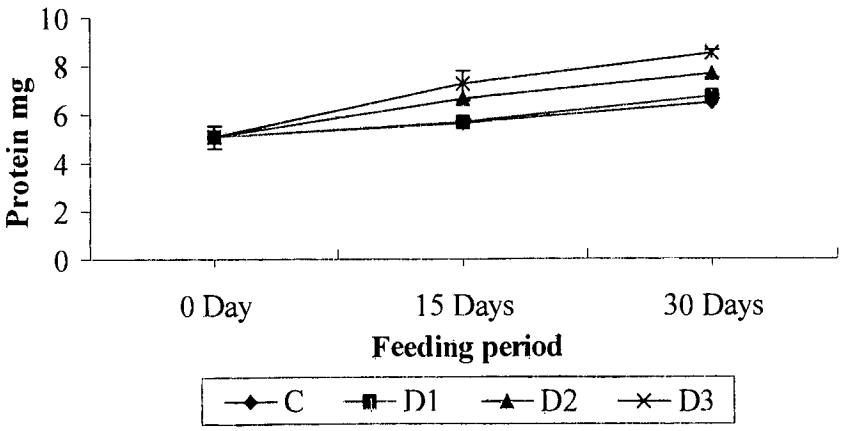
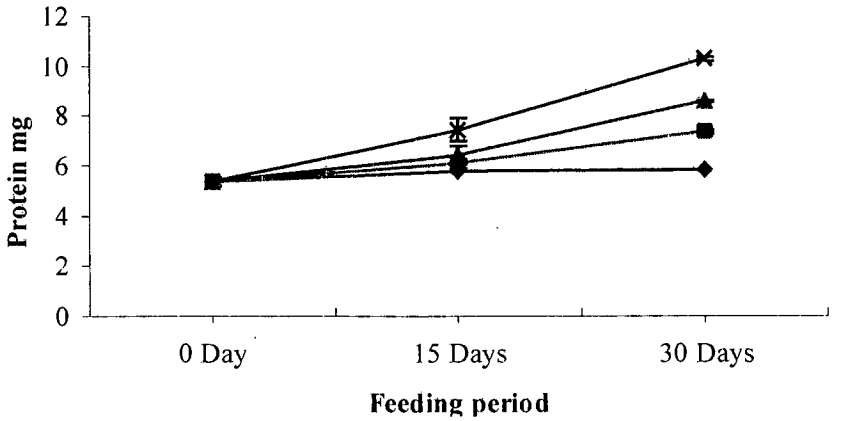
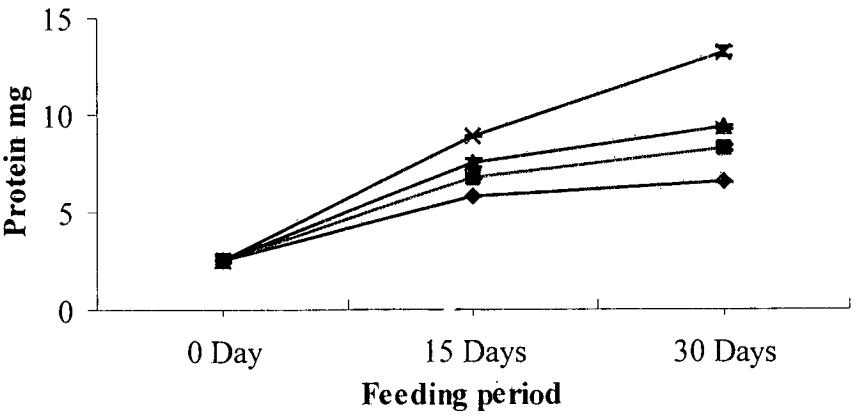


Figure C2: Changes in the total Protein (mg/100mg) concentration of the broiler chick *(Gallus domesticus)* supplemented with different doses of Sunflower oil along with the commercial diet during post hatching development

a: Liver



b: Muscle



c :Intestine

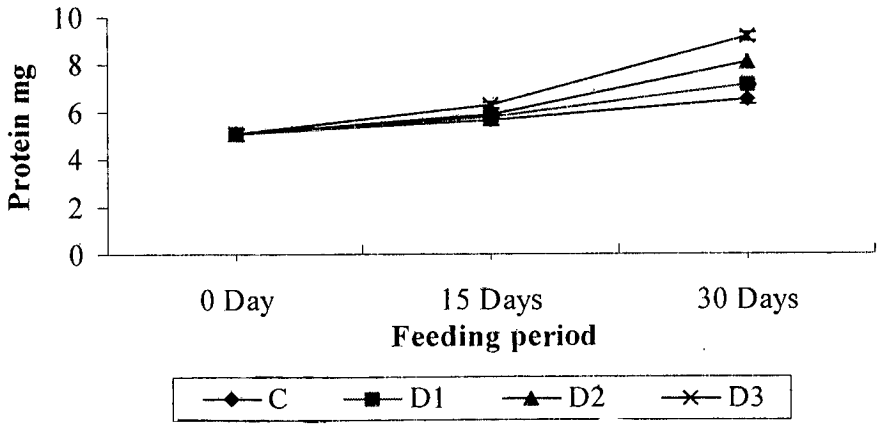
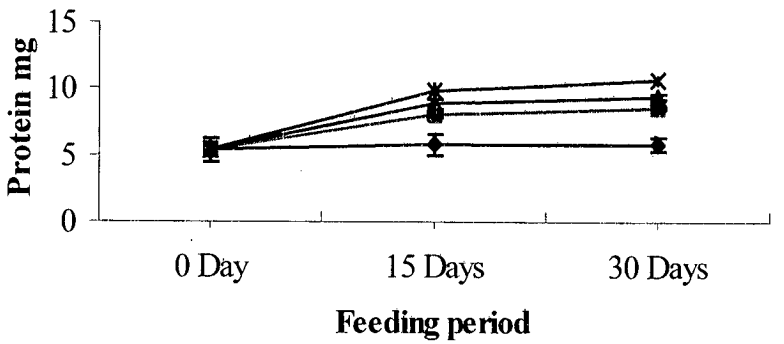
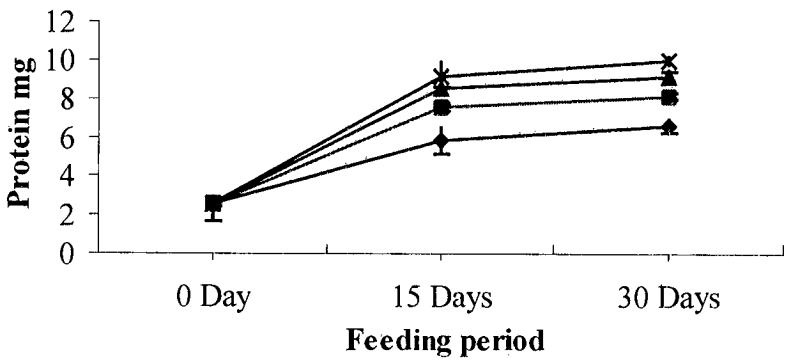


Figure C3: Changes in the total Protein (mg/100mg) concentration of various tissues of broiler chick*(Gallus domesticus)* supplemented with different doses of Fish oil along with the commercial diet during post hatching development

a:Liver



b: Muscle



c :Intestine

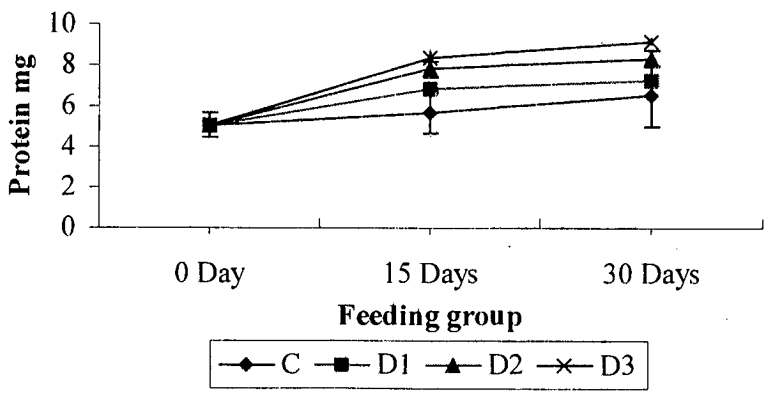
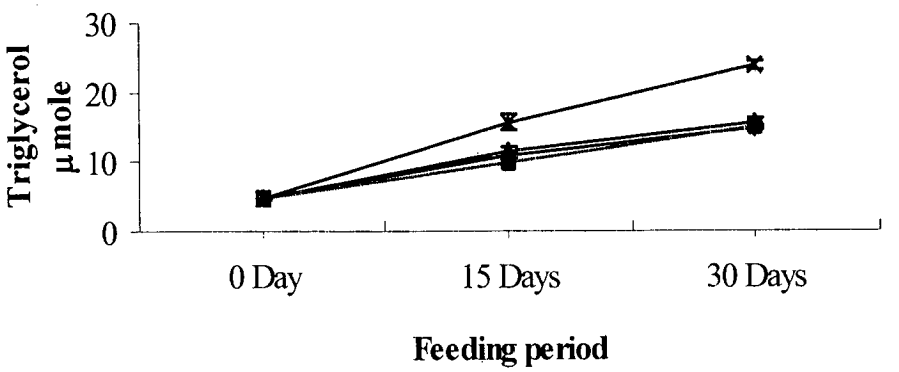
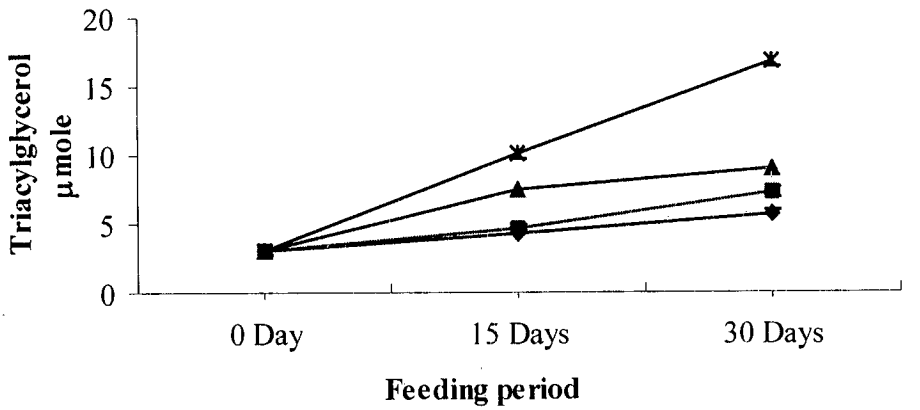


Figure D1: Changes in the total Trilglycerol (µmole/100mg of tissue) concentration of various tissues of broiler chick (*Gallus domesticus*) supplemented with Coconut oil alon with the commercial feed during post hatching development

a: Liver



b:Muscle



c :Intestine

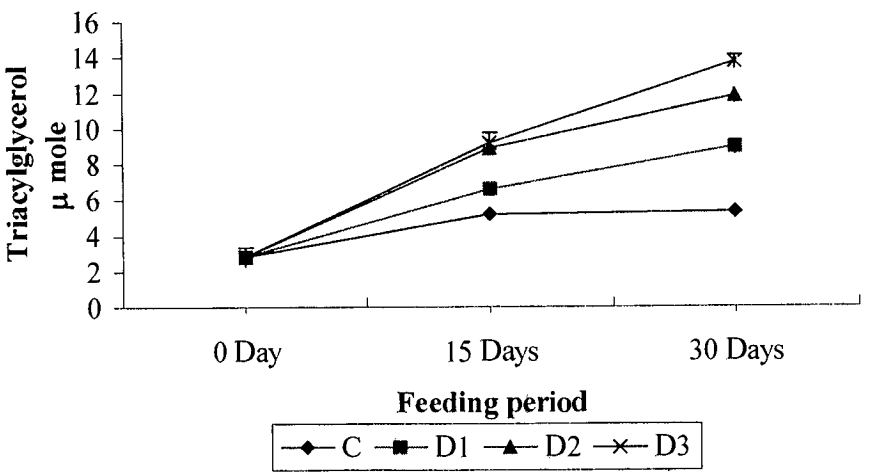
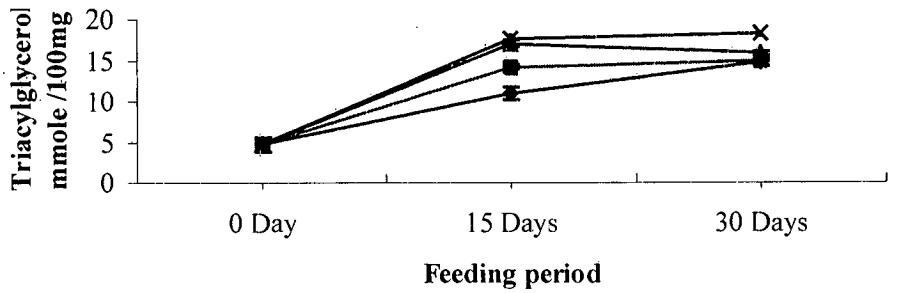
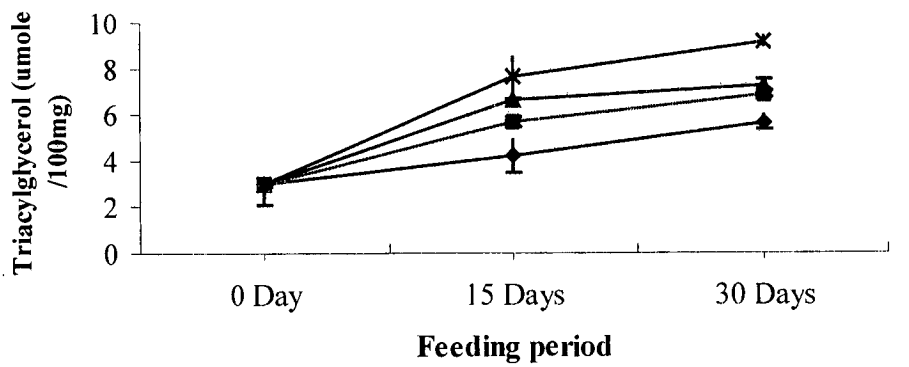


Figure D2: Changes in the total Triglycerol (μ mole/100mg of tissue) concentration of various tissues of broiler chick (*Gallus domesticus*) supplemented with sunflower oil along with the commercial feed during post hatching development

a:Liver



b : Muscle



c: Intestine

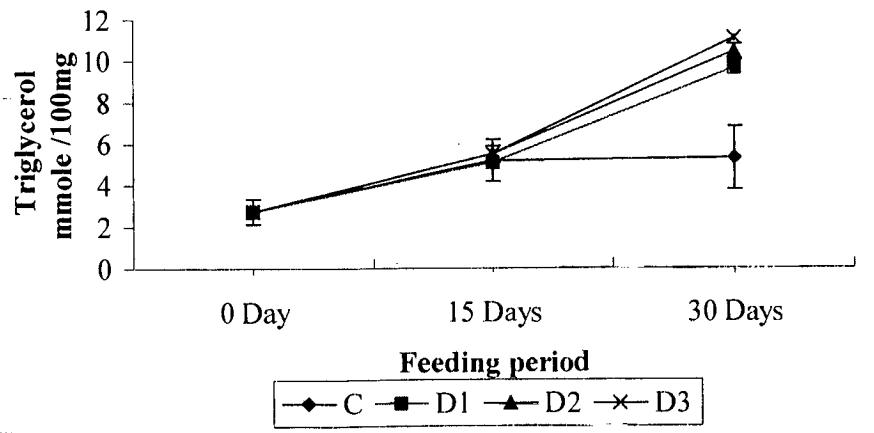
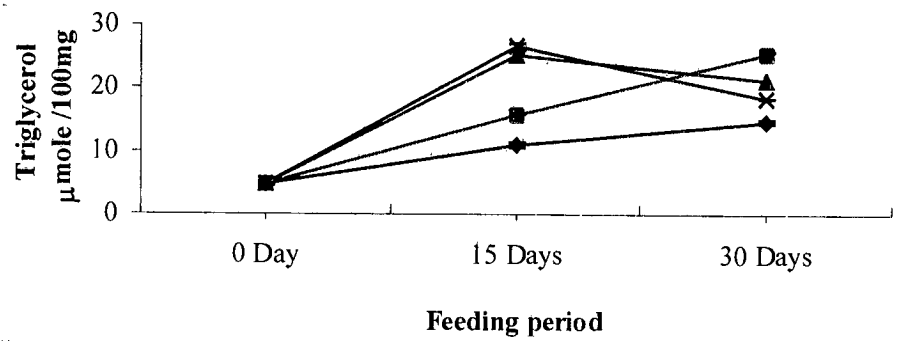
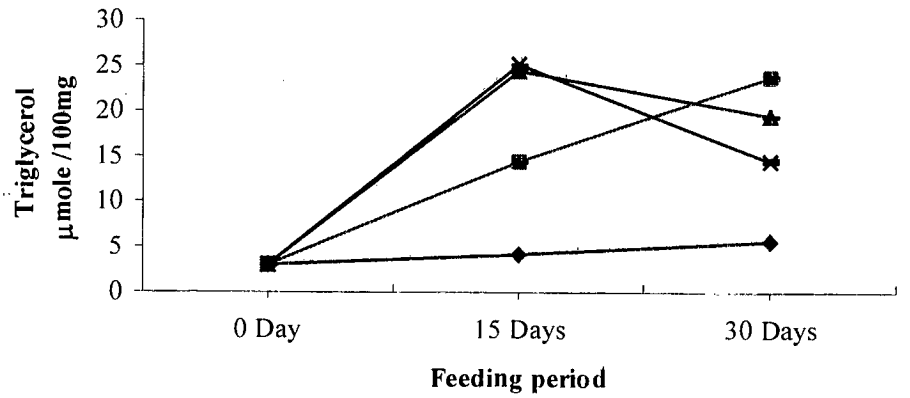


Figure D3: Changes in the total triglycerol (mg/100mg) concentration of the various tissues of the broiler chick (*Gallus domesticus*)supplemented with different doses of Fish oil along with the commercial feed during post hatching development

a:Liver



b : Muscle



c : Intestine

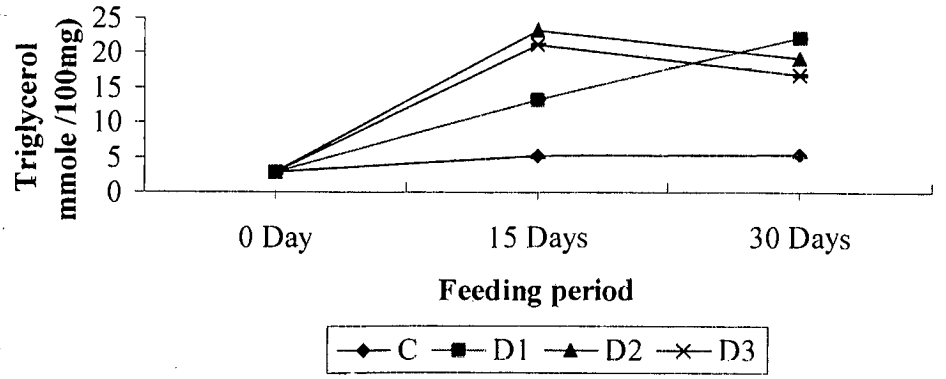
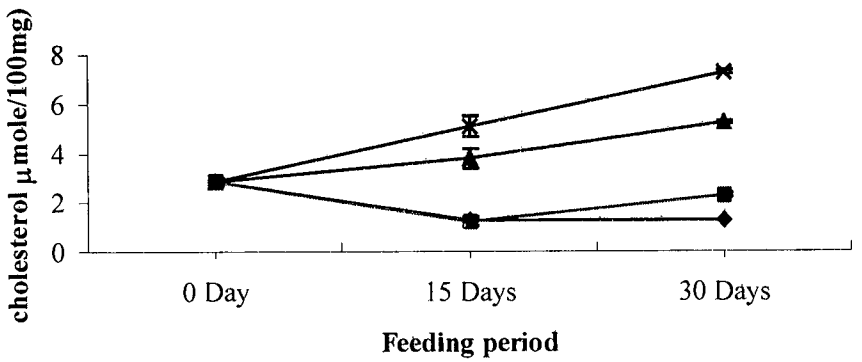
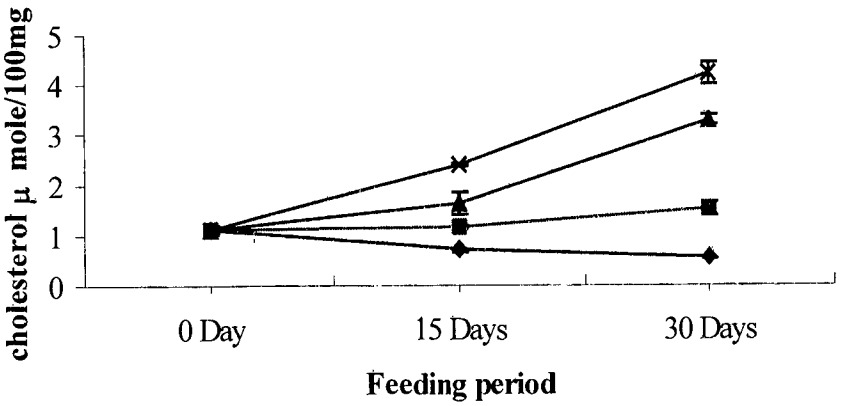


Figure E1: Changes in the total cholesterol (μ mole/100mg) Concentration of the Various tissue of broiler chick (*Gallus domesticus*)supplemented with different doses of Coconut oil along with the commercial diet during Post hatching development

a:Liver



b : Muscle



c : Intestine

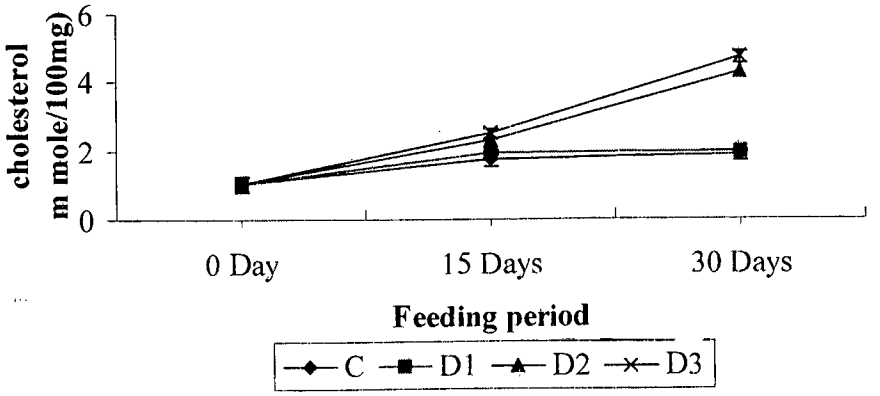
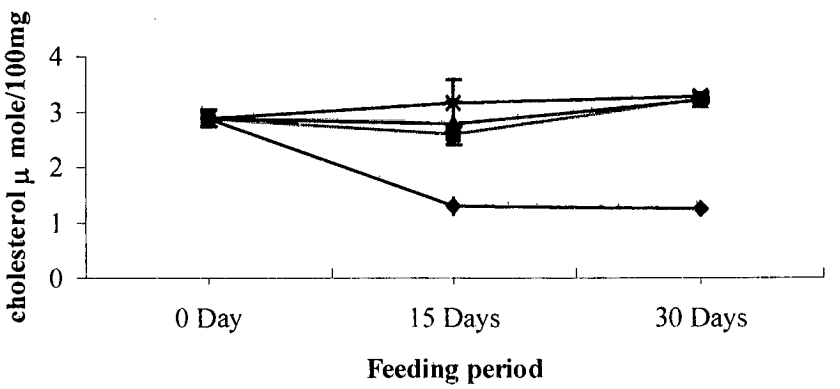
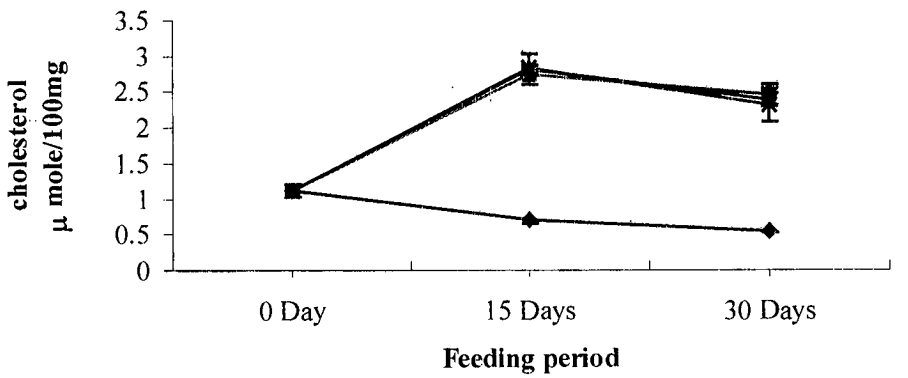


Figure E2: Changes in the total cholesterol (μ mole/100mg) Concentration of the various tissue of broiler chick (*Gallus domesticus*)supplemented with different doses of Sunflower oil along with the commercial diet during Post hatching development

a : Liver



b : Muscle



c : Intestine

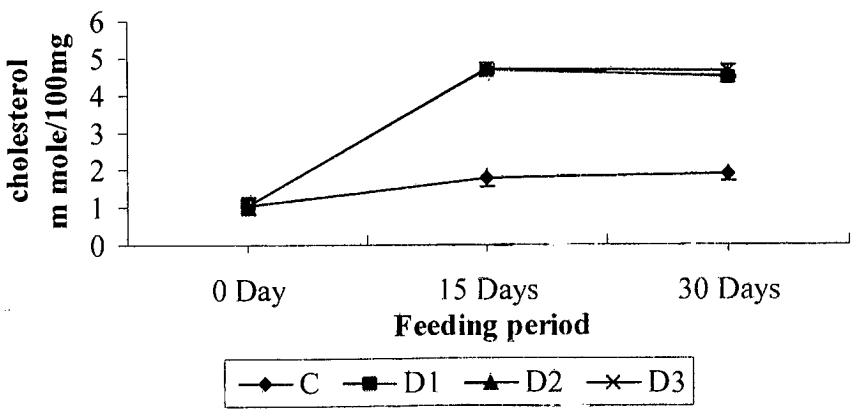
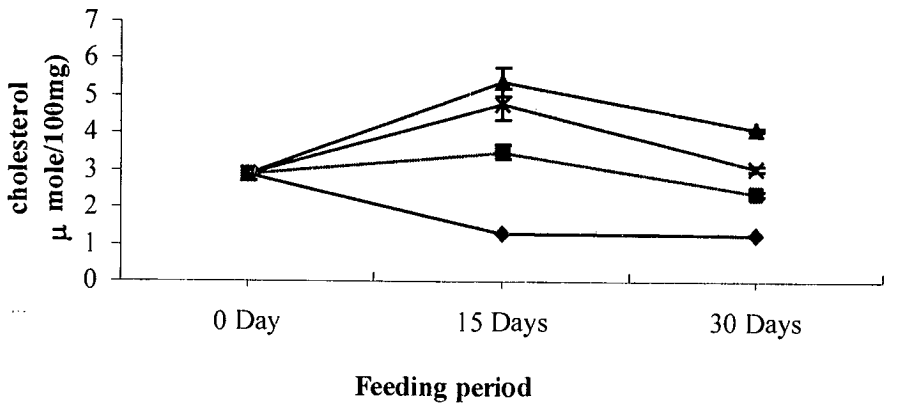
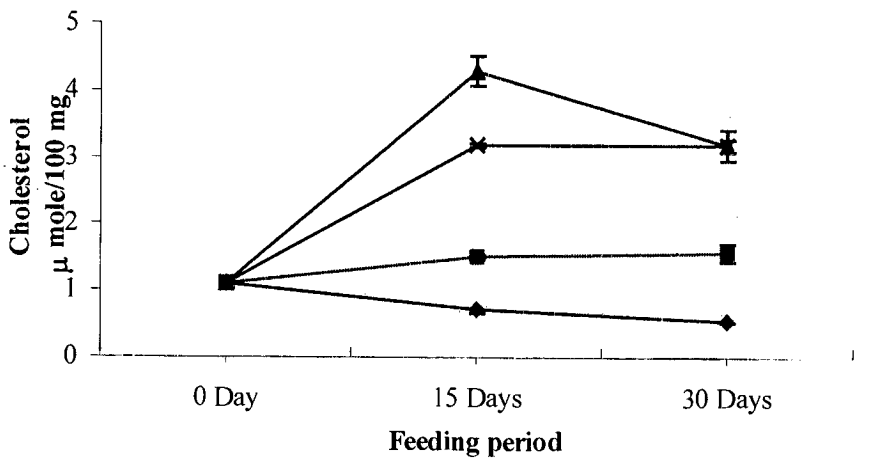


Figure E3: Changes in the total cholesterol (μ mole/100mg) Concentration of the various tissue of broiler chick (*Gallus domesticus*) supplemented with different doses of FishOil along with the commercial diet during Post hatching development

a: Liver



b : Muscle



c : Intestine

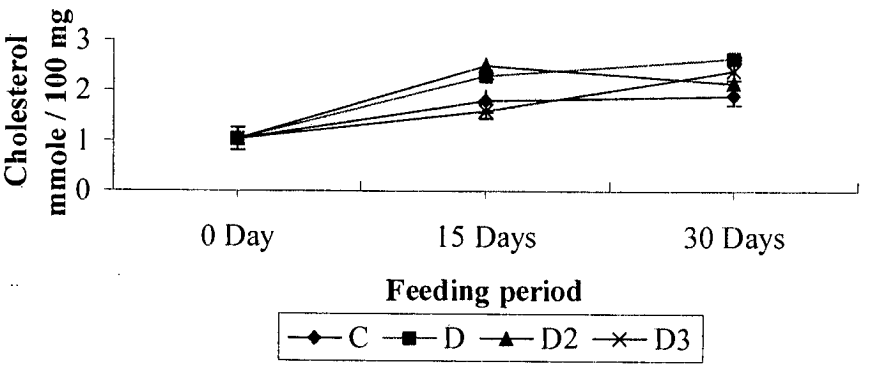
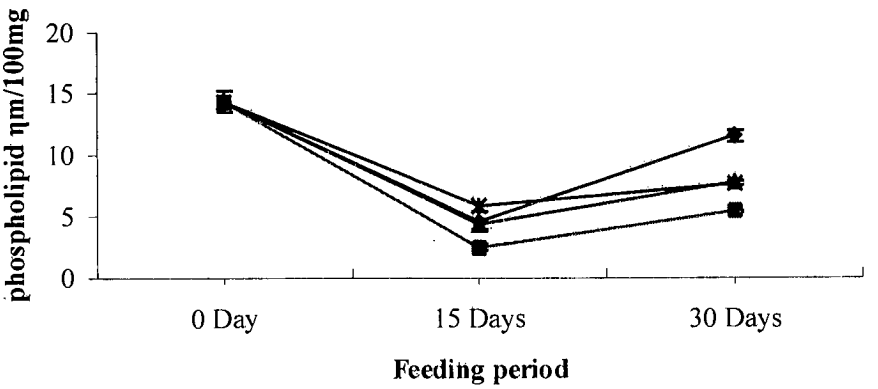
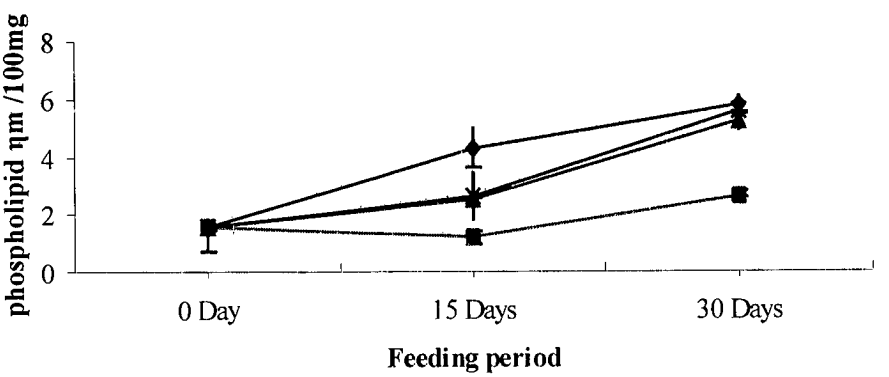


Figure F1: Changes in the total Phospholipid (ηm /100mg) Concentration of the various tissue of broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil along with the commercial feed during Post hatching development

a: Liver



b : Muscle



c: Intestine

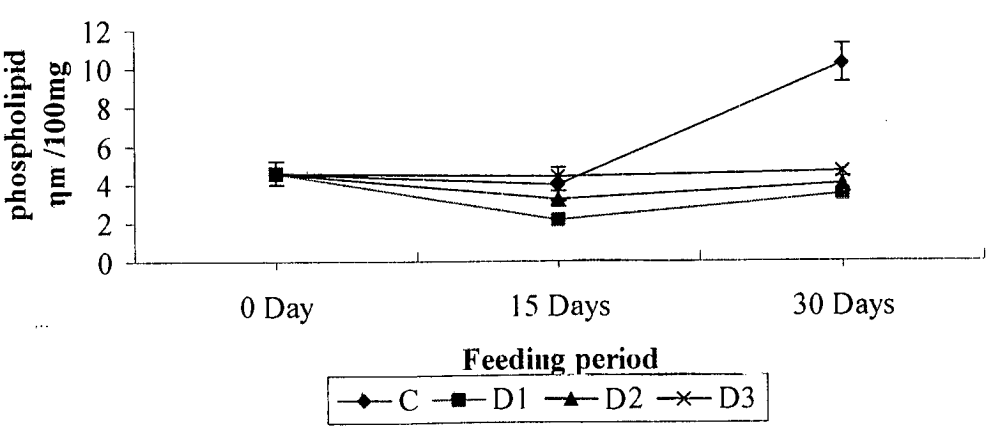
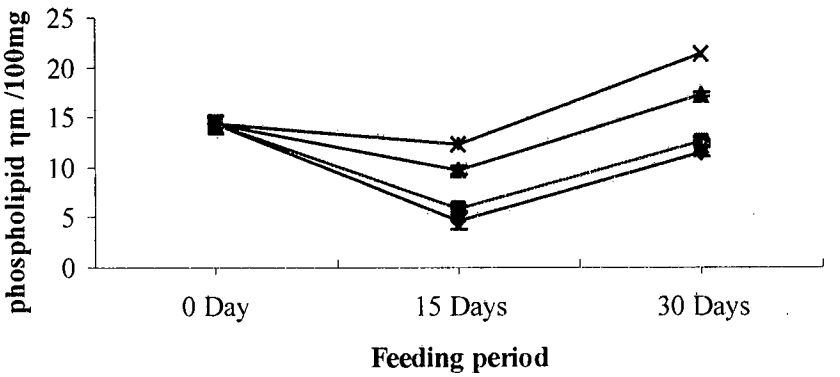
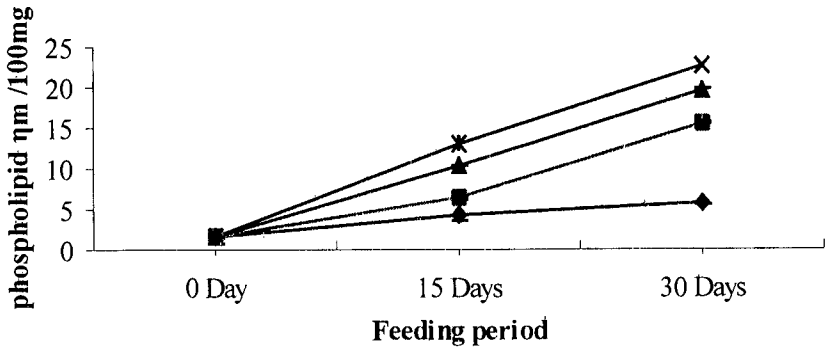


Figure F2: Changes in the total Phospholipid (ηm /100mg) Concentration of the various tissue of broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during Post hatching development

a : Liver



b: Muscle



c : Intestine

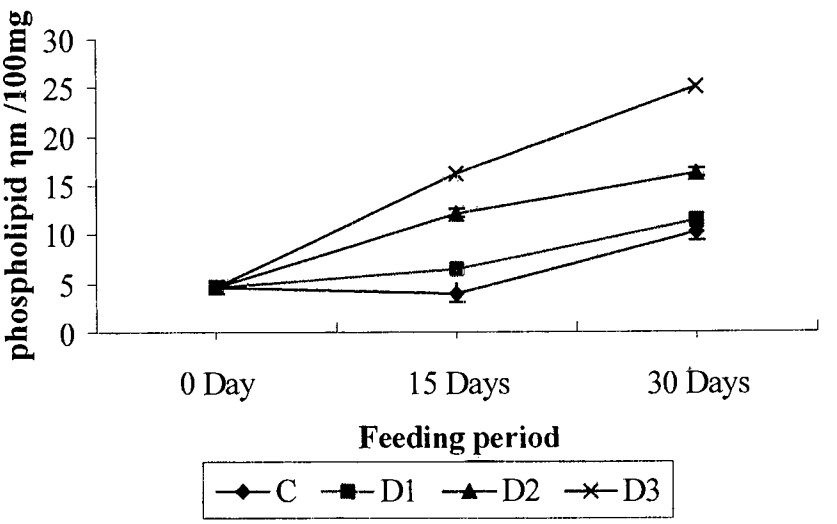
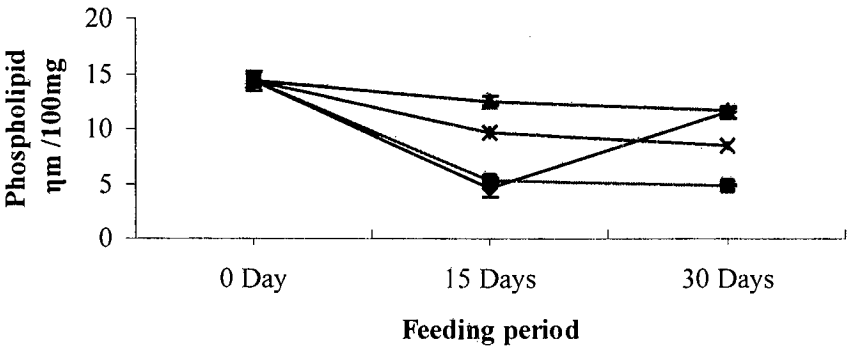
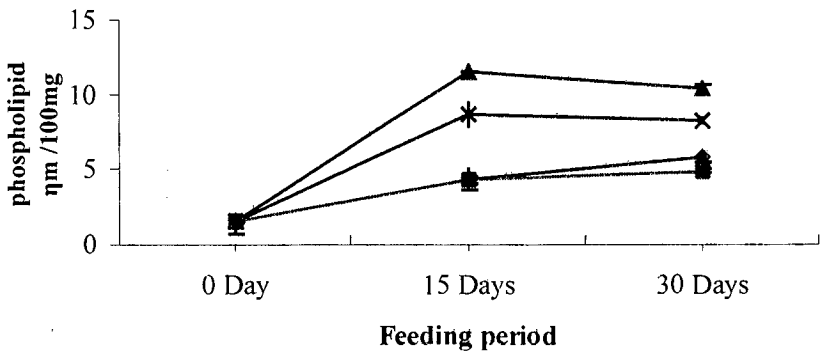


Figure F3: Changes in the total Phospholipid (ηm /100mg) Concentration of the various tissue of broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during Post hatching development a: Liver



b :Muscle



c : Intestine

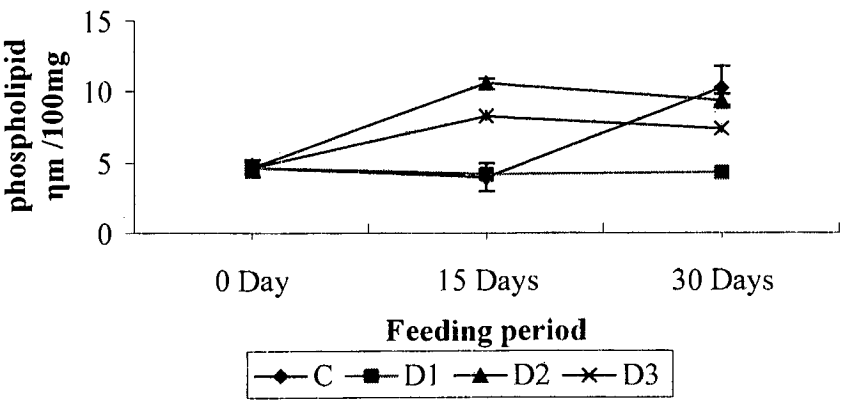
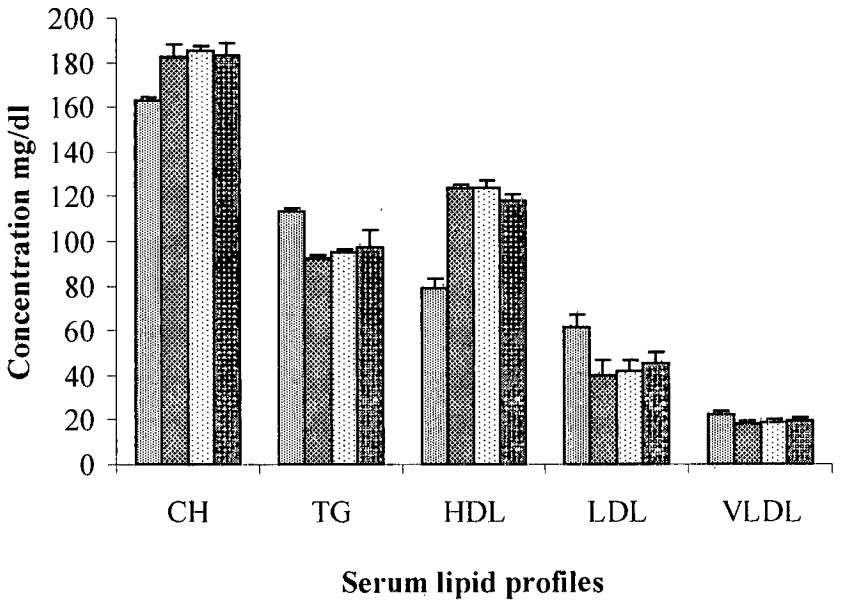


Figure G1: Changes in the serum lipid profiles of broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil for 15 and 30 days along with the commercial feed during post hatching development

a: 15 days feeding



b: 30 days feeding

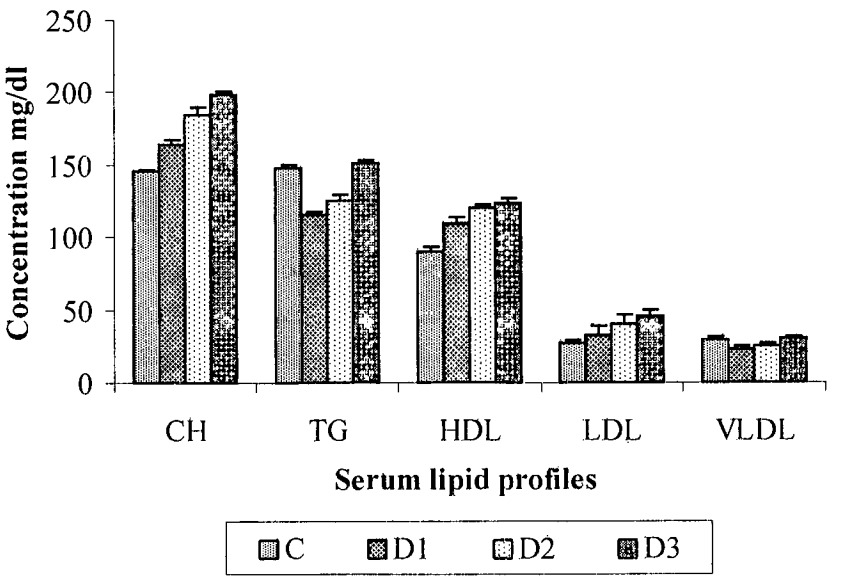
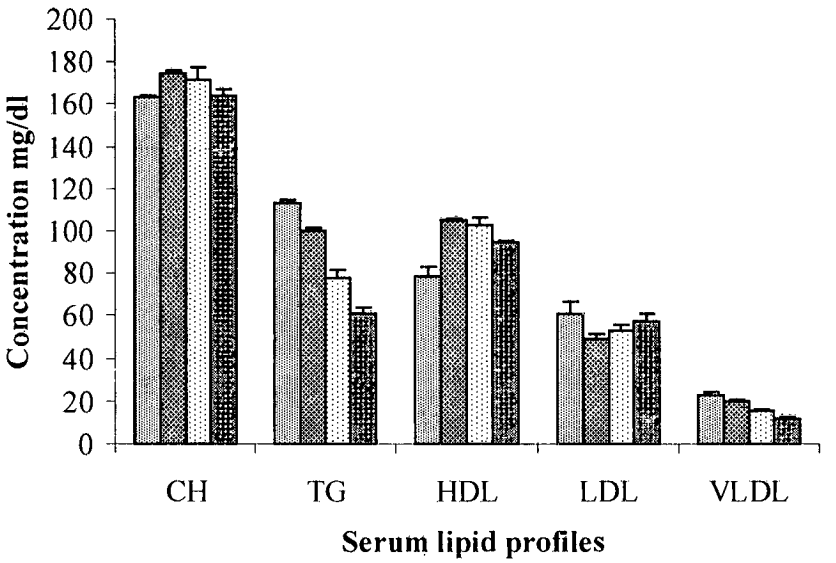


Figure G2: Changes in the serum lipid profiles of broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil for 15 and 30 days along with the commercial feed during post hatching development

a: 15 days feeding



b : 30 days feeding

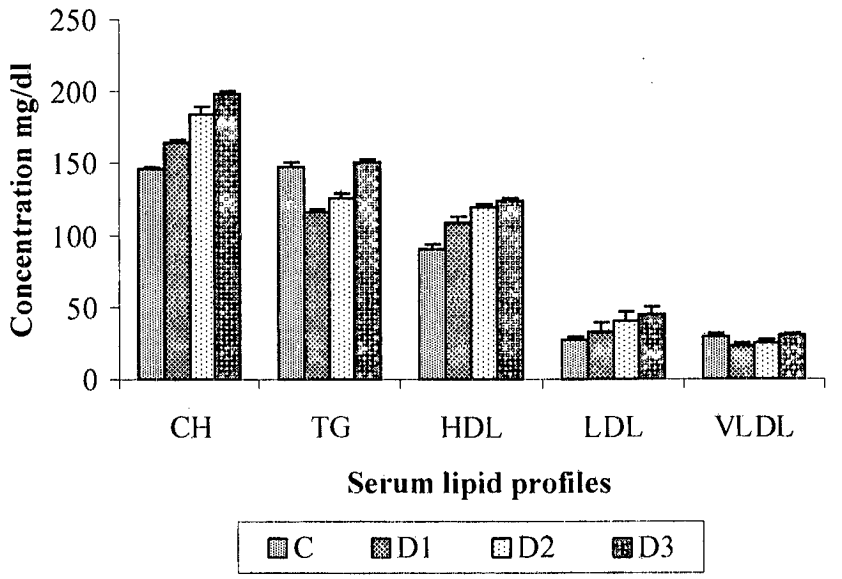
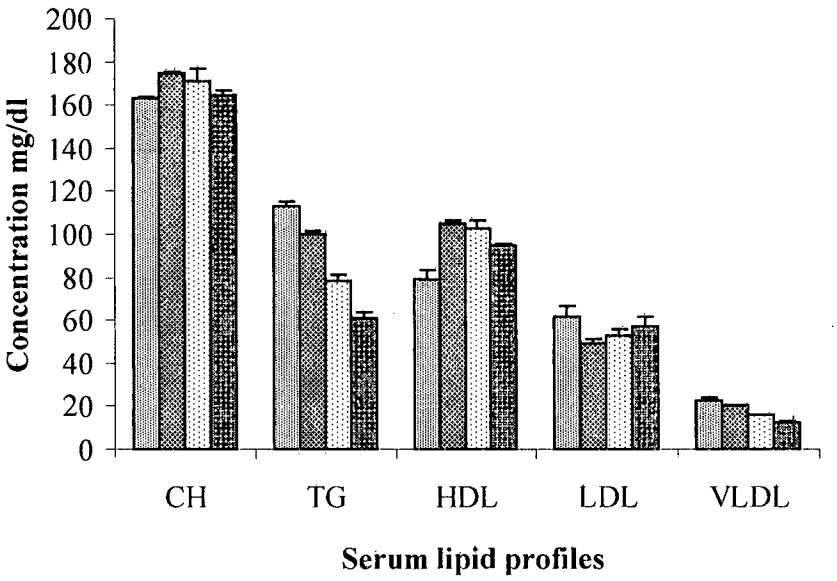


Figure G3: Changes in the serum lipid profiles of broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil for 15 and 30 days along with the commercial feed during post hatching development

a: 15 days feeding



b : 30 days feeding

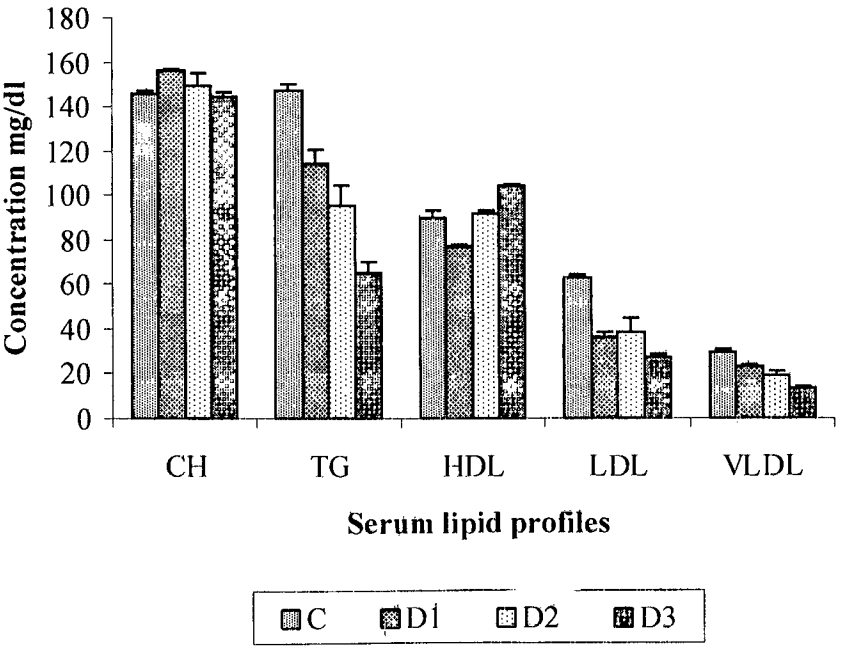
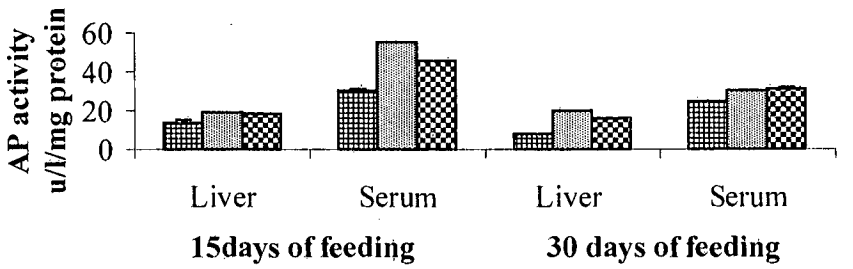


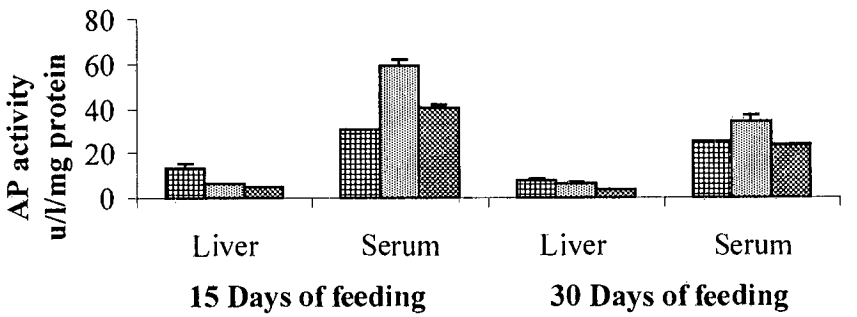
Figure H: Changes in the Alkaline Phosphatase (AP) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of different oils along with the commercial feed during post hatching development

(Liver --- μ mole/ mg /mg of protein; Serum --- μ mole / ml / mg of protein)

1: Coconut oil



2: Sunflower oil



3: Fish oil

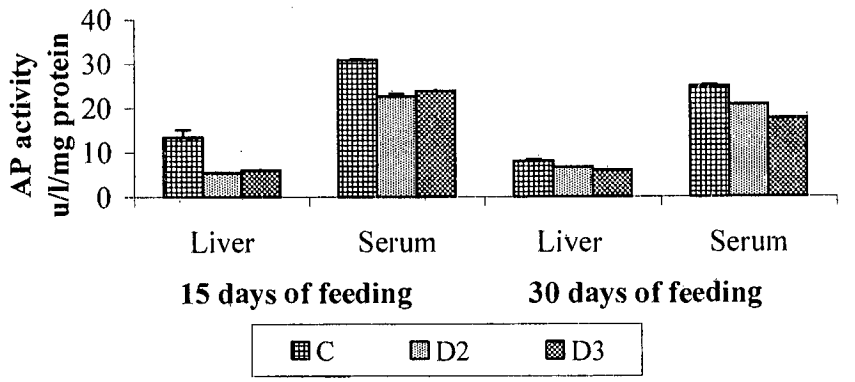
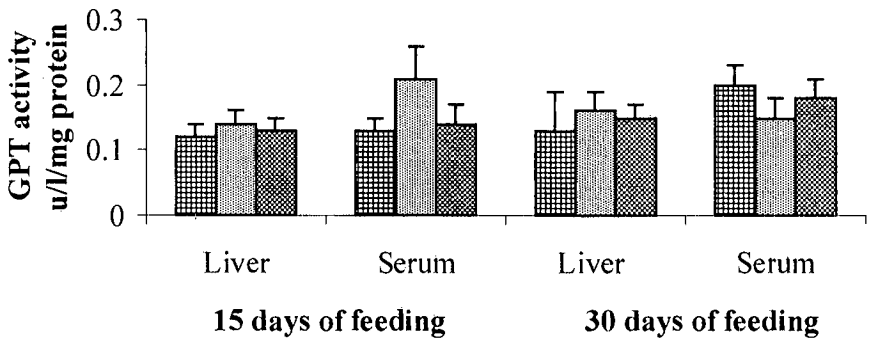


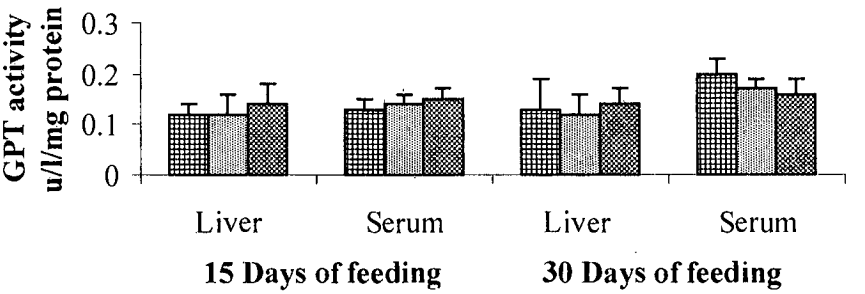
Figure I : Changes in the Glutamate Pyruvate Transaminase activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of different oilsalong with the commercial feed during post hatching development

(Liver ---µmole/ml / mg of protein; Serum ----µ mole/ ml /mg of protein)

1: Coconut oil



2: Sunflower oil



3: Fish oil

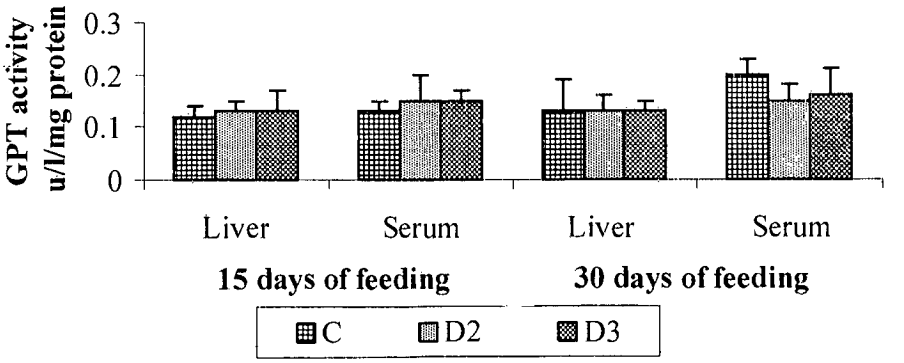
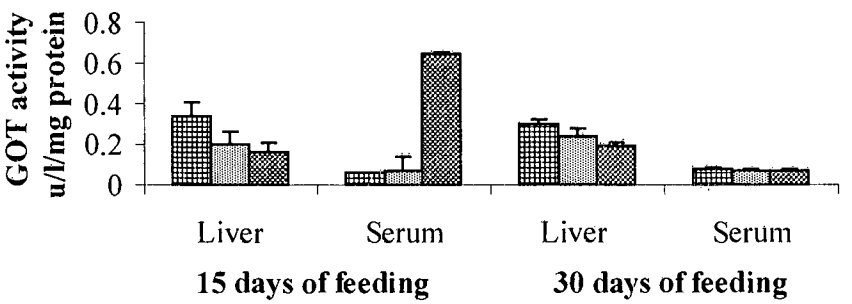


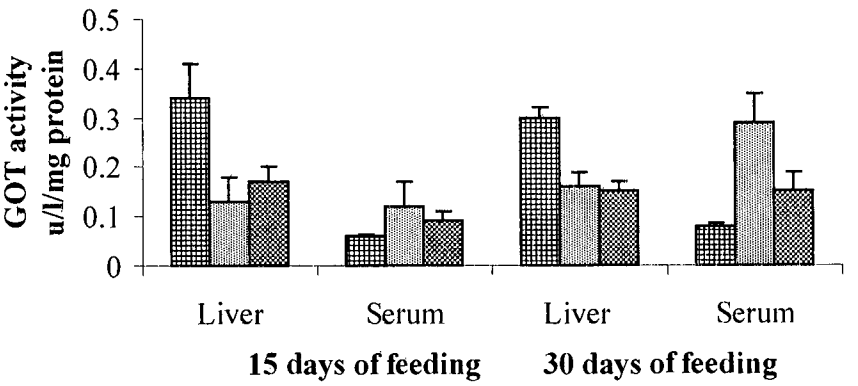
Figure J : Changes in the Glutamate Oxaloacetate Transaminase activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of different oils during post hatching development

Liver ---µmole/ml / mg of protein
Serum ----µ mole/ ml /mg of protein

1: Coconut oil



2: Sunflower oil



3: Fish oil

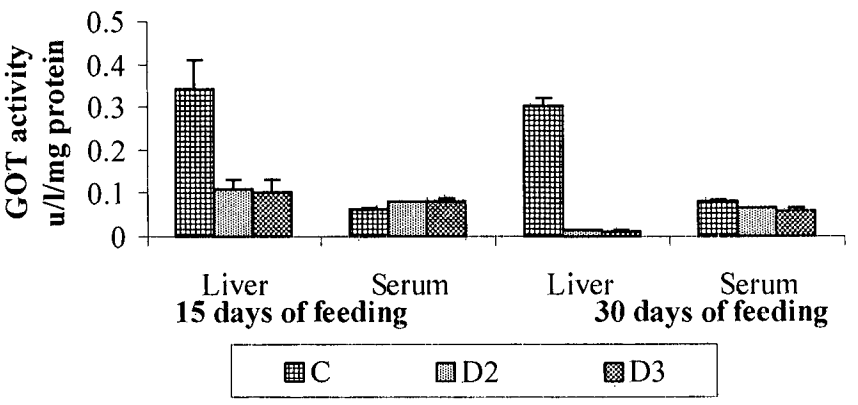
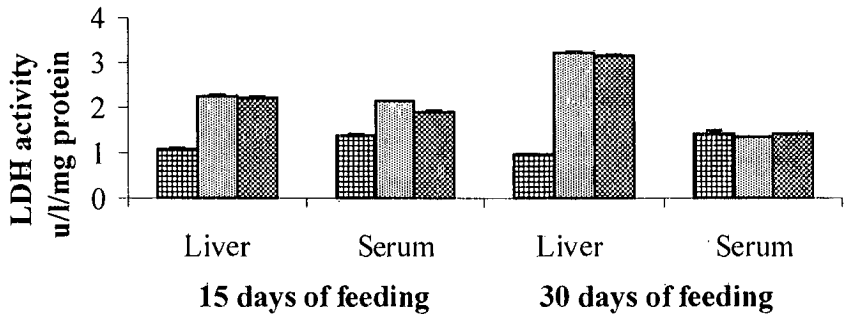


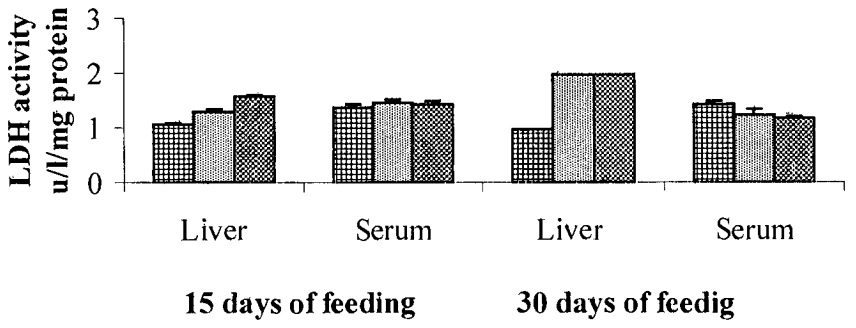
Figure K: Changes in the Lactate Dehydrogenase activity of the broiler chick (*Gallus domesticus*) supplemented with various doses of different oils during post hatching development

(Liver ---µmole/ml / mg of protein; Serum µ mole/ ml /mg of protein)

1: Coconut oil



2: Sunflower oil



3: Fish oil

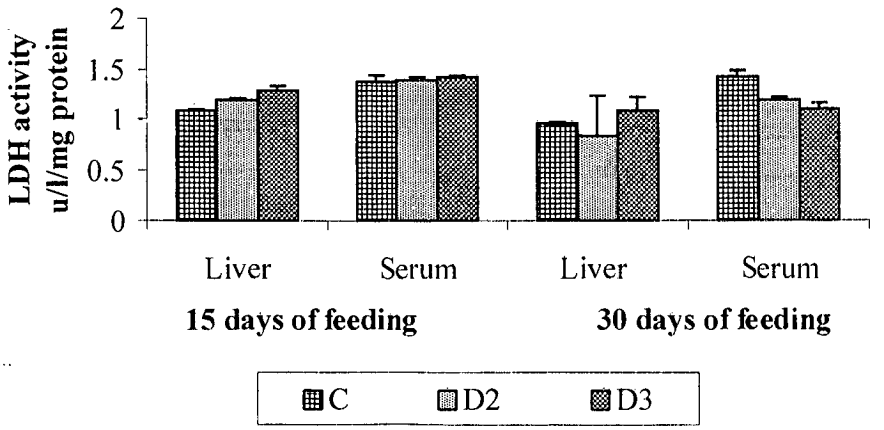
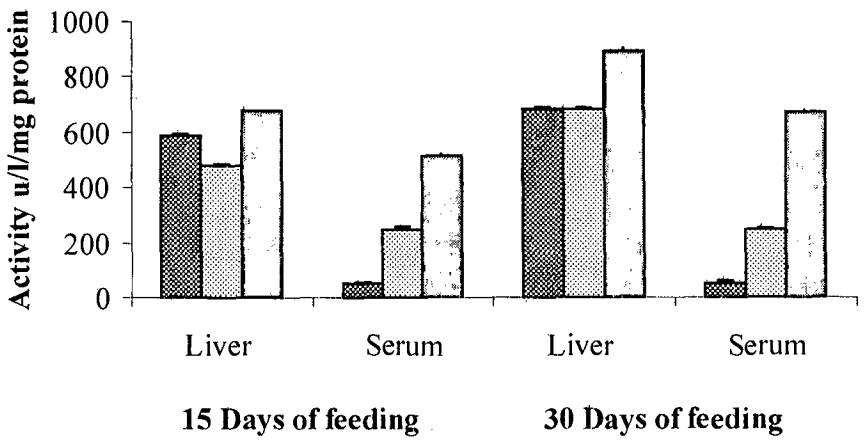


Figure L1 : Activity of regulatory enzymes of Cholesterol metabolism in broiler chick, (*Gallus domesticus*) supplemented with Coconut oil along with the commercial feed during post hatching development.

a: 3-HMG-CoA- Reductase



b: 3-HMG-CoA- Synthase

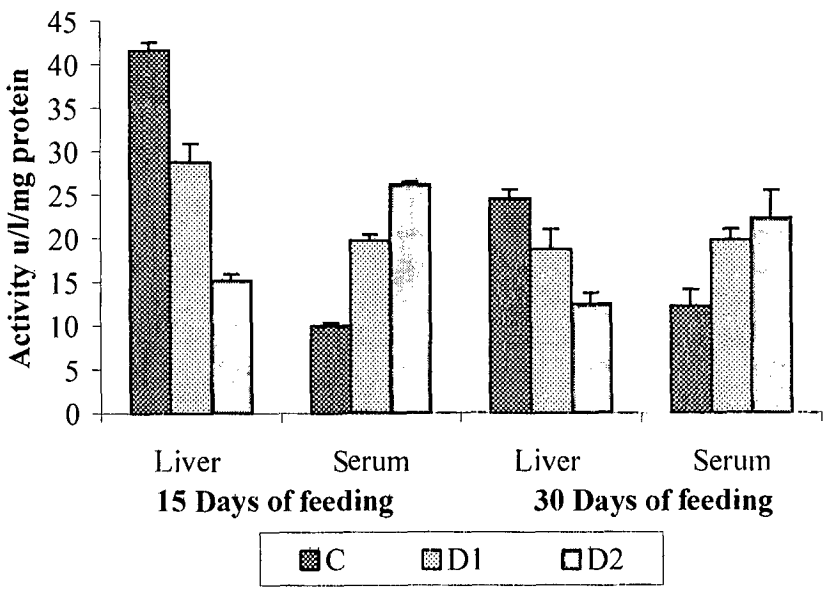
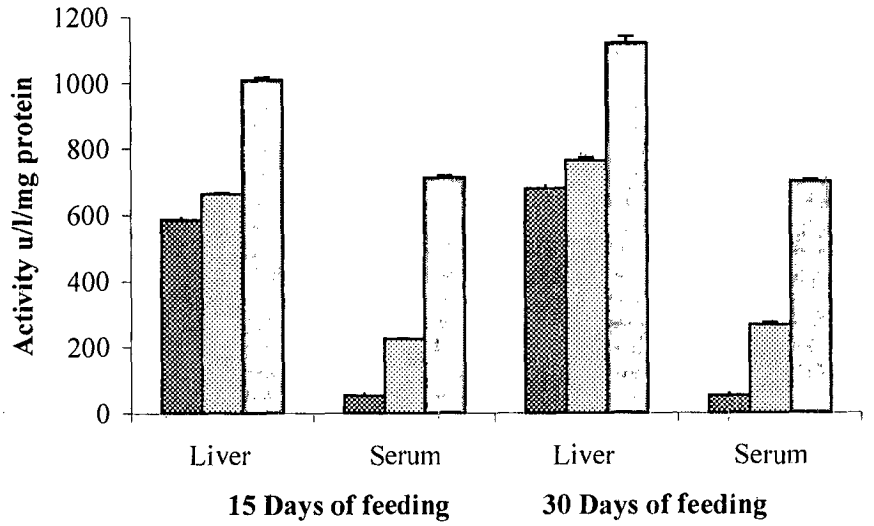


Figure L2 : Activity of regulatory enzymes of Cholesterol metabolism in broiler chick, *Gallus domesticus* supplemented with Sunflower oil along with the commercial feed during post hatching development

a: 3-HMG-CoA- Reductase



b: 3-HMG-CoA- Synthase

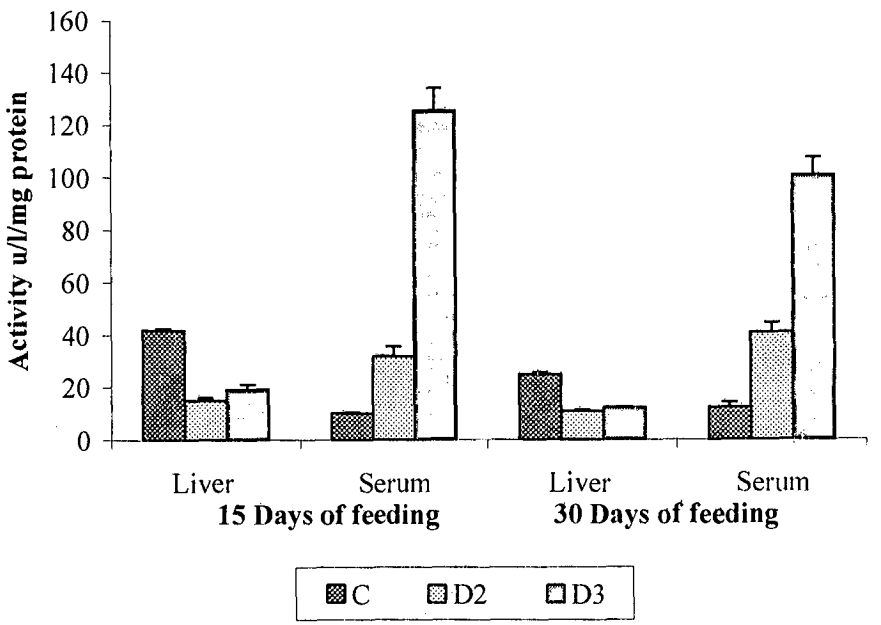
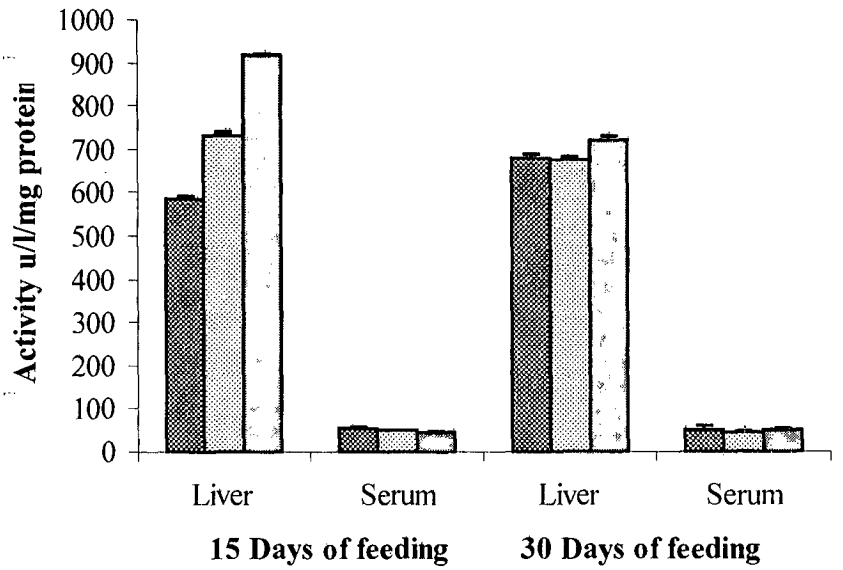


Figure L3 : Activity of regulatory enzymes of Cholesterol metabolism in broiler chick (*Gallus domesticus*)supplemented with Fish oil along with the commercial feed during post hatching development

a: 3-HMG-CoA- Reductase



b: 3-HMG-CoA- Synthase

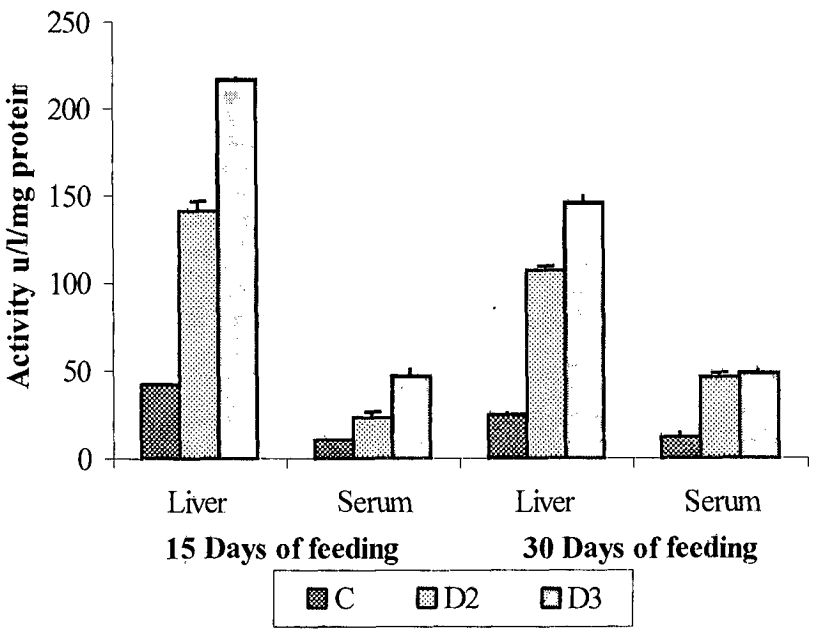


Table 9a: Relative composition of fatty acid profiles of different lipid sources used in the experiment
(Mean values of three estimates)

Fatty acid	Relative % composition					
	Coconut oil	Sunflower oil	Fish oil	B1	B2	B3
14:0	14.78	2.51	7.23	0.58	6.85	8.06
16:0	28.32	15.23	16.55	7.03	7.20	13.51
16:1	16.27	2.61	6.32	0.61	1.27	1.06
18:0	18.37	8.26	17.38	22.63	10.98	2.26
18:1	10.27	6.26	14.25	9.54	20.74	23.04
18:2	8.35	58.38	9.07	12.35	10.07	6.09
18:3	---	1.08	2.56	21.95	15.54	12.99
Others	3.64$	5.67$	26.64*	25.31#	27.35#	32.99#

* - n3 and n6 polyunsaturated fatty acids of C-20 and C-22 series
# - Short chained fatty acids of C-10 and C-12 series
$ - Unidentified fatty acids of C-16 and C-14 series

B1 - Staphylococcus *sp*
B2 - Streptococcus *sp*
B3 - Pseudomonas *sp*

Table 9b: Proximate composition of feeds used in the experiment
(Mean values of three estimates)

Parameters →	Dry matter	Crude fat	Ash	Fiber	% crude protein
Control feed (commercial)	92.09	6.50	11.48	2.83	36.28
Feed + 5% coconut oil	92.91	9.50	9.54	3.18	35.70
Feed + 5% sunflower oil	93.25	10.60	10.48	2.54	36.53
Feed + 5% fish oil	93.70	10.50	10.13	2.68	35.75
Feed + bacteria	93.50	9.25	9.15	2.76	45.63

Table 9c: Relative composition of fatty acid profiles of feed supplemented with different lipid sources used in the experiment
(Mean values of three estimates)

Fatty acid	Relative % composition						
	Control feed (commercial)	Feed + 5% Coconut oil	Feed + 5% Sunflower oil	Feed + 5% Fish oil	Feed + B1	Feed + B2	Feed + B3
14:0	7.50	10.87	5.00	7.26	4.22	7.45	7.75
16:0	16.00	22.36	15.50	15.26	11.50	11.62	15.26
16:1	2.30	14.37	2.20	4.32	1.45	1.75	1.65
18:0	8.30	12.32	8.00	6.36	15.30	19.26	5.67
18:1	2.50	8.37	4.74	10.26	6.23	11.25	13.28
18:2	55.50	35.37	56.78	32.36	38.25	32.64	30.14
18:3	0.50	---	0.98	1.86	11.00	7.85	7.50
Others	7.40$	5.24	6.80	22.32*	12.05#	8.18#	18.75#

* - n3 and n6 polyunsaturated fatty acids of C-20 and C-22 series
# - Short chained fatty acids of C-10 and C-12 series
$ - Unidentified fatty acids of C-16 and C-14 series

B1 - Staphylococcus *sp*
B2 - Streptococcus *sp*
B3 - Pseudomonas *sp*

Table 10a: Growth chart of broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Parameters	I	C	D1	D2	D3
15 days	Average weight of the birds (gm)	70.50 ± 2.81	284.67 ± 25.88	339.00 ± 24.55	364.17 ± 15.78	416.17 ± 12.04
	Net weight gain (gm)	---	214.167	268.50	293.67	345.67
	Daily instantaneous growth rate (Gw)	---	0.093	0.105	0.109	0.118
	FCR	---	0.123	0.120	0.109	0.088
30 days	Average weight of the birds	70.50 ± 2.81	725.50 ±27.12	721.67 ± 39.47	752.50 ± 23.56	794.17 ± 21.09
	Net weight gain	---	655.00	651.67	682.00	723.67
	Daily instantaneous growth rate Gw	---	0.078	0.077	0.079	0.080
	FCR	---	0.079	0.087	0.075	0.063

Table 10ai: Statistical calculation (comparison test by using student 't' test) of Table 10a.

Comparing group	15 days			30 days		
	% change	't' value	*p* value	% change	't' alue	*p* value
C vs D1	19.09↑	1.86	Equivocal	0.53↓	0.08	NS
C vs D2	27.93↑	3.65	<0.005	3.72↑	0.75	NS
C vs D3	46.19↑	6.55	<0.005	9.42↑	1.99	Equivocal
D1 vs D2	7.42↑	0.29	NS	4.27↑	0.59	NS
D2 vs D3	14.28↑	1.83	equivocal	5.53↑	1.32	NS

Table 10b: Growth chart of broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Parameters	I	C	D1	D2	D3
15 days	Average weight of the birds (gm)	70.50 ± 2.81	284.67 ± 15.88	344.50 ±18.09	368.00 ±6.74	492.00 ±2.57
	Net weight gain (gm)	---	214.167	274.00	297.50	421.50
	Daily instantaneous growth rate Gw	---	0.093	0.106	0.110	0.130
	FCR	---	0.123	0.130	0.135	0.109
30 days	Average weight of the birds (gm)	70.50 ± 2.81	725.50 ±27.12	736.00 ±51.80	762.67 ±16.33	798.00 ± 5.86
	Net weight gain (gm)	---	655.00	665.50	692.17	727.50
	Daily instantaneous growth rate Gw	---	0.078	0.078	0.080	0.081
	FCR	---	0.079	0.093	0.090	0.090

Table 10bi: Statistical calculation (comparison test by using student 't' test) of Table 10b.

Comparing group	15 days			30 days		
	% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	21.02↑	2.49	<0.025	1.45↑	0.18	NS
C vs D2	29.27↑	4.83	<0.005	5.12↑	1.17	NS
C vs D3	72.83↑	12.88	<0.005	9.99↑	2.61	<0.025
D1 vs D2	6.82↑	1.21	NS	3.62↑	0.49	NS
D2 vs D3	23.69↑	17.19	<0.005	4.32↑	2.04	<0.05

Table 10c: Growth chart of broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development
(Mean values of six samples and their standard error)

Feeding period	Parameters	I	C	D1	D2	D3
15 days	Average weight of the birds (gm)	70.50 ± 2.81	284.67 ± 15.88	356.33 ± 15.90	404.17 ±12.74	524.00 14.20
	Net weight gain (gm)	---	214.167	285.83	333.67	453.50
	Daily instantaneous growth rate Gw	---	0.093	0.108	0.116	0.134
	FCR	---	0.123	0.115	0.111	0.090
30 days	Average weight of the birds (gm)	70.50 ± 2.81	725.50 ±27.12	786.50 ± 13.01	825.17 ± 14.54	878.50 ±15.27
	Net weight gain (gm)	---	655.00	716.00	754.67	808.00
	Daily instantaneous growth rate Gw	---	0.078	0.080	0.082	0.084
	FCR	---	0.116	0.106	0.087	0.076

Table 10ci: Statistical calculation (comparison test by using student 't' test) of Table 10c.

Comparing group	15 days			30 days		
	% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	25.17↑	3.18	<0.005	8.40↑	2.02	<0.05
C vs D2	41.97↑	5.87	<0.005	13.73↑	3.23	<0.005
C vs D3	84.07↑	11.23	<0.005	21.08↑	4.91	<0.005
D1 vs D2	13.42↑	2.34	<0.025	4.91↑	1.98	<0.05
D2 vs D3	29.64↑	6.28	<0.005	6.46↑	2.52	<0.025

Table 11a : Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil during along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error.)

Feeding period	Parameters	C	D1	D2	D3
15 days	Hemoglobin Gm /dl	10.19 ± 0.39	11.76 ± 0.50	11.37 ± 0.23	10.75 ± 0.22
	Total erythrocytes x 10^6 /cubic mm	3.30 ±0.62	1.94 ± 0.23	1.73 ± 0.25	1.21 ± 0.10
	Total Leucocytes x 10^5/cubic mm	1.59 ± 0.18	0.87 ± 0.03	0.88 ± 0.11	0.83 ± 0.10
30 days	Hemoglobin Gm /dl	10.65 ± 0.68	10.03 ± 0.21	10.47 ± 0.32	10.49 ± 0.39
	Total erythrocytes x 10^6 /cubic mm	3.75 ± 0.48	1.76 ± 0.10	1.39 ± 0.03	1.18 ± 0.01
	Total Leucocytes x 10^5/cubic mm	0.96 ± 0.07	0.56 ± 0.04	0.57 ± 0.03	0.50 ± 0.02

Table 11ai: Statistical calculation (comparison test by using student 't' test) of Table 11a.

Comparing group	Parameters	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Hemoglobin gm /dl	15.40↓	2.48	<0.025	5.82 ↓	0.87	NS
C vs D2		11.58↓	2.60	<0.025	1.69↓	0.24	NS
C vs D3		5.49↓	1.25	NS	1.50↓	0.20	NS
D1 vs D2		3.32 ↑	0.70	NS	4.39↑	1.16	NS
D2 vs D3		3.45↓	1.95	<0.05	0.19↑	0.04	NS
C vs D1	Total erythrocytes $x\ 10^6$ /cubic mm	41.21↓	2.06	<0.05	53.07↓	4.06	<0.005
C vs D2		47.58↓	2.35	<0.025	62.93↓	4.92	<0.005
C vs D3		66.33↓	3.33	<0.005	68.53↓	5.35	<0.005
D1 vs D2		10.82↓	0.62	NS	21.02↓	12.66	<0.005
D2 vs D3		30.06↓	1.92	<0.05	15.10↓	7.00	<0.005
C vs D1	Total Leucocytes *x* 10^5/cubic mm	45.28↓	3.95	<0.005	41.67↓	4.96	<0.005
C vs D2		44.65↓	3.37	<0.005	40.63↓	5.12	<0.005
C vs D3		47.79↓	3.69	<0.005	47.92↓	6.32	<0.005
D1 vs D2		1.15↑	0.09	NS	1.78↑	0.20	NS
D2 vs D3		5.68↓	0.34	NS	12.28↓	1.94	<0.05

Table 11b: Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error.)

Feeding period	Parameters	C	D1	D2	D3
15 days	Hemoglobin Gm /dl	10.19 ± 0.39	10.93 ± 0.39	10.63 ± 0.29	10.67 ± 0.62
	Total erythrocytes x 10^6 /cubic mm	3.30 ±0.62	1.19 ± 0.03	1.44 ± 0.33	2.35 ± 0.32
	Total Leucocytes x 10^5/cubic mm	1.59 ± 0.18	0.83 ± 0.03	0.94 ± 0.03	0.98 ± 0.12
30 days	Hemoglobin Gm /dl	10.65 ± 0.68	10.90 ± 0.32	11.54 ± 0.18	11.04 ± 0.44
	Total erythrocytes x 10^6 /cubic mm	3.75 ± 0.48	1.04 ± 0.19	1.09 ± 0.07	1.25 ± 0.05
	Total Leucocytes x 10^5/cubic mm	0.96 ± 0.07	0.78 ± 0.19	1.08 ± 0.18	0.98 ± 0.009

Table 11bi: Statistical calculation (comparison test by using student 't' test) of Table 11b.

Comparing group	Parameters	15 days			30 days		
		% change	calculated 't' value	*p* value	% change	calculated 't' Value	*p* value
C vs D1	Hemoglobin gm /dl	7.26↑	1.35	NS	2.35↑	0.33	NS
C vs D2		4.32↑	0.89	NS	8.36↑	1.27	NS
C vs D3		4.71↑	0.66	NS	3.66↑	0.49	NS
D1 vs D2		2.74↓	0.61	NS	5.87↑	1.74	NS
D2 vs D3		0.38↑	0.06	NS	4.33↓	1.04	NS
C vs D1	Total erythrocytes $x\ 10^6$ /cubic mm	63.94↓	3.39	<0.005	72.27↓	5.25	<0.005
C vs D2		56.36↓	2.99	<0.01	70.93↓	5.48	<0.005
C vs D3		28.79↓	1.36	NS	66.67↓	5.18	<0.005
D1 vs D2		21.00↑	5.89	<0.005	4.80↓	0.25	NS
D2 vs D3		63.19↑	2.83	<0.01	14.68↓	1.86	<0.05
C vs D1	Total Leucocytes *x* 10^5/cubic mm	47.79↓	4.16	<0.005	18.75↓	0.89	NS
C vs D2		40.88↓	3.56	<0.005	12.50↑	0.62	NS
C vs D3		38.36↓	2.82	<0.01	2.08↑	0.18	NS
D1 vs D2		13.25↑	2.59	<0.025	38.46↑	1.15	NS
D2 vs D3		18.07↓	0.32	NS	25.64↑	0.49	NS

Table 11c : Changes in the hematological parameters of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error.)

Feeding period	Parameters	C	D1	D2	D3
15 days	Hemoglobin Gm /dl	10.19 ± 0.39	9.97 ± 0.39	10.93 ± 0.23	12.49 ± 0.11
	Total erythrocytes $x\ 10^6$ /cubic mm	3.30 ±0.62	3.67 ± 0.25	3.59 ± 0.31	3.73 ± 0.16
	Total Leucocytes $x\ 10^5$/cubic mm	1.59 ± 0.18	1.37 ± 0.04	1.36 ± 0.05	1.36 ± 0.09
30 days	Hemoglobin Gm /dl	10.65 ± 0.68	10.04 ± 0.51	12.29 ± 0.28	11.19 ± 0.55
	Total erythrocytes $x\ 10^6$ /cubic mm	3.75 ± 0.48	3.67 ± 0.42	3.53 ± 0.21	3.64 ± 0.21
	Total Leucocytes $x\ 10^5$/cubic mm	0.96 ± 0.07	1.17 ± 0.02	1.21 ± 0.02	1.20 ± 0.03

Table 11ci: Statistical calculation (comparison test by using student 't' test) of Table 11c.

Comparing group	Parameters	15 days			30 days		
		% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	**Hemoglobin gm /dl**	2.16↓	0.39	NS	5.73↓	0.72	NS
C vs D2		7.26↑	1.63	Equivocal	5.07↑	0.62	NS
C vs D3		22.57↑	5.68	<0.005	15.39↑	2.25	<0.025
D1 vs D2		9.63↑	2.12	<0.05	11.45↑	1.53	Equivocal
D2 vs D3		14.27↑	6.12	<0.005	9.83↑	1.77	Equivocal
C vs D1	**Total erythrocytes $x\ 10^6$/cubic mm**	11.21↑	0.55	NS	2.13↓	0.12	NS
C vs D2		8.79↑	0.42	NS	5.87↓	0.42	NS
C vs D3		13.03↑	0.67	NS	2.93↓	0.21	NS
D1 vs D2		2.18↓	0.20	NS	3.81↑	0.29	NS
D2 vs D3		3.89↑	0.40	NS	3.12↓	0.37	NS
C vs D1	**Total Leucocytes $x\ 10^5$/cubic mm**	13.84↓	1.19	NS	21.88↑	2.88	<0.01
C vs D2		14.46↓	1.23	NS	26.04↑	3.43	<0.005
C vs D3		14.46↓	1.14	NS	25.00↑	3.15	<0.01
D1vs D2		0.73↑	0.16	NS	3.42↑	1.41	Equivocal
D 2 vs D3		No change	---	---	2.56↑	0.28	NS

Table 12a: Changes in the total Protein (mg/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	5.38 ± 0.05	5.78 ± 0.37	5.40 ± 0.26	5.43 ± 0.29	5.87 ± 0.13	F = 0.79 $p = 0.51$
	Muscle	2.56 ± 0.17	5.85 ± 0.31	5.83 ± 0.19	6.67 ± 0.08	7.69 ± 0.14	F = 8.68 $p < 0.001$
	Intestine	5.07 ± 0.48	5.66 ± 0.20	5.72 ± 0.04	6.70 ± 0.10	7.30 ± 0.53	F = 5.91 $p = 0.005$
30 days	Liver	5.38 ± 0.05	5.83 ± 0.38	5.50 ± 0.29	5.50 ± 0.19	7.24 ± 0.23	F = 12.12 $p < 0.001$
	Muscle	2.56 ± 0.17	6.56 ± 0.12	6.35 ± 0.20	7.20 ± 0.40	8.47 ± 0.27	F = 12.38 $p < 0.001$
	Intestine	5.07 ± 0.48	6.52 ± 0.13	6.75 ± 0.06	7.71 ± 0.19	8.53 ± 0.16	F = 15.59 $p < 0.001$

Table12ai: Statistical calculation (comparison test by using student 't' test) of Table 12a.

Comparing group	Tissues	15 days			30 days		
		% change	calculated 't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	6.57 ↓	0.84	NS	5.66↓	0.69	NS
C vs D2		6.06 ↓	0.75	NS	5.66 ↓	0.77	NS
C vs D3		1.56 ↑	0.23	NS	24.18 ↑	3.17	< 0.005
D1 vs D2		0.56 ↑	0.08	NS	no change	---	---
D2 vs D3		8.10 ↑	1.38	Equivocal	31.64 ↑	5.83	< 0.005
C vs D1	Muscle	0.34 ↓	0.06	NS	3.20 ↓	0.90	NS
C vs D2		14.02 ↑	2.56	< 0.025	9.76 ↑	1.53	Equivocal
C vs D3		31.45 ↑	5.40	< 0.005	29.12 ↑	6.46	< 0.005
D1 vs D2		1.14 ↑	4.08	< 0.005	13.39 ↑	1.90	< 0.05
D2 vs D3		15.29 ↑	6.33	< 0.005	17.64 ↑	2.63	< 0.025
C vs D1	Intestine	1.06 ↑	0.29	NS	3.53 ↑	1.60	Equivocal
C vs D2		18.37 ↑	4.65	< 0.005	18.25 ↑	5.17	< 0.005
C vs D3		28.97 ↑	2.89	< 0.01	30.83 ↑	9.75	< 0.005
D1 vs D2		17.13 ↑	9.09	< 0.005	14.22 ↑	4.82	< 0.005
D2 vs D3		8.96 ↑	1.11	NS	10.64 ↑	3.30	< 0.005

Table 12b: Changes in the tissue total Protein (mg/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	5.38 ± 0.05	5.78 ± 0.37	6.10 ± 0.23	6.42 ± 0.25	7.46 ± 0.26	F = 11.77 $p < 0.001$
	Muscle	2.56 ± 0.17	5.85 ± 0.31	6.75 ± 0.08	7.53 ± 0.08	8.90 ± 0.10	F = 55.28 $p < 0.001$
	Intestine	5.07 ± 0.48	5.66 ± 0.20	5.81 ± 0.07	5.92 ± 0.02	6.30 ± 0.19	F = 2.35 $p = 0.103$
30 days	Liver	5.38 ± 0.05	5.83 ± 0.38	7.38 ± 0.06	8.58 ± 0.09	10.28 ± 0.47	F = 49.72 $p < 0.001$
	Muscle	2.56 ± 0.17	6.56 ± 0.12	8.26 ± 0.30	9.35 ± 0.07	13.25 ± 0.30	F = 157.79 $p < 0.001$
	Intestine	5.07 ± 0.48	6.52 ± 0.13	7.15 ± 0.29	8.10 ± 0.36	9.18 ± 0.19	F = 17.33 $p < 0.001$

Table12bi: Statistical calculation (comparison test by using student 't' test) of Table 12b.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	Liver	5.54 ↑	0.74	NS	26.58 ↑	4.03	<0.005
Cl vs D2		11.07 ↑	1.71	Equivocal	47.16 ↑	7.04	<0.005
Cl vs D3		29.06 ↑	3.72	<0.005	76.33 ↑	7.36	<0.005
D1 vs D2		5.24 ↑	1.36	NS	16.26 ↑	11.09	<0.005
D2 vs D3		16.19 ↑	3.93	<0.005	19.81 ↑	3.55	<0.005
C vs D1	Muscle	15.38↑	2.81	<0.01	25.91↑	5.26	<0.005
C vs D2		28.72 ↑	5.25	<0.005	42.53↑	20.08	<0.005
Cl vs D3		52.14↑	9.36	<0.005	101.98↑	20.70	<0.005
D1 vs D2		11.55↑	6.89	<0.005	13.19↑	3.54	<0.005
D2 vs D3		18.19 ↑	10.69	<0.005	41.71↑	12.66	<0.005
C vs D1	Intestine	2.65↑	0.71	NS	9.66 ↑	1.97	<0.05
C vs D2		4.59 ↑	1.30	NS	24.23 ↑	4.30	<0.005
C vs D3		11.30 ↑	1.81	Equivocal	40.79↑	8.36	<0.005
D1 vs D2		1.89 ↑	1.57	Equivocal	13.28↑	2.06	<0.05
D2 vs D3		6.42 ↑	4.75	<0.005	13.33↑	2.34	<0.025

Table 12c: Changes in the tissue total Protein (mg/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	5.38 ± 0.05	5.78 ± 0.37	8.03 ± 0.28	8.84 ± 0.05	9.85 ± 0.04	F = 53.45 *p < 0.001*
	Muscle	2.56 ± 0.17	5.85 ± 0.31	7.59 ± 0.05	8.53 ± 0.07	9.14 ± 0.05	F = 76.717 *p <0.001*
	Intestine	5.07 ± 0.48	5.66 ± 0.20	6.84 ± 0.27	7.83 ± 0.38	8.36 ± 0.05	F = 21.685 *p <0.001*
30 days	Liver	5.38 ± 0.05	5.83 ± 0.38	8.53 ± 0.27	9.37 ± 0.05	10.62 ± 0.13	F = 164.74 *p < 0.001*
	Muscle	2.56 ± 0.17	6.56 ± 0.12	8.09 ± 0.07	9.13 ± 0.22	9.99 ± 0.04	F = 54.820 *p <0.001*
	Intestine	5.07 ± 0.48	6.52 ± 0.13	7.24 ± 0.02	8.33 ± 0.11	9.16 ± 0.06	F = 29.885 *p <0.001*

Table12ci: Statistical calculation (comparison test by using student 't' test) of Table 12c.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	38.92↑	4.85	<0.005	46.31↑	5.79	<0.005
C vs D2		52.94↑	8.19	<0.005	60.72↑	9.24	<0.005
C vs D3		70.41↑	10.94	<0.005	82.16↑	11.93	<0.005
D1 vs D2		52.94↑	2.85	<0.01	9.84↑	3.06	<0.005
D2 vs D3		11.42↑	15.77	<0.005	13.34↑	8.97	<0.005
C vs D1	Muscle	29.74↑	5.54	<0.005	23.32↑	11.01	<0.005
C vs D2		45.81↑	8.43	<0.005	39.17↑	10.26	<0.005
C vs D3		56.23↑	10.48	<0.005	52.28↑	27.12	<0.005
D1 vs D2		12.38↑	10.93	<0.005	12.85↑	4.50	<0.005
D2 vs D3		7.15↑	7.09	<0.005	9.41↑	3.85	<0.005
C vs D1	Intestine	20.84↑	3.51	<0.005	11.04↑	5.47	<0.005
C vs D2		38.33↑	5.05	<0.005	27.76↑	10.63	<0.005
C vs D3		47.70↑	13.09	<0.005	40.49↑	18.44	<0.005
D1 vs D2		14.47↑	2.12	<0.05	15.05↑	9.75	<0.005
D2 vs D3		6.76↑	1.38	Equivocal	9.96↑	6.62	<0.005

Table 13a : Changes in the tissue total Triglycerol (μ mole/100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil during post hatching development
(Mean value of six samples and their standard error)

Feeding period	Tissues	I	C	D1	D2	D3	ANOVA
15 days	Liver	4.70 ± 0.24	10.96 ± 0.44	9.96 ± 0.45	11.55 ± 0.52	15.70 ± 1.21	F = 8.94 $p < 0.001$
	Muscle	2.98 ± 0.25	4.20 ± 0.21	4.60 ± 0.27	7.42 ± 0.29	10.14 ± 0.45	F = 76.17 $p < 0.001$
	Intestine	2.78 ± 0.52	5.22 ± 0.28	6.59 ± 0.29	8.88 ± 0.35	9.19 ± 0.56	F = 18.15 $p < 0.001$
30 days	Liver	4.70 ± 0.24	14.79 ± 0.26	14.91 ± 0.35	15.70 ± 0.47	23.82 ± 0.62	F = 64.67 $p < 0.001$
	Muscle	2.98 ± 0.25	5.64 ± 0.35	7.29 ± 0.36	8.92 ± 0.34	16.82 ± 0.42	F = 183.69 $p < 0.001$
	Intestine	2.78 ± 0.52	5.34 ± 0.32	8.91 ± 0.36	11.78 ± 0.39	13.72 ± 0.38	F = 101.13 $p < 0.001$

Table13ai: Statistical calculation (comparison test by using student 't' test) of Table 13a.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	9.12↓	1.59	Equivocal	0.81↑	0.28	NS
C vs D2		5.38↑	0.87	NS	6.15	1.69	Equivocal
C vs D3		43.25↑	3.68	<0.005	61.05	13.43	<0.005
D1 vs D2		15.96↑	2.31	<0.025	5.29↑	1.35	NS
D2 vs D3		34.80↑	3.15	<0.01	51.72↑	10.44	<0.005
C vs D1	Muscle	9.52↑	1.16	NS	29.25↑	3.29	<0.005
C vs D2		76.67↑	8.99	<0.005	58.16↑	6.72	<0.005
C vs D3		2.41 fold↑	11.96	<0.005	2.98fold↑	20.45	<0.005
D1 vs D2		61.30↑	17.57	<0.005	22.36↑	3.29	<0.005
D2 vs D3		36.66↑	5.08	<0.005	88.57↑	14.62	<0.005
C vs D1	Intestine	26.25↑	3.39	<0.005	66.85↑	7.41	<0.005
C vs D2		70.11↑	8.17	<0.005	120.59↑	12.77	<0.005
C vs D3		76.05↑	6.34	<0.005	2.56fold↑	16.87	<0.005
D1 vs D2		34.75↑	5.04	<0.005	32.21↑	5.40	<0.005
D2 vs D3		3.49↑	0.47	NS	16.47↑	3.56	<0.005

Table 13b: Changes in the tissue total Triglycerol (μ mole/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	4.70 ± 0.24	10.96 ± 0.44	14.18 ± 0.43	16.99 ± 0.42	17.59 ± 0.05	F = 15.87 $p < 0.001$
	Muscle	2.98 ± 0.25	4.20 ± 0.21	5.69 ± 0.09	6.62 ± 0.09	7.66 ± 0.03	F = 13.77 $p < 0.001$
	Intestine	2.78 ± 0.52	5.22 ± 0.28	5.14 ± 0.03	5.52 ± 0.16	5.50 ± 0.08	F =1.37 $p = 0.28$
30 days	Liver	4.70 ± 0.24	14.79 ± 0.26	14.95 ± 0.14	15.82 ± 0.13	18.24 ± 0.03	F = 16.79 $p < 0.001$
	Muscle	2.98 ± 0.25	5.64 ± 0.35	6.83 ± 0.12	7.24 ± 0.03	9.17 ± 0.04	F = 62.51 $p < 0.001$
	Intestine	2.78 ± 0.52	5.34 ± 0.32	9.62 ± 0.08	10.42 ± 0.26	11.09 ± 0.18	F = 130.58 $p < 0.001$

Table13bi: Statistical calculation (comparison test by using student 't' test) of Table 13b.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	Liver	29.38↑	5.19	<0.005	1.08↑	0.20	NS
C vs D2		55.02↑	9.14	<0.005	6.96↑	1.33	NS
C vs D3		60.49↑	15.07	<0.005	23.32↑	4.53	<0.005
D1 vs D2		19.81↑	4.68	<0.005	5.82↑	4.55	<0.005
D2 vs D3		3.53 ↑	1.42	Equivocal	22.00↑	18.19	<0.005
C vs D1	Muscle	35.47↑	6.53	< 0.005	21.09↑	2.00	<0.05
C vs D2		57.61↑	10.61	< 0.005	28.36↑	4.57	<0.005
C vs D3		82.38↑	16.32	< 0.005	62.58↑	10.08	<0.005
D1 vs D2		16.34↑	7.32	< 0.005	6.00↑	3.33	<0.005
D2 vs D3		15.70	11.55	< 0.005	26.65↑	38.60	<0.005
C vs D1	Intestine	1.53↓	0.28	NS	80.14↑	13.00	<0.005
C vs D2		5.74↑	0.93	NS	95.13↑	12.33	<0.005
C vs D3		5.36↑	0.96	NS	107.67↑	15.66	<0.005
D1 vs D2		7.39↑	2.34	<0.025	8.31↑	2.94	<0.01
D2 vs D3		0.36↓	0.11	NS	6.42↑	2.12	<0.05

Table 13c: Changes in the tissue total Triglycerol (μ mole/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	4.70 ± 0.24	10.96 ± 0.44	15.72 ± 0.22	25.32 ± 0.96	26.59 ± 0.59	F = 85.216 $p < 0.001$
	Muscle	2.98 ± 0.25	4.20 ± 0.21	14.36 ± 0.45	24.44 ± 0.49	25.13 ± 1.30	F = 177.76 $p < 0.001$
	Intestine	2.78 ± 0.52	5.22 ± 0.28	13.35 ± 0.20	23.30 ± 0.12	21.24 ± 0.30	F =1234.90 $p < 0.001$
30 days	Liver	4.70 ± 0.24	14.79 ± 0.26	25.36 ± 0.41	21.36 ± 0.60	18.40 ± 0.50	F = 54.642 $p < 0.001$
	Muscle	2.98 ± 0.25	5.64 ± 0.35	23.75 ± 0.55	19.39 ± 0.09	14.48 ± 0.64	F = 288.50 $p <0.001$
	Intestine	2.78 ± 0.52	5.34 ± 0.32	22.25 ± 0.38	19.36 ± 0.29	16.95 ± 0.20	F = 599.99 $p <0.001$

Table13ci: Statistical calculation (comparison test by using student 't' test) of Table 13c

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	Liver	43.43↑	2.87	<0.01	71.46↑	21.79	< 0.005
C vs D2		2.31fold↑	9.23	<0.005	44.42↑	10.10	<0.005
C vs D3		2.42fold↑	12.39	<0.005	24.40↑	6.45	<0.005
D1 vs D2		61.06↑	9.79	<0.005	15.77↓	5.48	<0.005
D2 vs D3		5.01↑	1.12	NS	13.86↓	3.79	<0.005
C vs D1	Muscle	3.41fold↑	20.73	<0.005	4.21fold↑	27.86	<0.005
C vs D2		5.81fold↑	38.19	<0.005	3.43fold↑	38.19	<0.005
C vs D3		5.98fold↑	12.09	<0.005	2.56fold↑	12.10	<0.005
D1 vs D2		70.19↑	15.04	<0.005	18.35↓	7.79	<0.005
D2 vs D3		2.82	0.49	NS	25.32↓	7.55	<0.005
C vs D1	Intestine	2.55fold↑	8.13	<0.005	4.16fold↑	34.51	<0.005
C vs D2		4.46fold↑	60.27	<0.005	3.62fold↑	32.60	<0.005
C vs D3		4.06fold↑	39.07	<0.005	3.17fold↑	30.55	<0.005
D1 vs D2		74.53↑	43.28	<0.005	12.98↓	6.02	<0.005
D2 vs D3		8.84↑	6.44	<0.005	12.44↓	6.89	<0.005

Table 14a: Changes in the tissue total Cholesterol (μ mole/100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil during post hatching development
(Mean value of six samples and their standard error)

Feeding period	Tissues	I	C	D1	D2	D3	ANOVA
15 days	Liver	2.88 ± 0.16	1.29 ± 0.14	1.23 ± 0.19	3.80 ± 0.39	5.08 ± 0.43	F = 40.32 $p < 0.001$
	Muscle	1.11 ± 0.09	0.71 ± 0.06	1.16 ± 0.03	1.62 ± 0.22	2.41 ± 0.03	F = 33.77 $p < 0.001$
	Intestine	1.03 ± 0.23	1.79 ± 0.22	1.97 ± 0.06	2.34 ± 0.14	2.53 ± 0.14	F = 4.98 $p = 0.01$
30 days	Liver	2.88 ± 0.16	1.25 ± 0.05	2.29 ± 0.08	5.25 ± 0.04	7.28 ± 0.07	F = 1898.30 $p < 0.001$
	Muscle	1.11 ± 0.09	0.55 ± 0.02	1.52 ± 0.14	3.30 ± 0.09	4.23 ± 0.23	F = 251.24 $p < 0.001$
	Intestine	1.03 ± 0.23	1.90 ± 0.19	1.98 ± 0.03	4.32 ± 0.08	4.74 ± 0.17	F = 98.09 $p < 0.001$

Table14ai: Statistical calculation (comparison test by using student 't' test) of Table 14a.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	4.65 ↓	0.25	NS	83.20 ↑	11.02	< 0.005
C vs D2		2.94fold↑	6.06	< 0.005	4.20fold↑	62.47	< 0.005
C vs D3		3.93fold↑	8.38	< 0.005	5.82fold ↑	70.09	< 0.005
D1 vs D2		3.08fold↑	5.92	< 0.005	2.29fold↑	33.09	< 0.005
D2 vs D3		33.68 ↑	2.20	<0.05	38.66 ↑	25.18	< 0.005
C vs D1	Muscle	63.38 ↑	6.70	< 0.005	2.76fold ↑	6.86	< 0.005
C vs D2		2.28fold↑	3.99	< 0.005	6.00↑	29.83	< 0.005
C vs D3		3.39fold↑	25.34	< 0.005	7.69fold↑	15.94	< 0.005
D1 vs D2		39.65 ↑	2.07	< 0.05	2.17fold↑	10.69	< 0.005
D2 vs D3		38.76 ↑	3.56	< 0.005	28.18↑	3.77	< 0.005
C vs D1	Intestine	10.05↑	0.79	NS	4.21↑	0.42	NS
C vs D2		30.72↑	2.10	< 0.05	2.27fold↑	11.74	< 0.005
C vs D3		41.34 ↑	2.84	< 0.01	2.49fold↑	11.14	< 0.005
D1 vs D2		18.78 ↑	2.43	< 0.025	2.18fold↑	27.39	< 0.005
D2 vs D3		8.11 ↓	0.96	NS	9.72↑	2.24	< 0.025

Table 14b: Changes in the tissue total Cholesterol (μ mole/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	2.88 ± 0.16	1.29 ± 0.14	2.58 ± 0.03	2.78 ± 0.04	3.15 ± 0.02	F = 72.75 $p < 0.001$
	Muscle	1.11 ± 0.09	0.71 ± 0.06	2.75 ± 0.04	2.81 ± 0.04	2.84 ± 0.05	F = 1.42 $p = 0.47$
	Intestine	1.03 ± 0.23	1.79 ± 0.22	4.73 ± 0.01	4.70 ± 0.02	4.72 ± 0.03	F =0.107 $p = 0.96$
30 days	Liver	2.88 ± 0.16	1.25 ± 0.05	3.23 ± 0.02	3.21 ± 0.02	3.26 ± 0.01	F = 54.642 $p < 0.001$
	Muscle	1.11 ± 0.09	0.55 ± 0.02	2.45 ± 0.02	2.39 ± 0.02	2.31 ± 0.03	F = 288.50 $p < 0.001$
	Intestine	1.03 ± 0.23	1.90 ± 0.19	4.49 ± 0.05	4.53 ± 0.04	4.66 ± 0.04	F = 599.99 $p < 0.001$

Table14bi: Statistical calculation (comparison test by using student 't' test) of Table 14b.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	2fold↑	4.96	<0.005	2.58fold↑	1.00	NS
C vs D2		2.15fold↑	3.51	<0.005	2.56fold↑	0.80	NS
C vs D3		2.44fold↑	0.99	NS	2.60fold↑	0.20	NS
D1 vs D2		7.75↑	4.00	<0.005	0.62↓	0.71	NS
D2 vs D3		13.30↑	8.40	<0.005	1.56↑	2.27	<0.025
C vs D1	Muscle	3.87fold↑	28.29	<0.005	4.45fold↑	3.57	<0.005
C vs D2		3.95fold↑	1.43	Equivocal	4.34fold↑	5.71	<0.005
C vs D3		4 fold↑	1.67	Equivocal	4.20fold↑	6.66	<0.005
D1 vs D2		2.18 ↑	1.07	NS	2.45↓	2.14	<0.05
D2 vs D3		1.07 ↑	0.50	NS	3.35↓	2.22	<0.05
C vs D1	Intestine	2.64fold↑	13.35	<0.005	2.36fold↑	13.18	<0.005
C vs D2		2.62fold↑	13.17	<0.005	2.38fold↑	13.54	<0.005
C vs D3		2.63fold↑	13.19	<0.005	2.45fold↑	14.21	<0.005
D1 vs D2		0.63↓	1.36	NS	0.89↑	0.66	NS
D2 vs D3		0.43↓	0.55	NS	2.87↑	2.32	<0.025

Table 14c: Changes in the tissue total Cholesterol (μ mole/100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	2.88 ± 0.16	1.29 ± 0.14	3.49 ± 0.37	5.37 ± 0.07	4.79 ± 0.10	F = 695.69 $p < 0.001$
	Muscle	1.11 ± 0.09	0.71 ± 0.06	1.50 ± 0.23	4.29 ± 0.09	3.18 ± 0.03	F =1443.53 $p < 0.001$
	Intestine	1.03 ± 0.23	1.79 ± 0.22	2.29 ± 0.08	2.50 ± 0.20	1.59 ± 0.15	F =588.863 $p < 0.001$
30 days	Liver	2.88 ± 0.16	1.25 ± 0.05	2.39 ± 0.09	4.12 ± 0.04	3.07 ± 0.20	F= 1522.51 $p < 0.001$
	Muscle	1.11 ± 0.09	0.55 ± 0.02	1.56 ± 0.06	3.18 ± 0.04	3.18 ± 0.04	F= 11102.8 $p <0.001$
	Intestine	1.03 ± 0.23	1.90 ± 0.19	2.65 ± 0.06	2.15 ± 0.04	2.39 ± 0.09	F= 1400.98 $p <0.001$

Table14ci: Statistical calculation (comparison test by using student 't' test) of Table 14c.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	Liver	2.7fold↑	5.56	<0.005	91.20↑	11.07	<0.005
C vs D2		4.16fold↑	26.06	<0.005	3.29fold↑	44.82	<0.005
C vs D3		3.71fold↑	20.34	<0.005	2.45fold↑	8.83	<0.005
D1 vs D2		58.87↑	4.99	<0.005	72.38↑	17.57	<0.005
D2 vs D3		10.80↓	4.75	<0.005	25.48↓	5.15	<0.005
C vs D1	Muscle	2.11fold↑	3.32	<0.005	2.83fold↑	15.97	<0.005
C vs D2		6.04fold↑	33.09	<0.005	5.78fold↑	58.80	<0.005
C vs D3		4.47fold↑	36.82	<0.005	5.78fold↑	58.80	<0.005
D1 vs D2		2.79fold↑	11.29	<0.005	2.03fold↑	22.47	<0.005
D2 vs D3		25.87↓	11.70	<0.005	no change	---	---
C vs D1	Intestine	27.93↑	2.14	<0.05	39.47↑	3.76	<0.005
C vs D2		39.66↑	2.39	<0.025	13.16↑	1.29	NS
C vs D3		11.17↑	0.75	NS	25.79↑	2.33	<0.025
D1 vs D2		9.17↑	0.97	NS	18.87↑	6.93	<0.005
D2 vs D3		36.40↓	3.64	<0.005	11.16↑	2.44	<0.025

Table 15a: Changes in the tissue total Phospholipid (ηmole /100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil during post hatching development
(Mean value of six samples and their standard error)

Feeding period	Tissues	I	C	D1	D2	D3	ANOVA
15 days	Liver	14.30 ± 0.90	4.60 ± 0.80	2.47 ± 0.24	4.35 ± 0.40	5.86 ± 0.50	F = 6.63 p =0.003
	Muscle	1.58 ± 0.10	4.30 ± 0.70	1.19 ± 0.20	2.54 ± 0.05	2.62 ± 0.80	F = 4.68 p = 0.012
	Intestine	4.60 ± 0.60	4.00 ± 0.90	2.14 ± 0.20	3.25 ± 0.40	4.41 ± 0.30	F = 2.94 p = 0.058
30 days	Liver	14.30 ± 0.90	11.50 ± 0.50	5.36 ± 0.08	7.69 ± 0.19	7.60 ± 0.24	F = 61.42 p < 0.001
	Muscle	1.58 ± 0.10	5.80 ± 0.30	2.63 ± 0.20	5.23 ± 0.30	5.58 ± 0.20	F = 198.89 p < 0.001
	Intestine	4.60 ± 0.60	10.30 ± 1.00	3.50 ± 0.08	4.01 ± 0.40	4.66 ± 0.13	F = 16.97 p < 0.001

Table15ai: Statistical calculation (comparison test by using student 't' test) of Table 15a.

Comparing group	Tissues	15 days			30 days		
		% change	calculated 't' value	*p* value	% change	calculated 't' Value	*p* value
C vs D1	Liver	46.30↓	2.56	< 0.005	53.39 ↓	4.29	< 0.005
C vs D2		5.43↓	0.28	NS	33.13 ↓	7.18	< 0.005
C vs D3		27.39↑	1034	NS	33.91↓	7.09	< 0.005
D1 vs D2		76.11↑	4.00	< 0.005	43.47 ↑	11.65	< 0.005
D2 vs D3		34.71 ↑	2.35	< 0.025	1.17 ↓	0.30	NS
C vs D1	Muscle	72.32 ↓	4.26	< 0.005	54.65↓	8.79	<0.005
C vs D2		40.93 ↓	2.51	< 0.025	3.82↓	1.34	NS
C vs D3		39.07 ↓	1.58	Equivocal	3.79↓	0.61	NS
D1 vs D2		2.13 fold↑	6.75	< 0.005	98.86 ↑	7.22	< 0.005
D2 vs D3		3.15↑	0.10	NS	6.69 ↑	0.97	NS
C vs D1	Intestine	46.50↓	2.02	< 0.025	66.02↓	4.53	< 0.005
C vs D2		18.75 ↓	0.76	NS	61.07 ↓	4.06	< 0.005
C vs D3		10.25 ↑	0.39	NS	54.76 ↓	3.63	< 0.005
D1 vs D2		51.87 ↑	2.78	< 0.01	14.57↑	1.28	NS
D2 vs D3		35.69↑	2.32	< 0.025	16.20↑	1.55	Equivocal

Table 15b: Changes in the tissue total Phospholipid (ηmole /100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	14.30 ± 0.90	4.60 ± 0.80	5.86 ± 0.10	9.67 ± 0.15	12.33 ± 0.03	F = 66.56 $p < 0.001$
	Muscle	1.58 ± 0.10	4.30 ± 0.70	6.48 ± 0.13	10.35 ± 0.30	13.10 ± 0.33	F = 84.27 $p < 0.001$
	Intestine	4.60 ± 0.60	4.00 ± 0.90	6.55 ± 0.22	12.18 ± 0.14	16.12 ± 0.02	F =87.24 *$p < 0.001$*
30 days	Liver	14.30 ± 0.90	11.50 ± 0.50	12.56 ± 0.41	17.24 ± 0.45	21.26 ± 0.49	F= 86.40 *$p < 0.001$*
	Muscle	1.58 ± 0.10	5.80 ± 0.30	15.56 ± 0.34	19.56 ± 0.70	22.66 ± 0.39	F= 123.04 $p <0.001$
	Intestine	4.60 ± 0.60	10.30 ± 1.00	11.50 ± 0.03	16.24 ± 0.58	25.14 ± 0.57	F= 63.79 $p <0.001$

Table15bi: Statistical calculation (comparison test by using student 't' test) of Table 15b.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	27.39↑	1.57	Equivocal	9.21↑	1.64	Equivocal
C vs D2		2.10fold↑	6.25	<0.005	49.91↑	8.54	<0.005
C vs D3		2.68fold↑	9.66	<0.005	84.86↑	13.94	<0.005
D1 vs D2		65.01↑	7.00	<0.005	37.26↑	7.80	<0.005
D2 vs D3		27.50↑	50.85	<0.005	23.31↑	6.04	<0.005
C vs D1	Muscle	50.69↑	3.07	<0.005	2.68fold↑	21.52	<0.005
C vs D2		2.40fold↑	7.96	<0.005	3.37fold↑	18.06	<0.005
C vs D3		2.04fold↑	11.38	<0.005	3.9 fold↑	34.26	<0.005
D1 vs D2		59.72↑	11.87	<0.005	25.70↑	5.14	<0.005
D2 vs D3		26.57↑	6.17	<0.005	15.84↑	3.87	<0.005
C vs D1	Intestine	63.75↑	2.49	<0.025	11.65↑	0.80	NS
C vs D2		3.04fold↑	8.18	<0.005	57.66↑	3.71	<0.005
C vs D3		4.03fold↑	12.12	<0.005	2.44fold↑	9.27	<0.005
D1 vs D2		85.95↑	21.65	<0.005	41.21↑	8.17	<0.005
D2 vs D3		32.34↑	27.94	<0.005	54.80↑	10.94	<0.005

Table 15c: Changes in the tissue total Phospholipid (ηmole /100mg) concentration of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	Initial	C	D1	D2	D3	ANOVA
15 days	Liver	14.30 ± 0.90	4.60 ± 0.80	5.30 ± 0.30	12.50 ± 0.40	9.62 ± 0.10	F = 56.12 $p < 0.001$
	Muscle	1.58 ± 0.10	4.30 ± 0.70	4.34 ± 0.16	11.50 ± 0.10	8.69 ± 0.08	F =89.099 $p < 0.001$
	Intestine	4.60 ± 0.60	4.00 ± 0.90	4.21 ± 0.09	10.54 ± 0.38	8.32 ± 0.07	F =27.672 $p < 0.001$
30 days	Liver	14.30 ± 0.90	11.50 ± 0.50	4.88 ± 0.07	11.67 ± 0.12	8.47 ± 0.07	F= 119.23 $p < 0.001$
	Muscle	1.58 ± 0.10	5.80 ± 0.30	4.85 ± 0.06	10.38 ± 0.31	8.24 ± 0.06	F= 148.70 $p <0.001$
	Intestine	4.60 ± 0.60	10.30 ± 1.00	4.34 ± 0.08	9.43 ± 0.23	7.35 ± 0.53	F= 11.246 $p <0.001$

Table15ci: Statistical calculation (comparison test by using student 't' test) of Table 15c.

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	15.21↑	0.82	NS	57.56↓	76.97	<0.005
C vs D2		2.71fold↑	8.88	<0.005	1.47 ↑	0.33	NS
C vs D3		2.09fold↑	6.28	<0.005	26.34 ↓	6.06	<0.005
D1 vs D2		2.35fold↑	14.40	<0.005	2.39fold↑	48.50	<0.005
D2 vs D3		23.04 ↓	7.02	<0.005	27.42 ↓	22.86	<0.005
C vs D1	Muscle	0.93↑	0.06	NS	16.37↓	3.10	<0.01
C vs D2		2.67fold↑	10.28	<0.005	78.96↑	10.61	<0.005
C vs D3		2.02fold↑	5.27	<0.005	42.06↑	7.97	<0.005
D1 vs D2		2.64fold↑	37.68	<0.005	2.14fold↑	17.51	<0.005
D2 vs D3		24.43↓	21.62	<0.005	20.61↓	6.77	<0.005
C vs D1	Intestine	5.25↑	26.25	<0.005	57.86↓	3.97	<0.005
C vs D2		2.63fold↑	6.11	<0.005	8.44↓	0.57	NS
C vs D3		2.08fold↑	4.32	<0.005	28.64↓	1.85	<0.05
D1 vs D2		2.5fold↑	15.44	<0.005	2.17fold↑	21.20	<0.005
D2 vs D3		21.06↓	5.45	<0.005	22.05↓	3.78	<0.005

Table 16a: Fatty acid profiles (relative percent composition) of Liver total lipid of chicks supplemented with different oils along with the commercial diet for 30 days

(Mean values of 3 set of samples are presented)

Fatty acid	Control	Coconut Oil		Sunflower Oil		Fish Oil	
		D1	D2	D2	D3	D2	D3
16:0	29.80	33.47[a]	33.00[a]	30.68	31.24	29.90	26.70[a]
16:1	4.20	2.20[a]	1.80[a]	4.20	3.80	3.20[ab]	2.10[ab]
18:0	18.20	21.05[a]	22.70[a]	11.24[a]	10.24[a]	19.20[a]	18.30[a]
18:1	15.20	9.37[a]	8.50[a]	14.37	11.37[a]	12.30	10.20[ab]
18:2(ω6)	15.30	14.47	14.44	20.25[ab]	23.69[ab]	11.30[ab]	9.20[ab]
18:3(ω3)	1.20	1.50	1.25	1.40	1.20	4.10[ab]	6.50[ab]
20:4 (ω6)	10.20	12.78[a]	13.38[a]	12.40[ab]	14.56[ab]	7.20[a]	6.20[a]
20:5(ω3)	2.40	1.50[a]	1.45[a]	1.60[ab]	0.80[ab]	4.60[ab]	7.80[ab]
22:6 (ω3)	1.40	1.36[a]	1.70[a]	1.06[ab]	0.50[ab]	4.60[a]	8.20[ab]
Others	2.10	2.30	1.78	2.80	2.60	3.60	4.80
ω3 / ω6	0.19	0.16	0.16	0.12	0.07	0.72	1.46

[a] These values are statistically significant (at p <0.05)over the same of control bird

[b] These values are statistically significant (at p<0.05)between the two treated groups of birds

Table 16b: Fatty acid profiles (relative percent composition) of Muscle total lipid of chicks supplemented with different oils along with the commercial diet for 30 days

(Mean values of 3 set of samples are presented

Fatty acid	Control	Coconut Oil		Sunflower Oil		Fish Oil	
		D1	D2	D2	D3	D2	D3
16:0	28.30	31.27[a]	32.37[a]	25.85[ab]	22.37[ab]	27.35	27.45
16:1	3.60	2.67[a]	2.53[a]	2.80	3.10	3.90	3.20
18:0	14.30	16.60[a]	17.04[a]	12.23[a]	12.78[a]	15.50	12.20[a]
18:1	19.30	16.50[a]	15.20[a]	16.15[ab]	14.25[ab]	16.30[ab]	14.50[ab]
18:2 (ω6)	14.00	16.20[a]	16.27[a]	18.37[ab]	22.45[ab]	13.20	13.60
18:3 (ω3)	2.50	0.75[a]	0.68[a]	1.80	2.10	5.80[ab]	7.80[ab]
20:4 (ω6)	9.30	10.80[a]	10.50[a]	14.30[a]	15.60[a]	7.50[ab]	5.80[ab]
20:5 (ω3)	2.20	1.37[a]	1.36[a]	2.10	1.80	4.50[ab]	6.20[ab]
22:6 (ω3)	2.60	0.95[a]	0.95[a]	1.20[a]	1.60[a]	4.40[ab]	6.30[ab]
Others	3.90	2.89	3.10[a]	5.20	3.95	1.55	2.95
ω3 / ω6	0.31	0.11	0.11	0.16	0.14	0.71	1.05

[a] These values are statistically significant (at $p < 0.05$)over the same of control bird

[b] These values are statistically significant (at $p<0.05$)between the two treated groups of birds

Table 16c: Fatty acid profiles (relative percent composition) of Intestine total lipid of chicks supplemented with different oils along with the commercial diet for 30 days.

(Mean values of 3 set of samples are presented

Fatty acid	Control	Coconut Oil		Sunflower Oil		Fish Oil	
		D1	D2	D2	D3	D2	D3
16:0	27.50	33.75[a]	35.16[a]	25.27	24.37	22.75[a]	22.10[a]
16:1	4.40	2.76[a]	2.36[a]	3.80	4.00	4.10	3.90
18:0	10.20	15.37[a]	15.30[a]	10.63	10.50	10.00	9.50
18:1	27.20	17.37[a]	17.68[a]	21.60[ab]	18.70[ab]	27.57[ab]	22.69[ab]
18:2 (ω6)	16.50	18.33[a]	17.37[a]	22.50[ab]	26.80[ab]	12.38[a]	13.01[a]
18:3 (ω3)	2.40	1.03[a]	1.08[a]	2.10	1.60[a]	4.60[a]	5.70[a]
20:4 (ω6)	7.50	7.25	7.75	8.00	7.40	6.90	5.80[a]
20:5 (ω3)	1.50	1.35	1.25	1.80	1.20	3.20[ab]	5.70[ab]
22:6 (ω3)	1.40	1.15[a]	1.05[a]	1.50	1.30	4.00[ab]	7.80[ab]
Others	1.40	1.64	1.00	2.80	4.13	4.50	3.80
ω3 / ω6	0.22	0.14	0.13	0.18	0.12	0.61	1.02

[a] These values are statistically significant (at $p < 0.05$)over the same of control bird

[b] These values are statistically significant (at $p < 0.05$)between the two treated groups of birds

Table 16d: Fatty acid profiles (relative percent composition) of Serum total lipid of chicks supplemented with different oils along with the commercial diet for 30 days.

(Mean values of 3 set of samples are presented

Fatty acid	Control	Coconut Oil		Sunflower Oil		Fish Oil	
		D1	D2	D2	D3	D2	D3
16:0	22.67	25.37[a]	25.27[a]	21.26	20.65	22.10	21.76
16:1	1.20	1.60	1.50	1.50	1.50	1.20	1.40
18:0	23.45	24.37	24.27	21.26[ab]	18.26[ab]	20.26[a]	20.24[a]
18:1	11.35	8.37[a]	8.86[a]	9.25[ab]	7.50[ab]	10.50[a]	10.00[a]
18:2 (ω6)	26.37	26.37	26.16	29.35[ab]	32.26[ab]	22.40[a]	20.70[a]
18:3 (ω3)	0.50	0.50	0.45	0.45	0.56	2.50[ab]	4.20[ab]
20:4 (ω6)	10.34	10.37	10.37	12.34[ab]	14.57[ab]	8.20[ab]	6.50[ab]
20:5 (ω3)	1.06	0.80	0.75[a]	1.10	1.40	4.34[a]	5.20[a]
22:6 (ω3)	1.30	1.25	1.35	1.20	1.30	4.30[ab]	6.50[ab]
Others	1.76	1.00	1.02	2.29	2.00	4.20	3.50
ω3 / ω6	0.08	0.07	0.07	0.07	0.07	0.36	0.58

[a] These values are statistically significant (at p <0.05)over the same of control bird

[b] These values are statistically significant (at p <0.05)between the two treated groups of birds

Table 17a: Changes in the serum lipid profiles of chick (*Gallus domesticus*) supplemented with different doses of coconut oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

	15 DAYS				30 DAYS			
	C	D1	D2	D3	C	D1	D2	D3
Total CH	163.00 ± 1.09	155.00 ± 1.83	187.67 ± 5.89	200.00 ± 4.76	146.00 ± 1.09	182.67 ± 4.23	203.00 ± 3.66	224.33 ± 5.60
Serum TG	113.33 ± 1.65	91.33 ± 8.25	93.67 ± 10.99	120.33 ± 9.10	147.33 ± 2.75	146.00 ± 7.33	159.33 ± 2.39	183.33 ± 2.75
HDL CH	79.00 ± 4.03	93.67 ± 3.48	96.67 ± 3.30	106.00 ± 1.09	89.67 ± 3.48	90.33 ± 2.92	95.67 ± 2.15	106.33 ± 7.88
LDLCH	61.33 ± 5.46	43.06 ±13.57	71.60 ± 11.20	69.93 ± 2.22	26.86 ± 1.83	63.14 ± 6.37	75.46 ± 5.25	81.33 ± 7.88
VLDL CH	22.67 ± 1.33	18.27 ± 1.65	18.73 ± 2.19	24.07 ± 1.82	29.47 ± 1.55	29.20 ± 1.47	31.87 ± 2.48	36.67 ± 2.55
CH:HDL	2.06	1.65	1.94	1.89	1.63	2.02	2.12	2.10
CH:TG	1.44	1.69	2.00	1.66	0.99	1.25	1.27	1.22

Table 17ai: Statistical calculation (comparison test by using student 't' test) of table 17a

Comparing group	Parameters	15 days			30 days		
		% change	't' value	p value	% change	't' value	p value
C v/s D1	**Total cholesterol**	4.90↓	3.75	<0.005	25.11↑	8.39	<0.005
C v/s D2		15.13↑	4.11	<0.005	39.04↑	14.92	<0.005
C v/sD3		22.69↑	7.57	<0.005	53.65↑	13.73	<0.005
D1 v/s D2		21.07↑	5.29	<0.005	11.12↑	3.63	<0.005
D2 v/s D3		6.57↑	1.62	Equivocal	10.50↑	3.18	<0.005
C v/s D1	**Total triglycerol**	19.41↓	2.61	<0.025	0.90↓	0.17	NS
C v/s D2		17.34↓	1.76	Equivocal	8.14↑	3.29	<0.005
C v/sD3		6.17↑	0.75	NS	24.43↑	9.25	<0.005
D1 v/s D2		2.56↑	0.17	NS	9.13↑	1.72	Equivocal
D2 v/s D3		28.46↑	1.86	Equivocal	15.06↑	6.58	<0.005
C v/s D1	**HDL**	18.56↑	2.75	<0.01	0.73↑	0.14	NS
C v/s D2		22.36↑	3.39	<0.005	6.69↑	1.46	<0.005
C v/sD3		34.17↑	4.46	<0.005	18.57↑	1.93	<0.05
D1 v/s D2		3.20↑	0.62	NS	5.91↑	1.47	Equivocal
D2 v/s D3		9.65↑	2.68	<0.025	11.14↑	1.30	NS
C v/s D1	**LDL**	29.78↓	1.24	NS	2.35 old↑	6.19	<0.005
C v/s D2		16.75↑	0.82	NS	2.8 fold↑	7.12	<0.005
C v/sD3		14.02↑	1.45	Equivocal	3 fold↑	10.30	<0.005
D1 v/s D2		66.27↑	1.62	Equivocal	19.51↑	8.65	<0.005
D2 v/s D3		2.33↑	0.14	NS	7.77↑	7.79	<0.005
C v/s D1	**VLDL**	19.40↓	2.07	<0.025	0.92↓	0.12	NS
C v/s D2		17.37↓	1.53	Equivocal	8.14↑	8.74	<0.005
C v/sD3		6.17↑	0.62	NS	24.43↑	6.73	<0.005
D1 v/s D2		2.51↑	0.16	NS	9.14↑	1.49	Equivocal
D2 v/s D3		28.51↑	1.87	Equivocal	15.06↑	0.62	NS

Table 17b: Changes in the serum lipid profiles of chick (*Gallus domesticus*) supplemented with different doses of sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

	15 DAYS				30 DAYS			
	C	**D1**	**D2**	**D3**	**C**	**D1**	**D2**	**D3**
Total CH	163.00 ± 1.09	182.33 ± 5.89	185.00 ± 2.56	183.00 ± 5.76	146.00 ± 1.09	164.00 ± 2.56	184.00 ± 5.73	198.00 ± 2.19
Serum TG	113.33 ± 1.65	92.00 ± 1.73	95.33 ± 1.29	97.00 ± 7.63	147.33 ± 2.75	115.33 ± 2.39	125.33 ± 3.85	150.67 ± 1.85
HDL CH	79.00 ± 4.03	124.00 ± 1.47	124.00 ± 3.29	118.33 ± 2.49	89.67 ± 3.48	108.67 ± 4.40	119.33 ± 2.02	123.00 ± 2.90
LDLCH	61.33 ± 5.46	39.93 ± 7.09	*41.93* ± 4.80	45.27 ± 5.20	26.86 ± 1.83	32.26 ± 6.49	39.6 ± 6.74	44.87 ± 4.97
VLDL CH	22.67 ± 1.33	18.40 ± 1.50	19.07 ± 1.26	19.40 ± 1.53	29.47 ± 1.55	23.07 ± 1.48	25.07 ± 1.77	30.13 ± 1.37
CH:HDL	2.06	1.47	1.49	1.55	1.63	1.50	1.54	1.60
CH:TG	1.44	1.98	1.94	1.89	0.99	1.42	1.47	1.31

Table 17bi: Statistical calculation (comparison test by using student 't' test) of table 17b

Comparing group	Parameters	15 days			30 days		
		% change	't' value	p value	% change	't' value	p value
C v/s D1	**Total cholesterol**	11.85↑	3.22	<0.005	12.32↑	6.47	<0.005
C v/s D2		13.43↑	7.90	<0.005	26.02↑	6.51	<0.005
C v/sD3		12.25↑	3.41	<0.005	35.61↑	21.25	<0.005
D1 v/s D2		1.46↑	0.41	NS	12.19↑	3.18	<0.005
D2 v/s D3		1.08↓	0.31	NS	7.60↑	2.28	<0.025
C v/s D1	**Total triglycerol**	18.82↓	8.92	<0.005	21.71↓	8.78	<0.005
C v/s D2		15.88↓	8.59	<0.005	14.93↓	4.65	<0.005
C v/sD3		14.40↓	2.09	<0.05	2.26↓	1.00	NS
D1 v/s D2		3.61↑	1.54	Equivocal	8.67↑	2.20	<0.05
D2 v/s D3		1.75↑	0.21	NS	20.21↑	5.93	<0.005
C v/s D1	**HDL**	56.96↑	10.49	<0.005	21.18↑	3.38	<0.005
C v/s D2		56.96↑	8.65	<0.005	33.07↑	7.37	<0.005
C v/sD3		49.78↑	8.30	<0.005	37.16↑	7.35	<0.005
D1 v/s D2		no change	---	---	9.80↑	2.20	<0.05
D2 v/s D3		4.57↓	1.37	NS	3.07↑	1.03	NS
C v/s D1	**LDL**	34.89↓	2.39	<0.025	20.10↑	0.80	NS
C v/s D2		31.63↓	2.66	<0.025	47.43↑	1.82	Equivocal
C v/sD3		26.18↓	2.12	<0.05	67.05↑	3.40	<0.005
D1 v/s D2		5.00↑	0.23	NS	22.75↑	0.78	NS
D2 v/s D3		7.36↑	0.47	NS	13.30↑	0.62	NS
C v/s D1	**VLDL**	18.83↓	2.13	<0.05	21.71↓14.	2.98	<0.025
C v/s D2		15.88↓	1.96	Equivocal	93↓	1.87	Equivocal
C v/sD3		14.42↓	1.61	Equivocal	2.23↑	0.31	NS
D1 v/s D2		36.41↑	0.34	NS	8.65↑	0.86	NS
D2 v/s D3		1.73↑	0.16	NS	20.13↑	2.26	<0.025

Table 17c: Changes in the serum lipid profiles of the chick (*Gallus domesticus*) supplemented with different doses of fish oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

	15 DAYS				30 DAYS			
	C	**D1**	**D2**	**D3**	**C**	**D1**	**D2**	**D3**
Total CH	163.00 ± 1.09	174.33 ± 1.29	171.33 ± 5.55	164.33 2.57	146.00 ± 1.09	156.33 ± 0.56	149.33 ± 5.32	144.33 ± 2.02
Serum TG	113.33 ± 1.65	100.00 ± 1.47	78.00 ± 3.29	60.67 ± 3.30	147.33 ± 2.75	114.33 ± 6.23	95.00 ± 9.53	65.00 ± 4.76
HDL CH	79.00 ± 4.03	105.33 ± 0.92	103.00 ± 3.66	94.67 ± 0.76	89.67 ± 3.48	77.00 ± 1.09	92.00 ± 1.32	104.33 ± 0.56
LDLCH	61.33 ± 5.46	49.00 ± 2.49	52.73 ± 3.00	57.53 ± 3.71	62.87 ± 1.83	36.47 ± 1.89	38.33 ± 6.36	27.00 ± 1.61
VLDL CH	22.67 ± 1.33	20.00 ± 0.29	15.60 ± 0.66	12.13 ± 0.66	29.47 ± 1.55	22.87 ± 1.25	19.00 ± 1.90	13.00 ± 0.95
CH:HDL	2.06	1.65	1.66	1.73	1.63	2.03	1.62	1.38
CH:TG	1.44	1.74	2.19	2.70	0.99	1.36	1.57	2.22

Table 17ci: Statistical calculation (comparison test by using student 't' test) of table 17c

Comparing group	Parameters	15 days			30 days		
		% change	't' value	p value	% change	't' value	p value
C v/s D1	Total cholesterol	6.95↑	6.71	<0.005	7.07↑	8.43	<0.005
C v/s D2		5.11↑	1.47	Equivocal	2.28↑	0.61	NS
C v/sD3		0.81↑	0.47	NS	1.14↓	0.72	NS
D1 v/s D2		1.72↓	0.52	NS	4.47↓	1.30	NS
D2 v/s D3		4.08↓	1.14	NS	3.34↓	0.88	NS
C v/s D1	Total triglycerol	11.76↓	6.03	<0.005	22.39↓	4.84	<0.005
C v/s D2		31.17↓	9.60	<0.005	35.51↓	5.27	<0.005
C v/sD3		46.46↓	14.27	<0.005	55.88↓	14.97	<0.005
D1 v/s D2		22.00↓	6.10	<0.005	16.90↓	1.69	Equivocal
D2 v/s D3		22.21↓	3.72	<0.005	31.57↓	2.81	<0.025
C v/s D1	HDL	33.33↑	6.37	<0.005	14.12↓	3.47	<0.005
C v/s D2		30.38↑	4.40	<0.005	2.59↑	0.63	NS
C v/sD3		19.83↑	3.82	<0.005	16.35↑	4.16	<0.005
D1 v/s D2		2.21↓	0.61	NS	19.48↑	8.77	<0.005
D2 v/s D3		8.08↓	2.22	<0.05	13.40↑	8.60	<0.005
C v/s D1	LDL	20.10↓	2.05	<0.05	41.59↓	10.03	<0.005
C v/s D2		14.02↓	1.38	NS	39.83↓	3.70	<0.005
C v/sD3		9.10↓	0.57	NS	57.46↓	14.72	<0.005
D1 v/s D2		7.61↑	0.95	NS	5.10↑	0.28	NS
D2 v/s D3		9.10↑	1.00	NS	25.55↓	1.72	Equivocal
C v/s D1	VLDL	11.77↓	1.96	Equivocal	22.39↓	3.31	<0.005
C v/s D2		31.11↓	4.76	<0.005	35.52↓	4.27	<0.005
C v/sD3		46.49↓	7.10	<0.005	55.88↓	9.06	<0.005
D1 v/s D2		22.00↓	6.11	<0.005	16.92↓	1.70	Equivocal
D2 v/s D3		22.24↓	3.72	<0.005	31.57↓	2.82	<0.025

Table 18a: Changes in the Alkaline Phosphatase activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut along with the commercial feed oil during post hatching development

(Mean value of six samples and their standard error)

Feeding period	Tissues	C	D1	D2
15 days	Liver	13.37 ± 1.64	19.15 ± 0.18	18.05 ± 0.009
	Serum	30.77 ± 0.13	55.12 ± 0.36	45.33 ± 0.25
30 days	Liver	8.00 ± 0.38	20.05 ± 0.17	15.79 ± 0.06
	Serum	24.79 ± 0.23	30.44 ± 0.28	31.50 ± 0.28

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	43.23↑	3.50	<0.005	2.5fold↑	28.94	<0.005
C vs D2		35.00↑	2.85	<0.01	97.37↑	20.24	<0.005
D1 vs D2		5.74↓	6.10	<0.005	21.24↓	23.63	<0.005
C vs D1	Serum	79.13↑	63.74	<0.005	22.79↑	15.59	<0.005
C vs D2		47.31↑	51.81	<0.005	27.06↑	18.52	<0.005
D1 vs D2		17.76↓	22.35	<0.005	3.48↑	2.68	<0.025

Table 18b: Changes in the Alkaline Phosphatase activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	13.37 ± 1.64	6.43 ± 0.07	4.87 ± 0.16
	Serum	30.77 ± 0.13	59.45 ± 2.29	40.19 ± 1.43
30 days	Liver	8.00 ± 0.38	6.56 ± 0.10	3.76 ± 0.06
	Serum	24.79 ± 0.23	33.82 ± 3.08	23.48 ± 0.14

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	51.90↓	4.22	<0.005	18.00↓	3.66	<0.005
C vs D3		63.50↓	5.15	<0.005	53.00↓	11.02	<0.005
D2 vs D3		24.41↓	8.93	<0.005	42.68↓	24.01	<0.005
C vs D2	Serum	93.20↑	12.50	<0.005	36.42↑	2.92	<0.01
C vs D3		30.60↑	6.56	<0.005	5.28↓	4.86	<0.005
D2 vs D3		32.41↓	7.13	<0.005	30.57↓	3.35	<0.005

Table 18c: Changes in the Alkaline Phosphatase activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Age	Tissues	C	D2	D3
15 days	Liver	13.37 ± 1.64	5.392 ± 0.104	5.924 ± 0.108
	Serum	30.77 ± 0.13	22.507 ± 0.54	23.785 ± 0.188
30 days	Liver	8.00 ± 0.38	6.615 ± 0.001	5.898 ± 0.019
	Serum	24.79 ± 0.23	20.663 ± 0.108	17.540 ± 0.120

Statistical calculation (comparison test by using student 't' test).

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	59.68↓	4.85	< 0.005	17.25↓	3.65	< 0.005
C vs D3		55.72↓	4.53	< 0.005	26.37↓	5.55	< 0.005
D2 vs D3		9.83↑	3.74	< 0.005	10.89↓	37.84	< 0.005
C vs D2	Serum	26.87↓	14.88	<0.005	16.65↓	16.25	<0.005
C vs D3		22.71↓	31.48	<0.005	29.40↓	27.94	<0.005
D2 vs D3		5.68↑	2.24	<0.025	15.10↓	19.32	<0.005

Table 19a: Changes in the Glutamate Pyruvate transaminase (GPT) activity of the broiler chick(*Gallus domesticus*) supplemented with different doses of Coconut oil along with the commercial feed during post hatching development

(Mean value of six samples and their standard error)

Feeding period	Tissues	C	D1	D2
15 days	Liver	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
	Serum	0.13 ± 0.02	0.21 ± 0.05	0.14 ± 0.03
30 days	Liver	0.13 ± 0.06	0.16 ± 0.03	0.15 ± 0.02
	Serum	0.20 ± 0.03	0.15 ± 0.03	0.18 ± 0.03

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	16.66↑	0.70	NS	23.07↑	0.44	NS
C vs D2		8.33↑	0.35	NS	15.38↑	0.31	NS
D1 vs D2		7.14↓	0.35	NS	6.25↓	0.27	NS
C vs D1	Serum	61.53↑	1.48	Equivocal	25.00↓	1.17	NS
C vs D2		7.69↑	0.27	NS	10.00↓	0.47	NS
D1 vs D2		33.33↓	1.20	NS	20.00↑	0.70	NS

Table 19b: Changes in the Glutamate Pyruvate transaminase (GPT) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	0.12 ± 0.02	0.12 ± 0.04	0.14 ± 0.04
	Serum	0.13 ± 0.02	0.14 ± 0.02	0.15 ± 0.02
30 days	Liver	0.13 ± 0.06	0.12 ± 0.04	0.14 ± 0.03
	Serum	0.20 ± 0.03	0.17 ± 0.02	0.16 ± 0.03

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	No change	---	--	7.69↓	0.13	NS
C vs D3		16.66↑	0.44	NS	7.69↑	0.14	NS
D2 vs D3		16.66↑	0.35	NS	16.66↑	0.40	NS
C vs D2	Serum	7.69↑	0.35	NS	15.00↓	0.83	NS
C vs D3		15.38↑	0.70	NS	20.00↓	0.94	NS
D2 vs D3		7.14↑	0.35	NS	5.88↓	0.27	NS

Table 19c: Changes in the Glutamate Pyruvate transaminase (GPT) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	0.12 ± 0.02	0.13 ± 0.02	0.13 ± 0.04
	Serum	0.13 ± 0.02	0.15 ± 0.05	0.15 ± 0.02
30 days	Liver	0.13 ± 0.06	0.13 ± 0.03	0.13 ± 0.02
	Serum	0.20 ± 0.03	0.15 ± 0.03	0.16 ± 0.05

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	8.33↑	0.35	NS	no change	---	---
C vs D3		8.33↑	0.22	NS	no change	---	---
D2 vs D3		no change	---	---	no change	---	---
C vs D2	Serum	15.38↑	0.37	NS	25.00↓	0.32	NS
C vs D3		15.38↑	0.70	NS	20.00↓	0.25	NS
D2 vs D3		no change	---	---	6.66↑	0.17	NS

Table 20a: Changes in the Glutamate Oxaloacetate Transaminase activity (GOT) of the broiler chick (*Gallusdomesticus*) supplemented with different doses of Coconut oil along with the commercial feed during post hatching development

(Mean value of six samples and their standard error)

Feeding period	Tissues	C	D1	D2
15 days	Liver	0.34 ± 0.07	0.20 ± 0.06	0.16 ± 0.05
	Serum	0.06 ± 0.004	0.07 ± 0.07	0.65 ± 0.006
30 days	Liver	0.30 ± 0.02	0.24 ± 0.04	0.19 ± 0.02
	Serum	0.08 ± 0.004	0.07 ± 0.007	0.07 ± 0.004

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	41.17↓	1.51	Equivocal	20.00↓	4.69	<0.005
C vs D2		52.94↓	2.09	<0.05	36.66↓	5.65	<0.005
D1 vs D2		20.00↓	0.51	NS	20.83↓	1.11	NS
C vs D1	Serum	1.16↑	1.24	NS	12.50↓	1.24	NS
C vs D2		no change	---	---	12.50↓	1.76	Equivocal
D1 vs D2		14.28↓	1.08	NS	no change	---	---

Table 20b: Changes in the Glutamate Oxaloacetate Transaminase activity (GOT)of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	0.34 ± 0.07	0.13 ± 0.05	0.17 ± 0.03
	Serum	0.06 ± 0.004	0.12 ± 0.05	0.09 ± 0.02
30 days	Liver	0.30 ± 0.02	0.16 ± 0.03	0.15 ± 0.02
	Serum	0.08 ± 0.004	0.29 ± 0.06	0.15 ± 0.04

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	32.35↓	2.44	<0.025	46.66↓	3.88	<0.005
C vs D3		50.00↓	2.23	<0.025	50.00↓	5.30	<0.005
D2 vs D3		26.08↓	0.68	NS	6.25↓	0.27	NS
C vs D2	Serum	2 fold↑	1.19	NS	3.6fold↑	3.49	<0.005
C vs D3		50.00↑	1.47	Equivocal	87.50↑	1.74	Equivocal
D2 vs D3		25.00↓	0.55	NS	48.27↓	1.94	Equivocal

Table 20c: Changes in the Glutamate Oxaloacetate Transaminase activity (GOT) of the broiler chick (*Gallusdomesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	0.34 ± 0.07	0.11 ± 0.02	0.10 ± 0.03
	Serum	0.06 ± 0.004	0.079 ± 0.002	0.078 ± 0.009
30 days	Liver	0.30 ± 0.02	0.013 ± 0.002	0.012 ± 0.003
	Serum	0.08 ± 0.004	0.065 ± 0.002	0.057 ± 0.009

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	67.64↓	3.15	<0.01	56.66↓	14.27	<0.005
C vs D3		70.58↓	3.15	<0.01	60.00↓	14.24	<0.005
D2 vs D3		9.09↓	0.27	NS	7.69↓	0.27	NS
C vs D2	Serum	31.66↑	4.24	<0.005	18.75↓	3.35	<0.005
C vs D3		30.00↑	1.82	Equivocal	28.75↓	2.33	<0.025
D2 vs D3		1.26↓	0.10	NS	12.30↓	0.86	NS

Table 21a : Changes in the Lactae Dehydrogenase(LDH) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Coconut oil along with the commercial feed during post hatching development

(Mean value of six samples and their standard error)

Feeding period	Tissues	C	D1	D2
15 days	Liver	1.07 ± 0.02	2.25 ± 0.04	2.19 ± 0.05
	Serum	1.37 ± 0.06	2.13 ± 0.011	1.88 ± 0.06
30 days	Liver	0.96 ± 0.01	3.20 ± 0.027	3.14 ± 0.020
	Serum	1.42 ± 0.06	1.34 ± 0.016	1.40 ± 0.02

Statistical calculation (comparison test by using student't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D1	Liver	2.1fold↑	26.38	<0.005	3.33fold↑	70.83	<0.005
C vs D2		2.04fold↑	20.79	<0.005	3.27fold↑	97.49	<0.005
D1 vs D2		2.66↓	0.93	NS	1.87↓	1.66	Equivocal
C vs D1	serum	55.47↑	12.49	<0.005	5.63↓	1.26	NS
C vs D2		37.22↑	6.01	<0.005	1.40↓	0.31	NS
D1 vs D2		11.73↓	4.11	<0.005	4.47↑	2.12	<0.05

Table 21b: Changes in the Lactae Dehydrogenase(LDH) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	1.07 ± 0.02	1.29 ± 0.05	1.58 ± 0.01
	Serum	1.37 ± 0.06	1.47 ± 0.05	1.44 ± 0.04
30 days	Liver	0.96 ± 0.01	1.96 ± 0.01	1.96 ± 0.02
	Serum	1.42 ± 0.06	1.23 ± 0.11	1.17 ± 0.02

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	20.56↑	4.08	<0.005	2.04fold↑	70.71	<0.005
C vs D3		47.66↑	22.80	<0.005	2.04fold↑	44.72	<0.005
D2 vs D3		22.48↑	5.68	<0.005	no change	---	---
C vs D2	Serum	7.29↑	1.28	NS	13.38↓	1.51	NS
C vs D3		5.10↑	0.97	NS	17.60↓	3.95	<0.005
D2 vs D3		2.04↓	0.47	NS	4.87↓	0.53	NS

Table 21c: Changes in the Lactae Dehydrogenase(LDH) activity of the broiler chick (*Gallus domesticus*) supplemented with different doses of Fish oil along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	D2	D3
15 days	Liver	1.07 ± 0.02	1.19 ± 0.01	1.28 ± 0.05
	Serum	1.37 ± 0.06	1.38 ± 0.04	1.41 ± 0.023
30 days	Liver	0.96 ± 0.01	0.83 ± 0.40	1.07 ± 0.15
	Serum	1.42 ± 0.06	1.18 ± 0.03	1.09 ± 0.06

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs D2	Liver	11.21↑	5.36	<0.005	13.54↓	0.32	NS
C vs D3		19.62↑	3.90	<0.005	11.45↑	0.73	NS
D2 vs D3		7.56↑	1.76	Equivocal	28.91↑	0.56	NS
C vs D2	Serum	0.72↑	0.13	NS	16.90↓	3.57	<0.005
C vs D3		3.64↑	0.63	NS	23.23↓	3.88	<0.005
D2 vs D3		2.89↑	0.67	NS	7.62↓	1.34	NS

Table 22a : Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler chick, *Gallus domesticus* supplemented with different doses of Coconut oil along with the commercial feed during post hatching development.

Feeding period		3-HMG-CoA- Reductase			HMG-CoA- Synthase		
	Tissues	C	D1	D2	C	D1	D2
15 days	Liver	585.97 ± 6.89	482.50 ± 5.54	678.17 ± 1.05	41.68 ± 0.75	28.68 ± 2.26	15.22 ± 0.69
	Serum	52.47 ± 5.92	249.76 ± 11.08	516.43 ± 1.39	9.99 ± 0.33	19.84 ± 0.72	26.28 ± 0.24
30 days	Liver	681.21 ± 8.17	685.60 ±5.70	892.37 ± 10.12	24.60 ± 1.03	18.68 ± 2.37	12.46 ± 1.27
	Serum	51.61 ± 7.92	250.80 ± 6.20	670.62 ± 5.26	12.18 ± 1.85	19.78 ± 1.27	22.28 ± 3.24

Table 22ai: Statistical calculation (comparison test by using student 't' test) of table 22a

Comparing group	Feeding period	Tissues	3-HMG-CoA- Reductase			HMG-CoA- Synthase		
			% change	't' value	*p* value	% change	't' Value	*p* value
C vs D1	15 days	Liver	17.65↓	11.70	<0.005	31.19↓	5.45	<0.005
C vs D2			15.73↑	13.22	<0.005	63.48↓	25.96	<0.005
D1 vs D2			40.55↑	34.70	<0.005	46.93↓	5.69	<0.005
C vs D1		Serum	4.76fold↑	15.70	<0.005	98.53↑	12.43	<0.005
C vs D2			9.84fold↑	76.29	<0.005	2.63fold↑	39.92	<0.005
D1 vs D2			2.06fold↑	23.88	<0.005	32.45↑	8.48	<0.005
C vs D1	30 days	Liver	0.64↑	0.44	NS	24.06↓	2.29	<0.025
C vs D2			30.99↑	16.23	<0.005	49.34↓	7.42	<0.005
D1 vs D2			30.15↑	17.80	<0.005	33.29↓	2.31	<0.025
C vs D1		Serum	4.85fold↑	19.80	<0.005	62.39↑	3.38	<0.005
C vs D2			12.99fold↑	65.10	<0.005	82.92↑	2.70	<0.025
D1 vs D2			2.67fold↑	51.63	<0.005	12.63↑	0.71	NS

Table 22b : Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler chick, *Gallus domesticus* supplemented with different doses of Sunflower oil along with the commercial feed during post hatching development.

Feeding period	Tissues	3-HMG-CoA- Reductase			3-HMG-CoA- Synthase		
		C	D2	D3	C	D2	D3
15 days	Liver	585.97 ± 6.89	664.45 ± 4.04	1009.24 ± 7.53	41.68 ± 0.75	24.88 ± 0.95	19.06 ± 2.10
	Serum	52.47 ± 5.92	225.23 ± 0.39	713.91 ± 5.68	9.99 ± 0.33	31.84 ± 3.72	125.32 ± 8.78
30 days	Liver	681.21 ± 8.17	764.56 ±8.26	1120.67 ±20.37	24.60 ± 1.03	10.76 ±0.26	10.16 ±0.26
	Serum	51.61 ± 7.92	267.22 ± 7.20	700.00 ± 6.57	12.18 ± 1.85	40.86 ±3.80	100.75 ±6.78

Table 22bi: Statistical calculation (comparison test by using student 't' test) of table 22b

Comparing group	Feeding period	Tissues	3-HMG-CoA- Reductase			HMG-CoA- Synthase		
			% change	't' value	*p* value	% change	't' Value	*p* value
C vs D2	15 days	Liver	13.39↑	9.82	<0.005	40.30↓	13.88	<0.005
C vs D3			72.23↑	41.47	<0.005	54.27↓	10.14	<0.005
D2 vs D3			51.89↑	40.34	<0.005	23.39↑	2.52	<0.05
C vs D2		Serum	4.29fold↑	29.11	<0.005	3.18fold↑	5.85	<0.005
C vs D3			13.60fold↑	80.62	<0.005	12.54fold↑	13.12	<0.005
D2 vs D3			3.16fold↑	85.83	<0.005	3.93fold↑	9.80	<0.005
C vs D2	30 days	Liver	12.23↑	7.17	<0.005	56.26↓	13.02	<0.005
C vs D3			64.51↑	20.03	<0.005	58.69↓	13.59	<0.005
D2 vs D3			46.57↑	16.20	<0.005	5.58↓	1.63	Equivocal
C vs D2		Serum	5.17fold↑	20.14	<0.005	3.35fold↑	6.78	<0.005
C vs D3			13.56fold↑	63.01	<0.005	8.27fold↑	12.60	<0.005
D2 vs D3			2.61fold↑	44.40	<0.005	2.46fold↑	7.70	<0.005

Table 22c : Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler broiler chick, *Gallus domesticus* supplemented with different doses of Fish oil along with the commercial feed during post hatching development.

Feeding period	Tissues	3-HMG-CoA- Reductase			3-HMG-CoA- Synthase		
		C	D2	D3	C	D2	D3
15 days	Liver	585.97 ± 6.89	732.36 ± 7.06	917.67 ± 2.53	41.68 ± 0.75	141.02 ± 5.98	216.27 ± 1.42
	Serum	52.47 ± 5.92	49.74 ± 2.13	43.38 ± 4.69	9.99 ± 0.33	23.38 ± 2.93	46.48 ± 4.26
30 days	Liver	681.21 ± 8.17	678.26 ±5.23	720.00 ± 10.37	24.60 ± 1.03	106.76 ± 2.78	146.20 ± 3.74
	Serum	51.61 ± 7.92	145.75 ± 2.36	50.76 ± 2.76	12.18 ± 1.85	46.38 ± 2.17	48.37 ± 1.56

Table 22ci: Statistical calculation (comparison test by using student 't' test) of table 22c

Comparing group	Feeding period	Tissues	3-HMG-CoA- Reductase			HMG-CoA- Synthase		
			% change	't' value	*p* value	% change	't' Value	*p* value
C vs D2	15 days	Liver	24.98↑	14.48	<0.005	3.38fold↑	16.48	<0.005
C vs D3			56.60↑	45.19	<0.005	5.18fold↑	108.71	<0.005
D2 vs D3			25.30↑	24.70	<0.005	53.36↑	12.24	<0.005
C vs D2		Serum	50.20↓	0.43	NS	2.34fold↑	72.37	<0.005
C vs D3			17.32↓	1.20	NS	4.65fold↑	8.54	<0.005
D2 vs D3			12.79↓	1.23	NS	98.80↑	34.21	<0.005
C vs D2	30 days	Liver	0.43↓	0.30	NS	4.33fold↑	27.71	<0.005
C vs D3			5.69↑	2.93	<0.01	5.94fold↑	31.34	<0.005
D2 vs D3			6.15↑	3.59	<0.005	36.94↑	8.46	<0.005
C vs D2		Serum	11.35↓	0.70	NS	3.80fold↑	11.99	<0.005
C vs D3			1.64↓	0.10	NS	3.97fold↑	14.95	<0.005
D2 vs D3			10.95↑	1.38	Equivocal	4.29↑	0.74	NS

3.4 Bacteria as a lipid source

3.4.1Growth:

With the supplementation of different strains of bacteria (B1, B2, B3) for 15 days the average weight of the chicks were increased (about 21-31%, $p<0.005$) (Table 23, 23a and figure M) followed by increase in the daily instantaneous growth rate (G_w). However, after 30 days of such feeding experiment 4 – 25% increase (equivocal - <0.005) was recorded in net weight gain of the bird. The maximum increase in the net weight gain was recorded with the birds supplemented with B2 bacterial strain.

3.4.2 Hematology

About 13 –17 % increase in the hemoglobin concentration was observed in the birds supplementated with B1 and B2 bacterial strains for 15 days. However, no significant change was recorded in the hemoglobin concentration of the chicks supplemented with all three strains of bacteria for 30 days. (Table 24 and figure N).

Supplementation of B1 and B2 strain of bacteria for 15 and 30 days did not alter the total erythrocyte count of the chicks. But, with the supplementation of B3 strain of bacteria the total erythrocyte count was decreased by about 46% (equivocal) and 29% ($p<0.005$) after 15 and 30 days of the feeding experiment respectively (Figure N).

There was an increase (about 26% - 3 fold, equivocal – $p<0.005$) in the total leukocyte count with the supplementation of all three strains of bacteria for 15 and 30 days (table 24a and figureN).

3.4.3 Biochemical changes

3.4.3.1 Total Protein

An increase (about 12 –68 %, equivocal – $p<0.025$) in the total protein concentration of the liver was noticed after 15 days and / or 30 days of supplementing B1, B2 and B3 strains of bacteria compared to control group (Table 25 and Figure O). The maximum increase in the liver protein concentration was recorded with B2 strain in comparison to other two strains (i.e. B1 and B3).

With the supplementation of B1 and B3 strains of bacteria for 15 days, no significant change in the total protein level of muscle was detected (Figure O). However, with the continuation of supplementation for another 15 days the protein concentration in muscle was decreased (about 10% - 20%, $p<$ 0.01- 0.05). Supplementation of B2 strain showed increase in the level of muscle protein content (18%, $p<0.01$) after 15 days, and it remained unaltered after 30 days (Table 25a).

The total protein concentration of the intestine decreased by 7% with the supplementation of B1 strain for 15 days, which was statistically equivocal, but remained unchanged after 30 days of the feeding experiment. Supplementation of B2 strain of bacteria for first 15 days did not change the protein concentration of the intestine but there was an increase by 9% ($p<$ 0.005) after 30 days (Table 25). With the supplementation of B3 strain for 15 days, about 26 % decrease was noticed in the protein concentration, which was significant at 0.25% level. However after 30 days, it remained unchanged (Figure O).

3.4.3.2 Total Triglycerol

A statistically significant increase (48% - 5.7 fold, $p<0.005$) was recorded in the level of total triglycerol in the liver, muscle and intestine of the chicks after 15 and 30 days of feeding all three strains of bacteria compared to the control group. The maximum elevation in the triglycerol content of liver and intestine was recorded in the chicks supplemented with B2 bacterial strain, but in the muscle, maximum increase in triglycerol concentration was recorded with B1 strain (Table 26 and figure P).

3.4.3.3 Total Cholesterol

Table 27 and figure Q demonstrates that there was statistically significant increase in the total cholesterol concentration in liver (about 5 - 15 fold, $p<0.005$), muscle (about 6 - 22 fold, $p<0.005$) and in intestine (about 3 - 6 fold, $p<0.005$) after15 and 30 days of feeding all three strains of bacteria. The highest amount of cholesterol accumulation in the liver, muscle and intestine was noticed with the supplementation of B3 strain of bacteria compared to the other two strains.

3.4.3.4 Total Phospholipid

A statistically significant increase (8% - 6 fold, $p<0.005 - 0.05$) in the level of total phospholipid in liver and muscle was observed with the supplementation of all three strains of bacteria for 15 days and / or 30 days except in the liver of birds supplemented with B2 bacterial strain for 30 days (Table 28a and figure R). In the intestine although the phospholipid concentration was elevated by 2 – 3 fold ($p<0.005$) after supplementing

the bacterial strains (B1, B2 and B3) for 15 days, only 7% increase in phospholipid level was noticed in the birds supplemented with B3 strain for 30 days (Table 28).

3.4.3.5 Fatty acid profiles

When the chicks were supplemented with different bacterial strains along with the commercial diet for 30 days, the liver showed about 42% increase in the relative concentration of palmitoleic acid along with 2.5 - 3 fold increase in linolenic acid, eicosapentaenoic acid and docosahexaenoic at the cost of about 10 – 30-% decrease in the same of palmitic acid , oleic acid, linoleic acid and arachidonic acid (Table 29a). In muscle the relative concentration of ω3 fatty acids viz., linolenic acid , eicosapentaenoic acid and docosahexaenoic acid increased almost by 2 fold along with about 25% decrease in ω6 fatty acidsviz., linoleic acid and arachidonic acid, 13% decrease in palmitic acid and about 26% decrease in oleic acid (Table 29b). Around 9.8% increase in stearic acid concentration and about 2 fold increase in linolenic acid , eicosapentaenoic acid and docosahexaenoic acid concentration at the cost of 23.89% decrease in oleic acid concentration, 12.12% decrease in linoleic acid concentration and 13.33% decrease in arachidonic acid concentration was observed in the intestine of the chicks supplemented with different bacteria along with the commercial feed (Table 29c). In serum (Table 29d), a decreasing trend of relative concentration of palmitic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid elevated the relative concentration of palmitoleic, linolenic acid, eicosapentaenoic acid and docosahexaenoic acid due to dietary supplementation of different strains of bacteria for a period of 30 days. All these

changes in the relative fatty acid composition of total lipid brought about a significant increase in n3/n6 ratio.

The maximum decrease in the relative percentage composition of fatty acid profiles of total lipid in tissues of *Gallus domesticus* was observed with respect to oleic acid (in liver), linoleic acid (in liver muscle and serum), arachidonic acid (in liver and muscle) and the maximum decrease in the same was observed with respect to eicosapentaenoic acid (in liver, muscle and serum), docosahexaenoic acid (in liver) with the supplementation of the B2 strain for 30 days as compared to the B1 and B3 strain.

3.4.4 Serum lipid profiles

When the broiler chicks were given diets containing different strains of bacteria (B1, B2, B3) for a period of 30 days , the total cholesterol level of serum was increased by 4.79% ($p < 0.005$) with the B1 strain and decreased by 8.09% ($p < 0.005$) with the B2 strain of bacteria, while no change in the same was detected with the supplementation of B3 strain (Table 30). The total triglycerol concentration in the serum was decreased significantly (68.70%, $p < 0.005$) with the B2 strain supplementation to the diet and was increased (54.75%,$p < 0.005$) with the B3 strain supplementation. However, there was no significant change observed with B1 strain supplementation. The HDL cholesterol concentration increased (10.07%, $p<0.025$) with the B2 strain while the same was decreased (about 2 – 18%, NS - $p < 0.005$) with the other two strains of bacteria. Supplementation of the B2 strain in the diet reduced the LDL cholesterol concentration insignificantly (6.63%). But with the supplementation of the other two strains, an

increase (32% - 2.14 fold, p <0.05- 0.005)was recorded (Figure S). Likewise the VLDL cholesterol concentration was also increased (about 54% - 3 fold, $p < 0.005 - 0.01$) with the supplementation of B1 and B3 strains of bacteria to the diet while the decrease (2.64%, NS) in the same was noticed with the B2 strain of bacteria.

3.4.5 Liver function and cardiac function test

Although three different strains of bacteria were selected for the present study, the liver function and cardiac function tests were checked with only one strain (B2).

With the supplementation of B2 strain of bacteria, there was increase (about 19% - 21%, equivocal - p<0.005) in the activity of alkaline phosphatase in the liver after 15 and / or 30 days. In serum, the activity of the said enzyme significantly decreased (about 17% - 59%, p<0.005) after 15 and 30 days of feeding the B2 strain of bacteria (Table 31 and figure T).

The activity of GPT in liver and serum remained unaltered with the supplementation of the B2 strain of bacteria for 15 and / or 30 days (Table 32 and figure U).

With the supplementation of the B2 bacterial strain the GOT activity remained unchanged in the liver after 15 days and in the serum after 15 and 30 days of feeding. However, the GOT level in the liver was decreased by about 23% (p<0.005) after 30 days of feeding the B2 bacterial strain (Table 33 and figure V).

The LDH activity in the serum did not show any significant alterations with the supplementation of the B2 strain of bacteria for 15 and / or 30 days (Figure W). However, in the liver, the LDH activity was increased by 42% (p <0.005) and 25% (NS) with the dietary supplementation of the B2 strain of bacteria for 15 and 30 days respectively (Table 34).

3.5 Cholesterol metabolism

Table 35 and figure X demonstrate the dynamics of the activity of 3 – HMG CoA Reductase and 3 – HMG CoA Synthase in chicks, when supplemented with the B2 strain of bacteria.

About 16% augmentation (p < 0.05) in liver HMG CoA Reductase activity was recorded in the chicks supplemented with the B2 bacterial strain for 15 days compared to the same of control birds. However, no change in Liver HMG CoA Reductase activity was detected with extended feeding period. About 2.5 – 3 fold increase of serum HMG CoA Reductase activity was detected with bacterial supplementation in the chick's dietfor 15 and / or 30 days (Figure X).

88% - 2.3 fold increase in the liver HMG CoA Synthase activity was detected with the supplementation of the B2 strain of bacteria for 15 and/or 30 days. Although about 3 fold increase in serum HMG CoA Synthase activity was detected in the first phase of the feeding experiment with the supplementation of B2 strain of bacteria, no such change was detected in the prolonged period of the feeding experiment (Table 35).

Figure M: Growth chart (net weight gain in grams) of chick (*Gallus domesticus*) supplemented with different strains of bacteria along with the commercial diet during post hatching development.

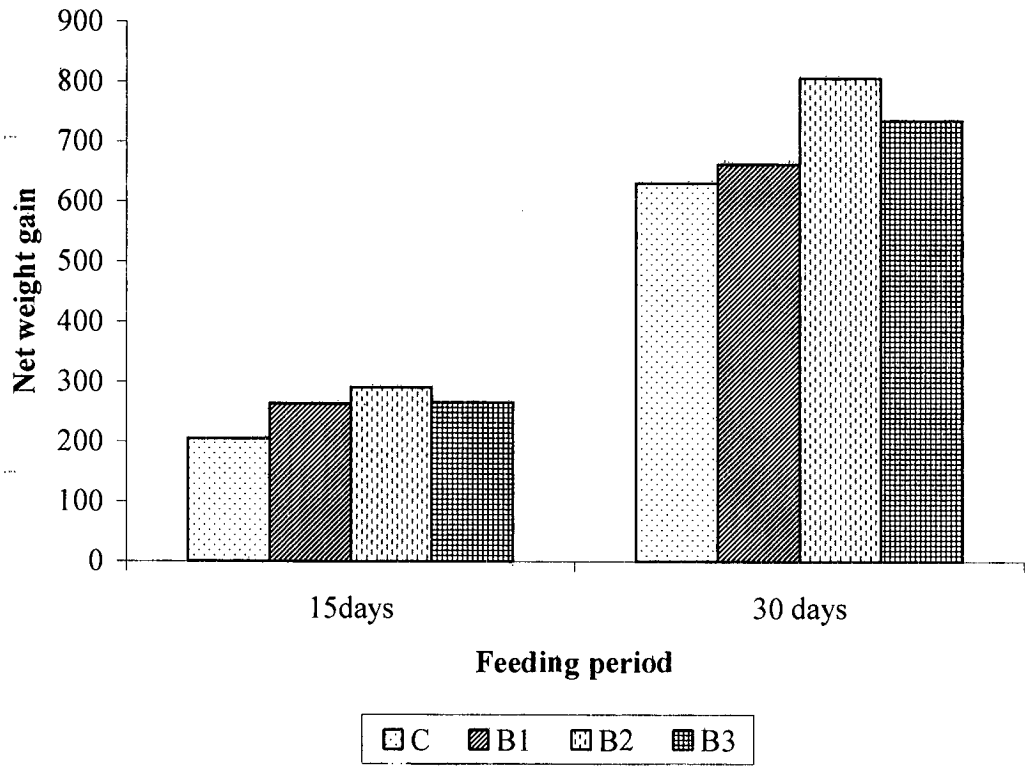
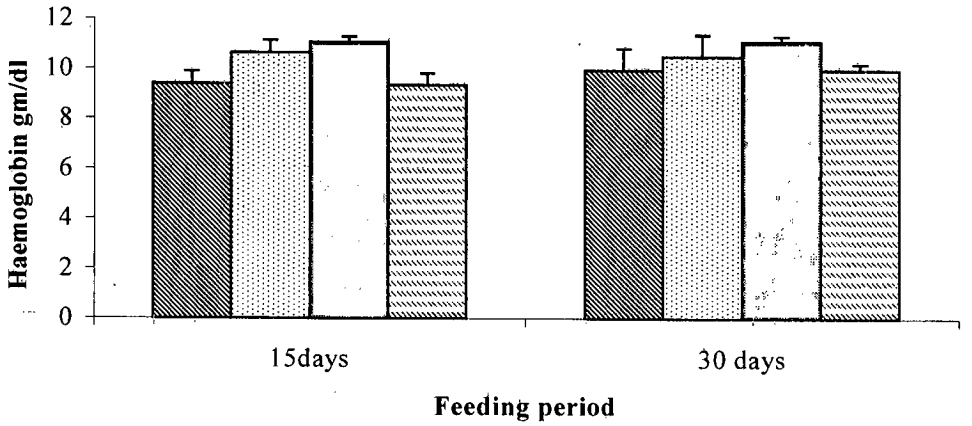
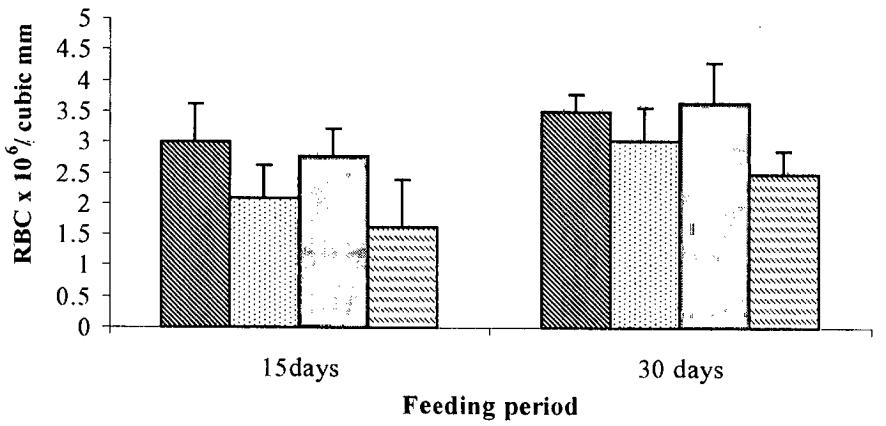


Figure N: Changes in the hematological parameters of chick (*Gallus domesticus*) supplemented with different strains of bacteria along with the commercial feed during post hatching development.

1: Haemoglobin concentration



2: Erythrocyte (RBC) count



3: Leucocyte (WBC) count

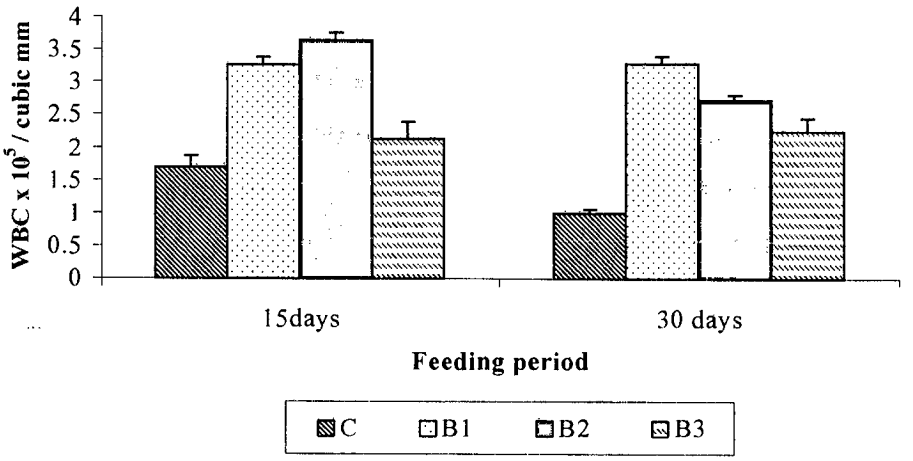
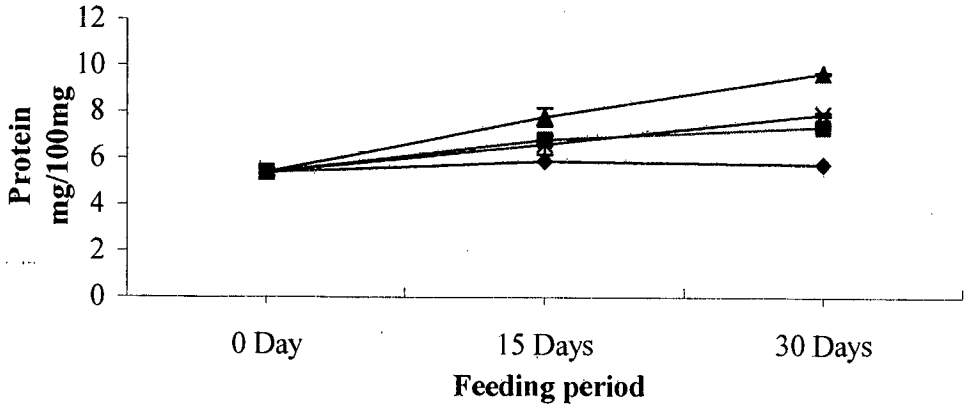
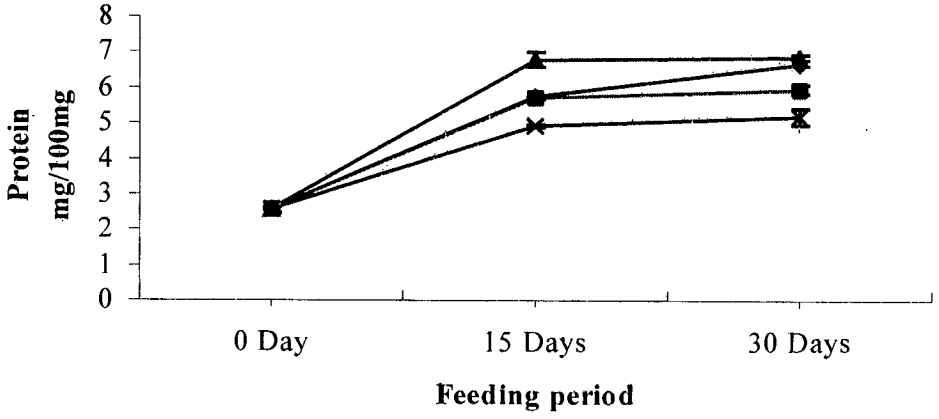


Figure O : Changes in the total Protein (mg/100mg) concentration of various tissues of broiler chick *(Gallus domesticus)* supplemented with different strains of Bacteria along with the commercial feed during post hatching development.

1: Liver



2:Muscle



3: Intestine

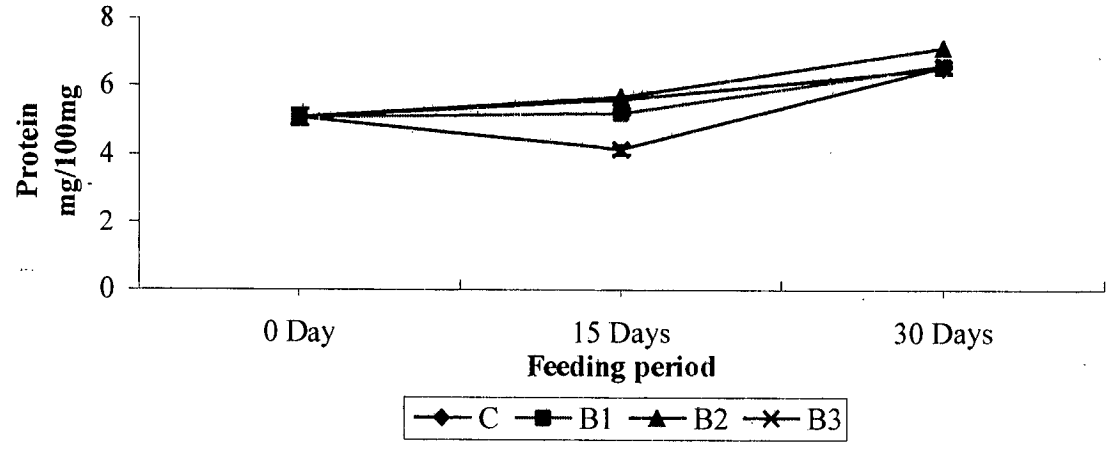
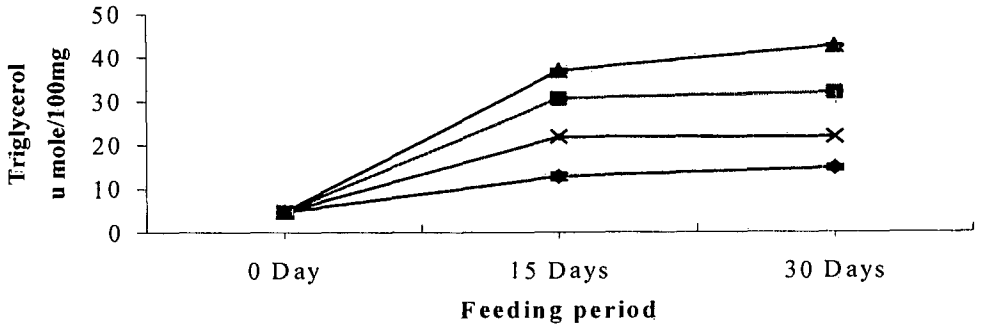
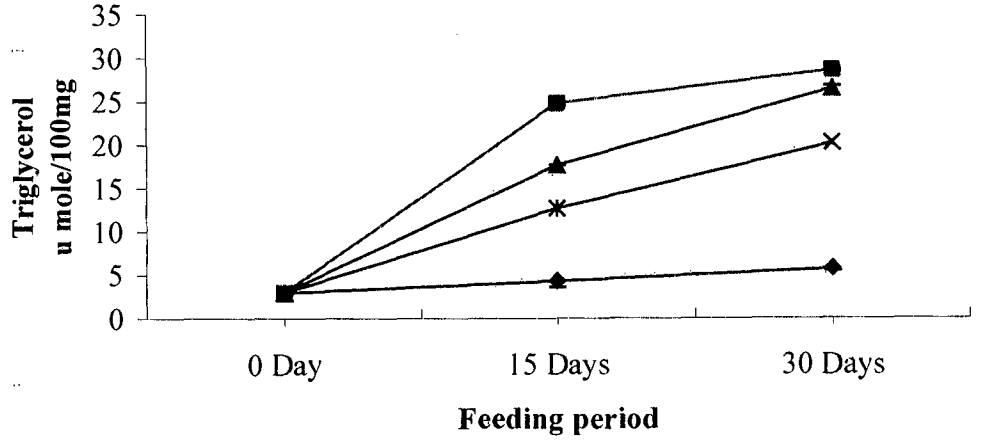


Figure P: Changes in the total Triglycerol (μ mole/100mg) concentration of various tissues of broiler chick *(Gallus domesticus)* supplemented with different Bacteria along with the commercial feed during post hatching development.

1: Liver



2: Muscle



3: Intestine

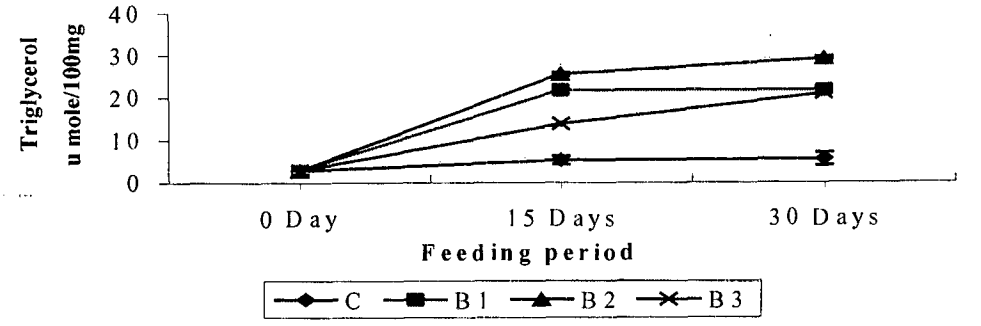
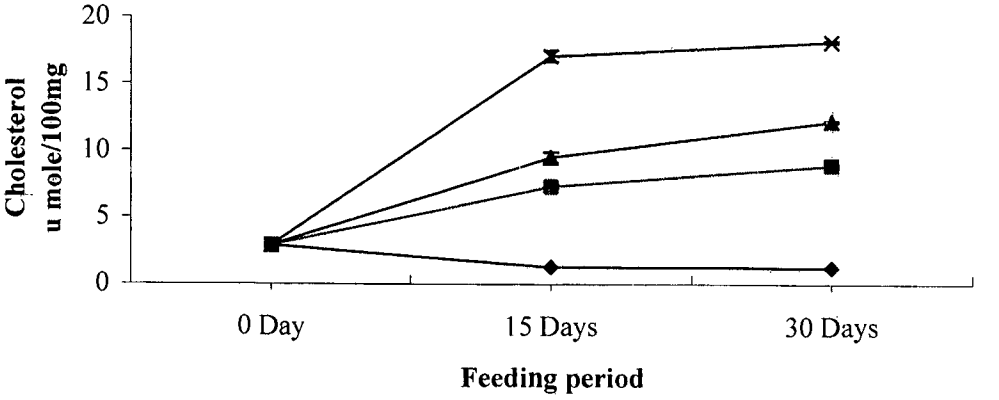
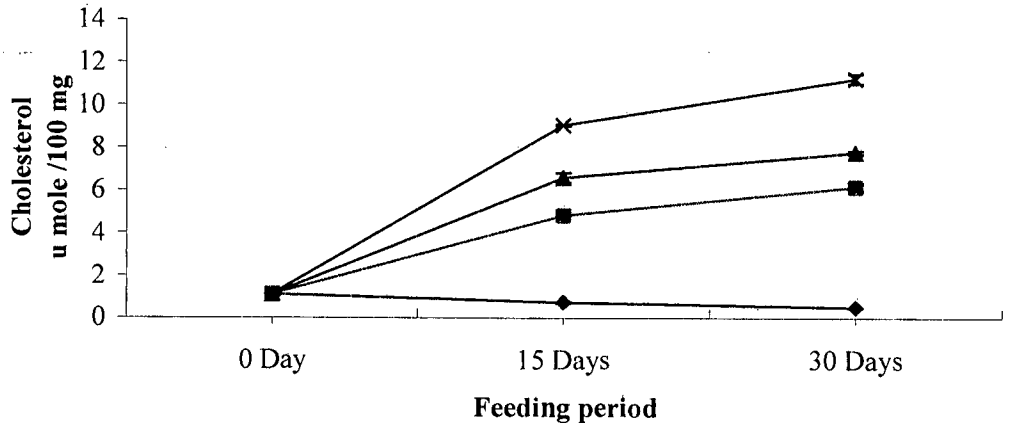


Figure Q: Changes in the total Cholesterol (μ mole/100mg) Concentration of various tissues of broiler chick *(Gallus domesticus)* supplemented with different strains of Bacteria along with the commercial feed during post hatching development.

1: Liver



2: Muscle



3: Intestine

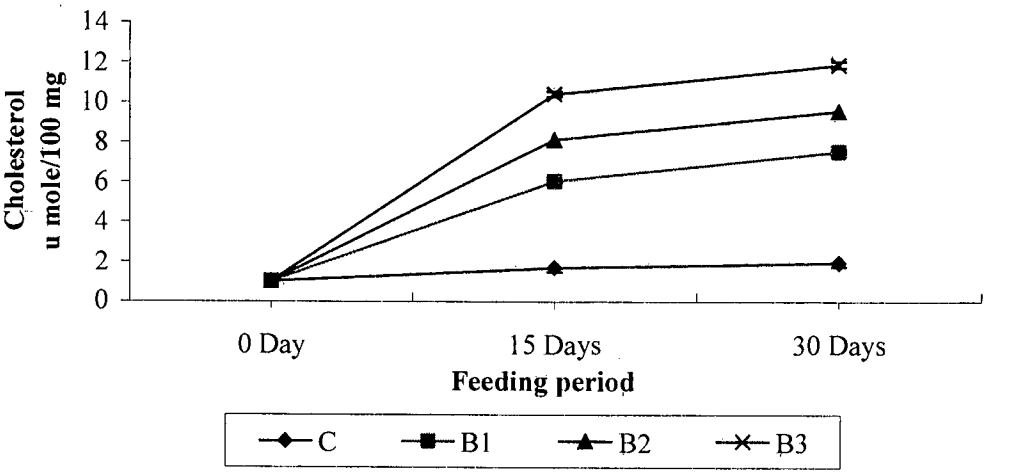
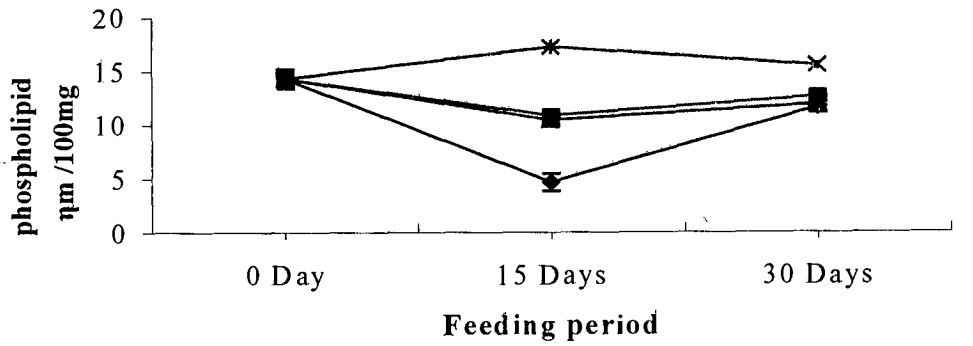
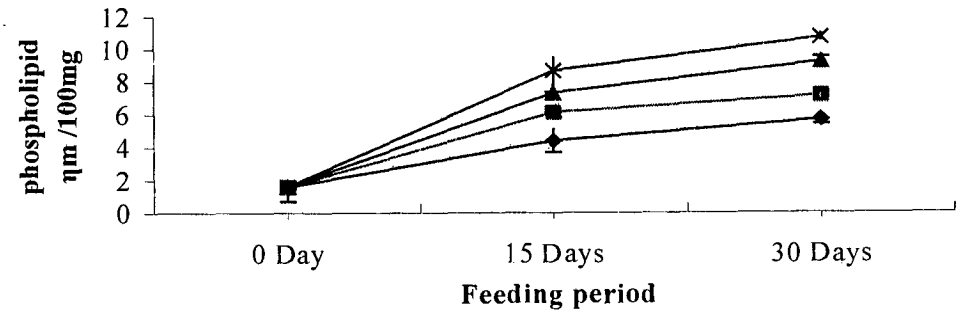


Figure R: Changes in the total phospholipid(ηmole/100mg)concentration of various tissues of broiler chick*(Gallus domesticus)* supplemented with different strains of Bacteria along with the commercial feed during post hatching development.

1: Liver



2: Muscle



3: Intestine

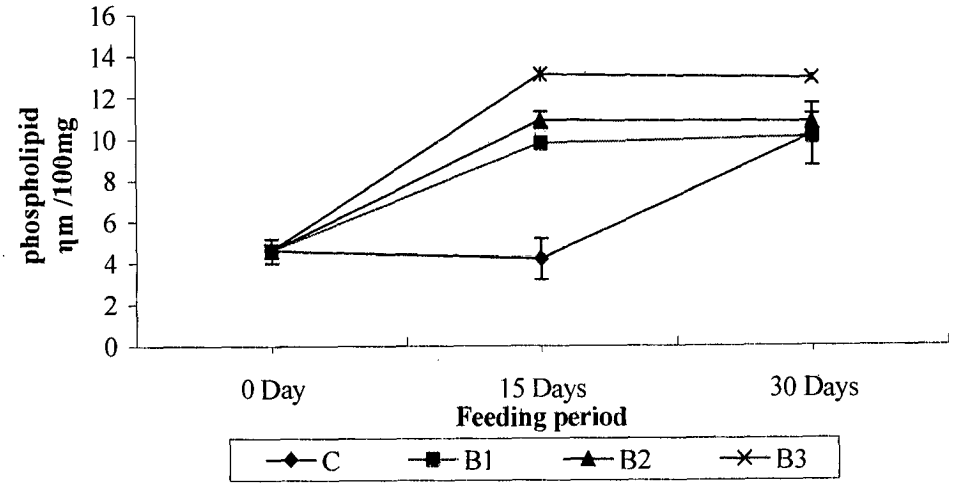


Figure S: Changes in the Serum lipid profiles of the chick (*Gallus domesticus*) supplemented with different strains of Bacteria for 30 days along with the commercial feed during post hatching development.

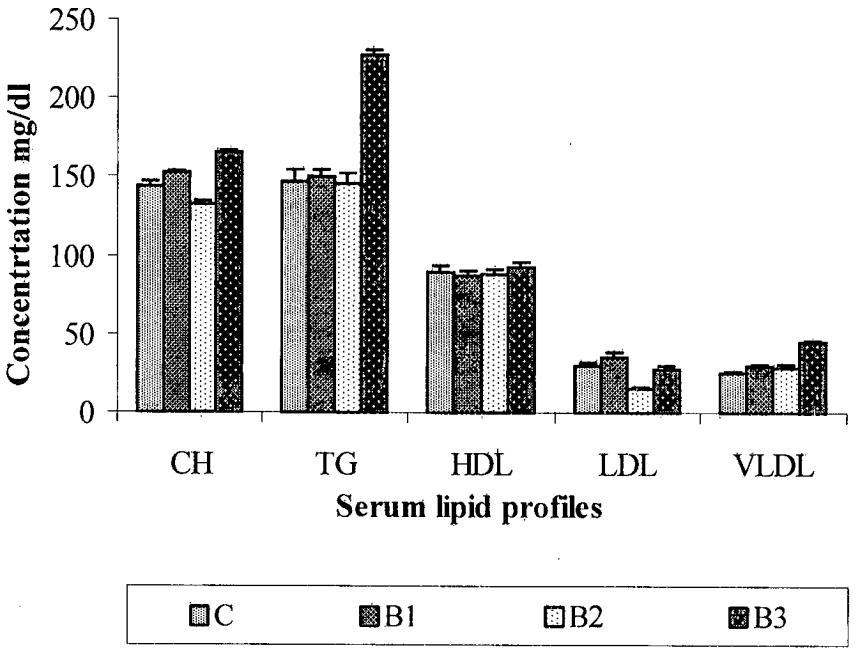


Figure T: Activity of Alkaline Phosphatase (ALP) of broiler chick (*Gallus domesticus*) supplemented with B2 bacterial strain along with the commercial diet during post hatching development.

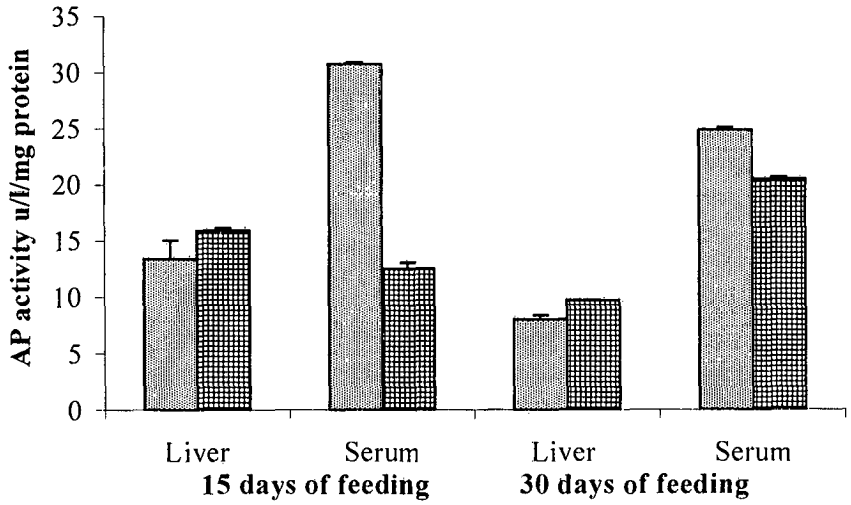


Figure U: Activity of Glutamate Pyruvate Transaminase (GPT) of broiler chick (*Gallus domesticus*) supplemented with B2 bacterial strain along with the commercial feed during post hatching development.

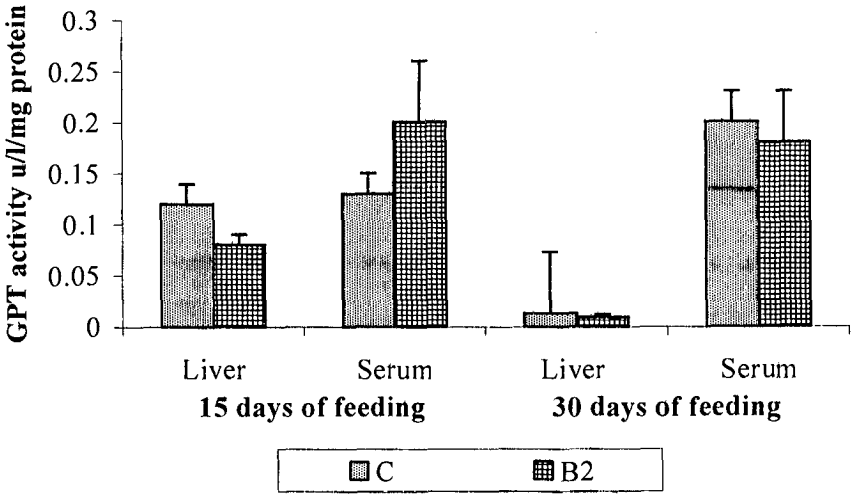


Figure V: Activity of Glutamate Oxaloacetate Transaminase (GOT) of the broiler chick (*Gallus domesticus*) supplemented with B2bacterial strain along with the commercial feed during post hatching development.

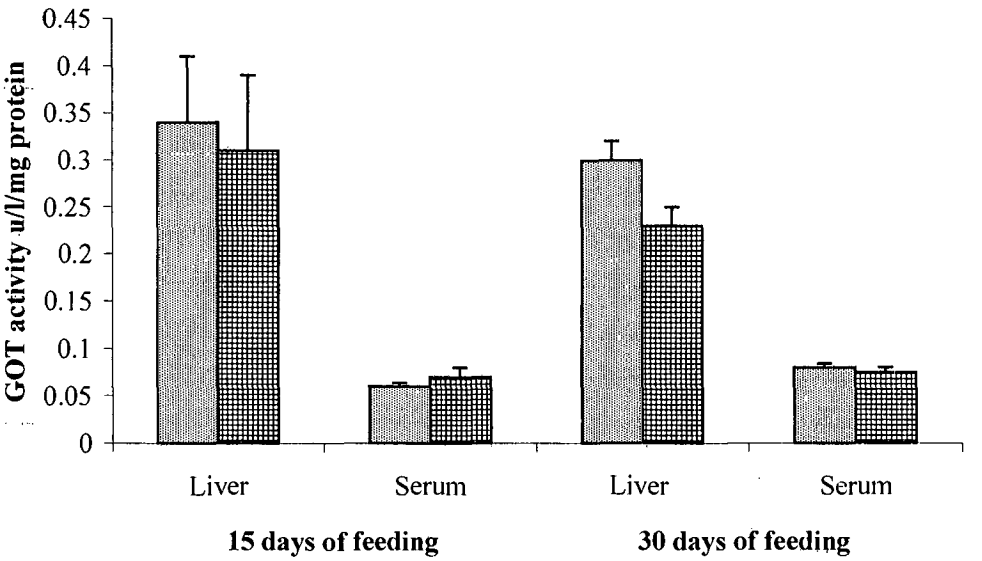


Figure W: Activity of Lactate Dehydrogenase (LDH) of the broilerchick (*Gallus domesticus*) supplemented with B2 bacterial strain along with the commercial feed during post hatching development.

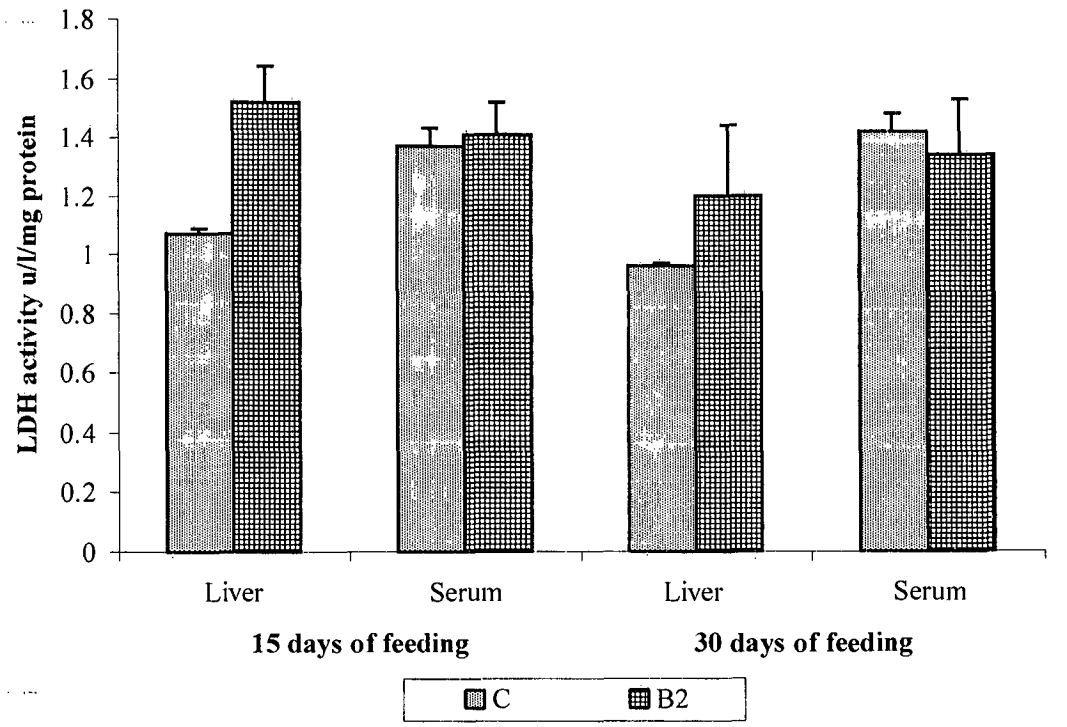
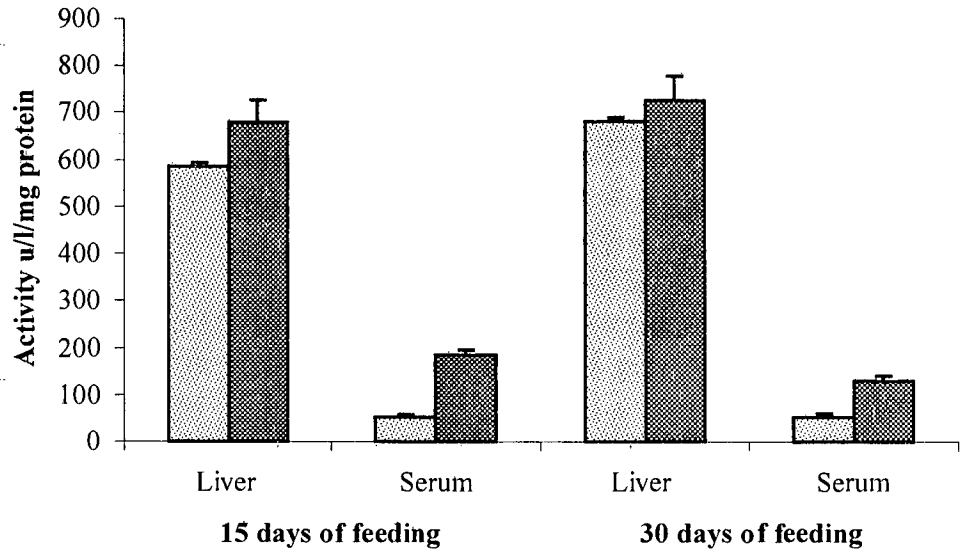


Figure X: Activity of regulatory enzymes of Cholesterol metabolism in broiler chick (*Gallus domesticus*) supplemented with B2 bacterial strain along with the commercial feed during post hatching development.

1: 3-HMG-CoA- Reductase



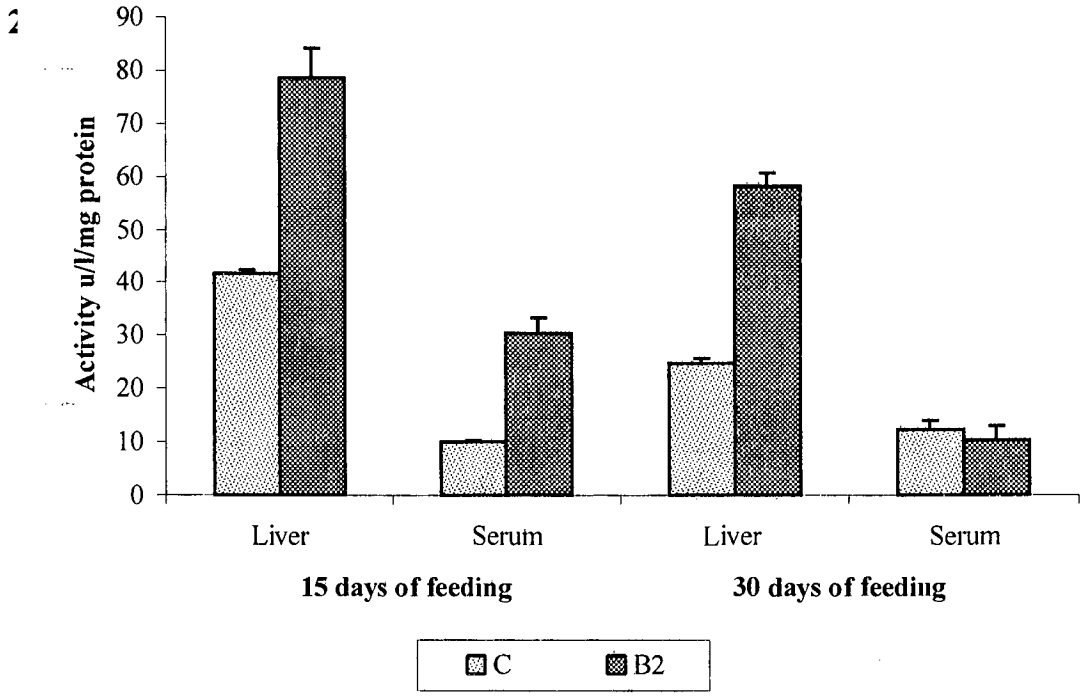


Table 23: Growth chart of broiler chick (*Gallus domesticus*) Supplemented with different strains of Bacteria along with the commercial feed during post hatching development

(Mean values of six samples and their standard error)

Feeding period	Parameters	I	C	B1	B2	B3
15 days	Average weight of the birds (gm)	68.27 ± 3.86	272.76 ± 10.23	331.25 ± 9.25	358.95 ± 10.23	334.68 ± 8.25
	Net weight gain (gm)	---	204.49	262.98	290.68	266.41
	Daily instantaneous growth rate G_w	---	0.092	0.105	0.110	0.105
30 days	Average weight of the birds (gm)	68.27 ± 3.86	700.00 ±15.67	731.30 ± 15.67	875.70 ± 20.30	805.40 ± 10.27
	Net weight gain (gm)	---	631.73	663.03	807.43	737.13
	Daily instantaneous growth rate Gw	---	0.077	0.079	0.085	0.082

Table 23a : Statistical calculation (comparison test for by using student 't' test) of table 23

Comparing group	15 days			30 days		
	% change	't' value	*p* value	% change	't' value	*p* value
C vs B1	21.44↑	4.24	<0.005	4.47↑	1.41	Equivocal
C vs B2	31.59↑	5.95	<0.005	25.10↑	6.85	<0.005
C vs B3	22.70↑	4.71	<0.005	15.05↑	5.62	<0.005
B1 vs B2	8.36↑	2.00	<0.05	19.74↑	5.63	<0.005
B2 vs B3	6.76↓	1.84	<0.05	8.02↓	3.09	<0.01

Table 24: Changes in the hematological parameters of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error.)

Feeding period	Parameters	C	B1	B2	B3
15 days	Hemoglobin Gm /dl	9.38 ± 0.49	10.64 ± 0.12	11.06 ± 0.21	9.34 ± 0.46
	Total erythrocytes $x\ 10^6$ /cubic mm	3.00 ±0.62	2.08 ± 0.56	2.76 ± 0.45	1.62 ± 0.78
	Total Leucocytes $x\ 10^5$/cubic mm	1.69 ± 0.18	3.26 ± 0.12	3.64 ± 0.13	2.13 ± 0.25
30 days	Hemoglobin Gm /dl	9.94 ± 0.88	10.48 ± 0.22	11.08 ± 0.21	9.94 ± 0.24
	Total erythrocytes $x\ 10^6$ /cubic mm	3.50 ± 0.28	3.02 ± 0.55	3.65 ± 0.65	2.48 ± 0.38
	Total Leucocytes $x\ 10^5$/cubic mm	1.00 ± 0.05	3.29 ± 0.11	2.72 ± 0.08	2.25 ± 0.19

Table 24a: Statistical calculation (comparison test by using student 't' test) of table 24

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	' 't' value	*p* value
C vs B1	Hemoglobin Gm /dl	13.43↑	2.50	<0.025	5.43↑	0.59	NS
C vs B2		17.91↑	3.15	<0.01	11.46↑	1.19	NS
C vs B3		0.42↓	0.06	NS	no change	---	---
B1 vs B2		3.94↑	1.74	Equivocal	5.72↑	1.97	Equivocal
B2 vs B3		15.55↓	3.40	<0.005	10.28↓	3.58	<0.005
C vs B1	Total erythrocytes $x\ 10^6$ /cubic mm	30.66↓	1.10	NS	13.71↓	0.77	NS
C vs B2		8.00↓	0.31	NS	4.28↑	0.21	NS
C vs B3		46.00↓	1.38	Equivocal	29.14↓	2.16	<0.05
B1 vs B2		32.69↑	0.94	NS	20.86↑	0.74	NS
B2 vs B3		41.30↓	1.26	NS	32.05↓	1.55	Equivocal
C vs B1	Total Leucocytes $x\ 10^5$/cubic mm	92.89↑	7.26	<0.005	3.29fold↑	19.08	<0.005
C vs B2		2.15fold↑	8.78	<0.005	2.72fold↑	18.29	<0.005
C vs B3		26.03↑	1.42	Equivocal	2.25fold↑	6.37	<0.005
B1 vs B2		11.65↑	2.15	<0.05	17.32↓	4.19	<0.005
B2 vs B3		41.48↓	5.37	<0.005	17.28↓	2.28	<0.025

Table 25: Changes in the tissue total Protein (mg/100mg) concentration of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development.
(Mean values of six samples and their standard error)

Feeding period	Tissues	C	B1	B2	B3
15 days	Liver	5.88 ± 0.37	6.79 ± 0.08	7.78 ± 0.05	6.60 ± 0.25
	Muscle	5.75 ± 0.35	5.72 ± 0.07	6.80 ± 0.05	4.92 ± 0.49
	Intestine	5.60 ± 0.25	5.20 ± 0.04	5.69 ± 0.14	4.13 ± 0.53
30 days	Liver	5.73 ± 0.28	7.36 ± 0.12	9.66 ± 0.12	7.90 ± 0.70
	Muscle	6.66 ± 0.22	5.97 ± 0.08	6.86 ± 0.20	5.20 ± 0.70
	Intestine	6.55 ± 0.15	6.60 ± 0.15	7.16 ± 0.12	6.58 ± 0.40

Table 25a: Statistical calculation (comparison test by using student 't' test) of table 25

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	' 't' value	*p* value
C vs B1	Liver	15.47↑	2.40	<0.025	28.44↑	5.36	<0.005
C vs B2		32.31↑	5.09	<0.005	68.58↑	12.92	<0.005
C vs B3		12.24↑	1.61	Equivocal	37.87↑	2.88	<0.01
B1 vs B2		14.58↑	11.00	<0.005	31.25↑	13.60	<0.005
B2 vs B3		15.16↓	4.64	<0.005	18.22↓	2.47	<0.025
C vs B1	Muscle	0.52↓	0.08	NS	10.36↓	2.95	<0.01
C vs B2		18.26↑	2.97	<0.01	3.00↑	0.67	NS
C vs B3		14.43↓	1.37	NS	21.92↓	1.99	<0.05
B1 vs B2		18.88↑	12.55	<0.005	14.90↑	4.13	<0.005
B2 vs B3		27.64↓	3.82	<0.005	24.19↓	2.28	<0.025
C vs B1	Intestine	7.14↓	1.58	Equivocal	0.76↑	0.23	NS
C vs B2		1.60↑	0.31	NS	9.31↑	3.17	<0.005
C vs B3		26.25↓	2.50	<0.025	0.45↑	0.07	NS
B1 vs B2		9.42↑	3.37	<0.005	8.48↑	2.91	<0.01
B2 vs B3		27.41↓	2.84	<0.01	8.10↓	1.39	Equivocal

Table 26 : Changes in the total Triacylglycerol (µ mole/100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development.
(Mean value of six samples and their standard error)

Feeding period	Tissues	C	B1	B2	B3
15 days	Liver	12.86 ± 0.84	30.69 ± 1.71	36.96 ± 1.36	21.94 ± 2.90
	Muscle	4.30 ± 0.25	24.77 ± 1.33	17.66 ± 1.09	12.68 ± 0.55
	Intestine	5.20 ± 0.20	21.75 ± 1.20	25.48 ± 1.40	13.78 ± 0.01
30 days	Liver	14.69 ± 0.66	32.02 ± 1.00	42.61 ± 1.70	21.80 ± 0.40
	Muscle	5.74 ± 0.35	28.62 ± 0.75	26.48 ± 1.00	20.24 ± 0.05
	Intestine	5.44 ± 0.35	21.64 ± 1.48	29.18 ± 0.57	20.86 ± 0.13

Table 26a: Statistical calculation (comparison test by using student 't' test) of table 26

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs B1	Liver	2.38 fold ↑	9.35	<0.005	2.17fold↑	14.46	<0.005
C vs B2		2.87 fold ↑	15.08	<0.005	2.9 fold↑	15.31	<0.005
C vs B3		70.60↑	3.00	<0.005	48.40↑	9.21	<0.005
B1 vs B2		20.43↑	2.87	<0.01	33.07↑	5.36	<0.005
B2 vs B3		40.63↓	4.68	<0.005	48.83↓	11.91	<0.005
C vs B1	Muscle	5.76 fold↑	15.12	<0.005	4.98fold↑	28.35	<0.005
C vs B2		4.10 fold ↑	11.94	<0.005	4.61fold↑	19.86	<0.005
C vs B3		2.94 fold↑	13.87	<0.005	3.52fold↑	47.69	<0.005
B1 vs B2		28.70↓	4.13	<0.005	7.47↓	1.71	Equivocal
B2 vs B3		29.19↓	4.08	<0.005	23.56↓	6.24	<0.005
C vs B1	Intestine	4.18 fold↑	13.61	<0.005	3.97fold↑	10.62	<0.005
C vs B2		4.9 fold ↑	14.34	<0.005	5.36fold↑	35.53	<0.005
C vs B3		2.65 fold ↑	42.90	<0.005	3.83fold↑	41.34	<0.005
B1 vs B2		17.14↑	2.02	<0.05	34.84↑	4.75	<0.005
B2 vs B3		45.91↓	8.35	<0.005	28.51↑	14.24	<0.005

Table 27 : Changes in the tissue total Cholesterol (μ mole/100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development
(Mean value of six samples and their standard error)

Feeding period	Tissues	C	B1	B2	B3
15 days	Liver	1.25 ± 0.10	7.28 ±0.58	9.46 ±1.02	17.05 ±0.73
	Muscle	0.75 ± 0.06	4.82 ±0.31	6.59 ±0.14	9.05 ± 0.54
	Intestine	1.70 ± 0.20	6.05 ±0.01	8.14 ±0.96	10.44 ± 1.40
30 days	Liver	1.20 ± 0.05	8.88 ±0.40	12.15 ±0.04	18.14 ±1.50
	Muscle	0.50 ± 0.02	6.16 ±0.28	7.78 ±0.41	11.25 ±0.11
	Intestine	1.95 ± 0.19	7.55 ±0.25	9.59 ±0.14	11.91 ±1.50

Table 27a: Statistical calculation (comparison test by using student 't' test) of table 27

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs B1	Liver	5.82fold↑	10.25	<0.005	7.4fold↑	19.05	<0.005
C vs B2		7.56fold↑	8.01	<0.005	10.12fold↑	171.09	<0.005
C vs B3		13.64fold↑	21.46	<0.005	15.11fold↑	11.29	<0.005
B1 vs B2		29.94↑	1.85	Equivocal	36.82↑	8.15	<0.005
B2 vs B3		80.23↑	6.05	<0.005	49.30↑	3.99	<0.005
C vs B1	Muscle	6.42 fold↑	12.92	<0.005	12.32fold↑	20.21	<0.005
C vs B2		8.78fold↑	38.42	<0.005	15.56fold↑	17.75	<0.005
C vs B3		12.06fold↑	15.28	<0.005	22.5fold↑	96.84	<0.005
B1 vs B2		36.72↑	5.20	<0.005	26.29↑	3.26	<0.005
B2 vs B3		37.32↑	4.41	<0.005	44.60↑	8.18	<0.005
C vs B1	Intestine	3.55fold↑	21.75	<0.005	3.87fold↑	17.83	<0.005
C vs B2		4.78 fold↑	6.57	<0.005	4.91fold↑	32.37	<0.005
C vs B3		6.14 fold↑	6.18	<0.005	6.10fold↑	6.59	<0.005
B1 vs B2		34.54↑	2.17	<0.05	27.01↑	7.13	<0.005
B2 vs B3		28.25↑	1.35	NS	24.19↑	1.54	<0.005

Table 28 : Changes in the tissue total Phospholipid ((ηmole/100 mg of tissue) concentration of the broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development
(Mean value of six samples and their standard error)

Feeding period	Tissues	C	B1	B2	B3
15 days	Liver	4.65 ± 0.70	10.80 ± 0.16	10.40 ± 0.10	17.23 ± 0.88
	Muscle	4.40 ± 0.70	6.14 ± 0.02	7.31 ± 0.03	8.67 ± 0.80
	Intestine	4.20 ± 1.00	9.80 ± 0.08	10.90 ± 0.05	13.10 ± 0.01
30 days	Liver	11.60 ± 0.50	12.60 ± 0.23	11.89 ± 0.15	15.50 ± 0.24
	Muscle	5.70 ± 0.30	7.16 ± 0.03	9.22 ± 0.02	10.70 ± 0.12
	Intestine	10.20 ± 1.50	10.10 ± 0.10	10.82 ± 0.12	12.92 ± 0.01

Table 28a: Statistical calculation (comparison test by using student 't' test) of table 28

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs B1	Liver	2.32fold↑	8.56	<0.005	8.62↑	1.81	<0.05
C vs B2		2.23fold↑	8.13	<0.005	2.50↑	0.55	NS
C vs B3		3.70fold↑	11.18	<0.005	33.62↑	7.03	<0.005
B1 vs B2		3.70↓	2.12	<0.05	5.63↑	2.58	<0.025
B2 vs B3		65.67↓	7.71	<0.005	30.36↑	12.75	<0.005
C vs B1	Muscle	39.54↑	2.48	<0.025	4.21fold↑	4.84	<0.005
C vs B2		66.13↑	4.15	<0.005	5.42fold↑	11.70	<0.005
C vs B3		97.04↑	4.01	<0.005	6.29fold↑	15.47	<0.005
B1 vs B2		19.05↑	32.45	<0.005	28.77↑	57.13	<0.005
B2 vs B3		18.60↑	1.69	Equivocal	16.05↑	12.16	<0.005
C vs B1	Intestine	2.33fold↑	5.58	<0.005	0.98↑	0.06	NS
C vs B2		2.59fold↑	6.69	<0.005	6.07↑	0.41	NS
C vs B3		3.11fold↑	8.90	<0.005	7.05↑	1.81	<0.05
B1 vs B2		11.22↑	11.66	<0.005	7.12↑	4.60	<0.005
B2 vs B3		20.18↑	43.16	<0.005	19.40↑	17.44	<0.005

Table 29a : Fatty acid profiles (relative percent composition) of Liver total lipid of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed for 30 days.
(Mean values of 3 set of samples are presented)

Fatty acid	Control	B1	B2	B3
16:0	29.8 [a]	26.2	27.1	28.00
16:1	4.20 [a]	5.80	6.00	5.50
18:0	18.2 [c]	19.00	20.50	19.50
18:1	15.2 [a]	12.50	10.50	11.80
18:2 (ω6)	15.3 [a]	14.00	12.00 [b]	13.50
18:3 (ω3)	1.20 [a]	3.50	3.00	3.00
20:4 (ω6)	10.20 [a]	8.20	7.00 [b]	8.00
20:5 (ω3)	2.40 [a]	4.80	6.50 [b]	4.00
22:6(ω3)	1.40 [a]	3.60	4.50 [b]	3.00
Others	2.10	2.40	2.90	3.70
n3/n6	0.19	0.54	0.74	0.46

a – significant change ($p < 0.05$) over the same of B1, B2 and B3
b - significant change ($p < 0.05$) over the same of B1 and B3
c - significant change ($p < 0.05$) over the same of B2

Table 29b: Fatty acid profiles (relative percent composition) of Muscle total lipid of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed for 30 days.
(Mean values of 3 set of samples are presented)

Fatty acid	Control	B1	B2	B3
16:0	28.30[a]	25.20	24.50	26.10
16:1	3.60	3.20	4.10	3.90
18:0	14.30	15.20	14.60	15.00
18:1	19.30[a]	15.20	14.20	15.00
18:2 (ω6)	14.00[a]	12.00	10.50[b]	11.50
18:3 (ω3)	2.50[a]	4.00	5.00	4.00
20:4 (ω6)	9.30[a]	8.00	7.0[b]	8.50
20:5 (ω3)	2.20[a]	4.40	5.80[b]	5.00
22:6(ω3)	2.60[a]	4.20	5.50	4.80
Others	3.90	8.60	8.80	6.20
n3/n6	0.31	0.63	0.93	0.69

a – significant change ($p < 0.05$) over the same of B1, B2 and B3
b - significant change ($p < 0.05$) over the same of B1 and B3
c - significant change ($p < 0.05$) over the same of B2

Table 29c: Fatty acid profiles (relative percent composition) of Intestine total lipid of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed for 30 days.
(Mean values of 3 set of samples are presented)

Fatty acid	Control	B1	B2	B3
16:0	27.50	25.90	26.50	27.00
16:1	4.40	5.60	4.80	4.50
18:0	10.20[a]	12.00	11.20	11.00
18:1	27.20[a]	22.50	20.70	21.30
18:2 (ω6)	16.50[a]	15.00	14.50	15.50
18:3 (ω3)	2.40[a]	4.80	5.20	4.60
20:4 (ω6)	7.50[c]	7.00	6.50	7.20
20:5 (ω3)	1.50[c]	2.50	3.00	2.80
22:6(ω3)	1.40[c]	2.10	3.80	2.50
Others	1.40	2.60	3.80	3.60
n3/n6	0.22	0.43	0.57	0.44

a – significant change ($p < 0.05$) over the same of B1, B2 and B3
b - significant change ($p < 0.05$) over the same of B1 and B3
c - significant change ($p < 0.05$) over the same of B2

Table 29d: Fatty acid profiles (relative percent composition) of Serum total lipid of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed for 30 days.
(Mean values of 3 set of samples are presented)

Fatty acid	Control	B1	B2	B3
16:0	22.67[a]	21.50	20.78	21.50
16:1	1.20[a]	3.50	3.50	4.20
18:0	23.45[a]	20.10	22.12	20.56
18:1	11.35[a]	9.20	8.54	8.78
18:2 (ω6)	26.37[a]	22.45	21.00[b]	23.20
18:3 (ω3)	0.50[a]	1.20	2.50	2.00
20:4 (ω6)	10.34[a]	8.20	7.80	8.50
20:5 (ω3)	1.06[a]	4.35	5.70[b]	4.10
22:6(ω3)	1.30[a]	4.60	4.80	4.00
Others	1.76	4.90	3.26	3.18
n3/n6	0.08	0.33	0.45	0.32

a – significant change ($p < 0.05$) over the same of B1, B2 and B3
b - significant change ($p < 0.05$) over the same of B1 and B3
c - significant change ($p < 0.05$) over the same of B2

Table 30: Changes in the serum lipid profiles of the broier chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed for 30 days of post hatching development.

(Mean values of six samples and their standard error)

Serum Lipid Profiles	30 days feeding			
	C	B1	B2	B3
Total CH	146.00 ± 1.09	153.00 ± 1.05	133.00 ±2.25	146.00 ±1.27
Serum TG	147.33 ± 2.75	150.00 ± 2.65	46.10 ± 1.10	228.00 ±3.26
HDL CH	89.67 ± 3.48	87.50 ± 2.30	98.70 ± 1.45	73.00 ±1.50
LDLCH	26.86 ±1.83	35.50 ± 3.35	25.08 ± 1.05	57.40 ± 2.16
VLDL CH	9.47 ± 1.55	30.00 ± 1.06	9.22 ± 0.75	14.60 ± 0.95
CH:HDL	1.63	1.75	1.35	2.00
CH:TG	0.99	1.02	2.88	0.64

Table 30a : Statistical calculation (comparison test by using student 't' test) of Table 30

Comparing groups	Parameters	% change	't' value	*p* value
C v/s B1		4.79↑	4.63	<0.005
C v/s B2		8.09↓	5.20	<0.005
C v/s B3	**Total**	no chnage	---	---
B1 v/s B2	**Cholesterol**	13.07↓	8.06	<0.005
B2 v/s B3		9.77↑	5.04	<0.005
C v/s B1		1.81↑	0.69	NS
C v/s B2		68.70↓	34.19	<0.005
C v/s B3	**Total**	54.75↑	18.94	<0.005
B1 v/s B2	**Triglycerol**	69.26↓	36.20	<0.005
B2 v/s B3		4.94 fold↑	52.88	<0.005
C v/s B1		2.42↓	0.52	NS
C v/s B2		10.07↑	2.39	<0.025
C v/s B3	**HDL**	18.59↓	4.39	<0.005
B1 v/s B2		12.8↑	4.12	<0.005
B2 v/s B3		26.04↓	12.29	<0.005
C v/s B1		32.17↑	2.26	<0.05
C v/s B2		6.63↓	0.85	NS
C v/s B3	**LDL**	2.14 fold↑	10.79	<0.005
B1 v/s B2		29.35↓	2.97	<0.01
B2 v/s B3		2.29 fold ↑	13.47	<0.005
C v/s B1		3.17 fold↑	10.92	<0.005
C v/s B2		2.64↓	0.15	NS
C v/s B3	**VLDL**	54.17↑	2.82	<0.01
B1 v/s B2		69.27↓	16.10	<0.005
B2 v/s B3		58.35↑	4.45	<0.005

Table 31: Changes in the Alkaline Phosphatase (μ mole/ml/mg protein)concentration of broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development. (Mean values of six samples and their standard error)

Feeding period	Tissues	C	B2
15 days	Liver	13.37 ± 1.64	15. 92 ± 0.20
	Serum	30.77 ± 0.13	12.507 ± 0.57
30 days	Liver	8.00 ± 0.38	9.69 ± 0.04
	Serum	24.79 ± 0.23	20.46 ± 0.16

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*P* value	% change	't' value	*p* value
C vs B2	Liver	19.07↑	1.54	Equivocal	21.12↑	4.42	<0.005
C vs B2	Serum	59.37↓	31.25	<0.005	17.46↓	15.45	<0.005

Table 32: Changes in the Glutamate Pyruvate Transaminase (μ mole/ml/mg protein) concentration of the broiler chick (*Gallus domesticus*) supplemented with different strains Bacteria along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	B2
15 days	Liver	0.12 ± 0.02	0.08 ± 0.1
	Serum	0.13 ± 0.02	0.20 ± 0.006
30 days	Liver	0.013 ± 0.06	0.009 ± 0.003
	Serum	0.20 ± 0.03	0.18 ± 0.05

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs B2	Liver	33.33↓	0.39	NS	30.76↓	0.59	NS
C vs B2	Serum	53.84↑	0.11	NS	10.00↓	0.34	NS

Table 33: Changes in the Glutamate Oxaloacetate Transaminase (μ mole/ml/mg protein) concentration of the broiler chick (*Gallus domesticus*) supplemented with different Bacteria along with the commercial feed during post hatching development.

(Mean values of six samples and their standard error)

Feeding period	Tissues	C	B2
15 days	Liver	0.34 ± 0.07	0.31 ± 0.08
	Serum	0.06 ± 0.004	0.07 ± 0.01
30 days	Liver	0.30 ± 0.02	0.23 ± 0.02
	Serum	0.08 ± 0.004	0.075 ± 0.005

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*p* value
C vs B2	Liver	8.82↓	0.28	NS	23.33↓	3.48	<0.005
C vs B2	Serum	16.66↑	0.93	NS	6.25↓	0.78	NS

Table 34: Changes in the Lactate Dehydrogenase (μ mole/ml/mg protein) concentration of the broiler chick (*Gallus domesticus*) supplemented with different strains of Bacteria along with the commercial feed during post hatching development

(Mean value of six samples and their standard error)

Feeding period	Tissues	C	B2
15 days	Liver	1.07 ± 0.02	1.52 ± 0.12
	Serum	1.37 ± 0.06	1.41 ± 0.11
30 days	Liver	0.96 ± 0.01	1.20 ± 0.24
	Serum	1.42 ± 0.06	1.34 ± 0.19

Statistical calculation (comparison test by using student 't' test)

Comparing group	Tissues	15 days			30 days		
		% change	't' value	*p* value	% change	't' value	*P* value
C vs B2	Liver	42.00↑	3.69	<0.005	25.00↑	0.99	NS
C vs B2	serum	2.92↑	0.32	NS	5.63↓	0.40	NS

Table 35: Activity of 3-HMG-CoA- Reductase (u/l/mg protein) and 3-HMG-CoA- Synthase (u/l/mg protein) in broiler chick (*Gallus Domesticus)* supplemented with different strains of Bacteria along withthe commercial feed during post hatching development.

Feeding period	Tissues	3-HMG-CoA- Reductase		3 - HMG-CoA- Synthase	
		C	B2	C	B2
15 days	Liver	585.97 ± 6.89	679.82 ± 46.76	41.68 ± 0.75	78.43 ± 5.67
	serum	52.47 ± 5.92	184.19 ± 12.28	9.99 ± 0.33	30.36 ± 2.79
30 days	Liver	681.21 ± 8.17	727.25 ± 50.28	24.60 ± 1.03	58.27 ± 2.27
	serum	51.61 ± 7.92	130.26 ± 10.37	12.18 ± 1.85	10.37 ± 2.56

Statistical calculation (comparison test by using student 't' test)

Comparing group	Feeding period	Tissues	3-HMG-CoA- Reductase			HMG-CoA- Synthase		
			% change	't' value	*p* value	% change	't' Value	*p* value
C vs B2	15 days	Liver	16.00 ↑	1.97	<0.05	88.00↑	6.43	<0.005
C vs B2		Serum	3.51 fold↑	9.66	<0.005	3.03 fold↑	7.25	<0.005
C vs B2	30 days	Liver	67.00↑	0.90	NS	2.36 fold↑	13.50	<0.005
C vs B2		Serum	2.52 fold↑	6.03	<0.005	14.86 ↓	0.57	NS

Discussion

ABBREVIATIONS

PUFA	Polyunsaturated fatty acid
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein
GPT	Glutamate pyruvate transaminase
GOT	Glutamate oxaloacetate transaminase
LDH	Lactate dehydrogenase
ALP	Alkaline phosphatase
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
FCR	Feed conversion ratio
Gw	Daily instantaneous growth rate
N	Normality
CH	Cholesterol
TG	Triglycerol
HMG CoA	Hydroxy methyl glutaryl Coenzyme A
RNA	Ribonucleic acid
LCAT	Lecithine cholesterol acyl transferase
SCP	Single cell protein
SREBP	Serum regulatory element binding protein

Poultry farming in rural India has boosted the economy of rural India by solving the problem of unemployment. Tremendous demand for poultry products ranging from poultry meat to egg to processed poultry food draws the attention of nutritionists and researchers towards proper poultry management. This semi-intensive growth in poultry farming also addresses the needs and health of the consumers. As far as human nutrition is concerned, not the dietary cholesterol, but the intake of fat in terms of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids is to be looked forward. Polyunsaturated fatty acids of ω3 and ω6 series influence the plasma ratio of various lipoproteins viz., Low density lipoprotein (LDL) Cholesterol and High density lipoprotein (HDL) Cholesterol. Both dietary cholesterol (Rudel *et al.*, 998) and fatty acid pattern with regards to total n3/n6 ratio of PUFA, the dietary lipid fraction are in a close relationship to serious chronic diseases in humans. (Lands, 1987).

4.1 Changes in tissue lipid profiles during post hatching development

Lipid metabolism in animals can no longer be considered to be simply a matter of dietary fatty acids. The interrelationship between dietary fatty acids, membrane fluidity and membrane integrity and metabolic pathways in animals are evident from the work of various authors like Farkas *et al,* (2001); Roy *et al.*, (1997); Dey *et al.,* (1993). The state of the lipids in the animals is in a constant flux.

For the developing chicks within the egg, yolk lipid represents the primary nutrient source by providing the required energy for ongoing developmental processes as well as for supplying the structural component for membrane biogenesis. The various tissues of a newly hatched chick display a range of highly characteristic lipid and fatty

acid composition in accordance with their functions but this may also reflect the intensity and complexity of lipid transfer processes in a growing chick (Speake *et al.*, 1998). The results presented in tables 4 – 7 and figures IV – VI once again support these views. Various tissues viz., liver, pectoral muscle, large intestine and total blood showed a distinct tissue specific pattern of variations in the lipid profiles in terms of total cholesterol, total triglycerol, total phospholipid and fatty acid profiles of total lipid right from the 1st to the 35th day of post hatching developmental period. This indicates that the lipid metabolism plays a very important role in post hatching development and growth of the bird. Similar observations were also made by Lin *et al.*,(1992) on the mule ducklings. In order to study the effect of age on the lipid metabolism in broiler chick, *Gallus domesticus,* they were maintained from the 1st to the 35th day of post hatch development with the same commercial diet with a constant proximate composition, so that the tissue biochemical changes as observed in present study reflects only the endogenous metabolism pattern of the bird.

Dietary fat did not affect plasma levels of triiodothyronine (T3), thyroxin (T4) or insulin like growth factor I (Rosebrough *et al.*, 1999). Dietary protein levels modulate metabolic effects of dietary fat and vice versa. The dietary fat spares proteins and amino acids from the energy yielding processes which is in very high demand during post hatching development and direct them towards the growth of the animal. Accumulation of protein along with triglycerol and phospholipid particularly in muscle and intestine and to some extent in liver (figure III, IV and VI) supports the increase in the net weight gain (Table 1) of *Gallus domesticus* during post hatching developmental period. The linear

growth rate of the bird (figure I) till 28 days of post hatching development and the reduction in the feed conversion ratio (FCR) till 28 days is in accordance with the normal growth of animals as observed in animals like fishes (Bell *et al.,* 1994; Peres and OlivaTeles, 1999).

The complete blood count and hemoglobin concentration of blood is a good sensitive indicator to understand general health and the growth pattern of a bird. Any change in the haemogram at a particular age of a bird might be noted when the abnormalities in the growth are detected. It is the single most important test performed to understand the growth of birds during post hatching development (McDonald, 1996). The haematocrit values during post hatching development of a bird become almost stable from the 28^{th} day onwards of post hatching development (Table 2 and figure II). The haematocrit values reported in the present dissertation are in a similar range as those reported for other birds (Peinado *et al.,* 1992, McDonald, 1996). The younger birds have comparatively higher hemoglobin concentration and total leukocyte count and comparatively lower erythrocyte count compared to older birds (28 days to 35 days old). These observations, more particularly about hemoglobin concentration, are contradictory to those made by Kundu *et al.,* 1993 for Japanese quail. The energy demand for birds is high during the initial phase of post hatching development and growth and a higher amount of oxygen molecules are required for the catabolic processes to yield required demand for energy. Thus, the increase in hemoglobin concentration in blood during initial phase of post hatching development and growth indicates a higher oxygen binding capacity to the porphyrin ring of the hemoglobin molecule. However the lower value of

RBC count in the blood during early stages of post hatching development along with the higher concentrations of hemoglobin in blood might indicate the over expression of mRNA gene for heme protein during early stages of post hatching development. This needs further verification in the future work.

The data presented in Table 7 clearly indicates the increase in the relative concentration of arachidonic acid in liver, muscle, intestine and blood and linoleic acid (except in muscle) at the cost of decrease in oleic acid (except in intestine). This suggests the augmentation of desaturase activity of oleic acid towards the production of ω6 series fatty acids during post hatching development. The fatty acid profiles of chicks as presented in table 7 are quite similar to those observed by other workers like Ortiz *et al.,* (1998); Komprda *et al.,* (1999 & 2000). Accumulation of long chain PUFA has been related to neonatal growth and development (Marin *et al.,* 2000; Patricx and Gerard, 2002). The ratio of unsaturated to saturated fatty acid in breast muscle increases with the increased growth intensity of chicks (Komprda *et al.,* 1999). The fatty acid composition of the bird carcass lipid is generally a reflection of fatty acid profiles of diet (Yau *et al.,* 1991; Ochrimenko *et al.,* 1997; Zollitch *et al.,* 1997). A relatively constant cholesterol concentration in liver and muscle particularly from 14 days onwards of post hatching development of *Gallus domesticus* (table 5) is in accordance with the fact that cholesterol is an integral compound of cell membranes and organisms have to maintain their homeostasis (Falkenberg *et al.,* 1995). The most prominent characteristic of one day hatched bird liver is the high amount of cholesterol in the form of cytosolic lipid droplets as compared to other tissues (Noble and Cocchi, 1990; Shand *et al.,* 1994). A

progressive depletion of liver and plasma cholesterol deposits was detected within one to two weeks of post hatching development of chicks (Aguilera *et al.*, 1984). Gradual depletion of HMG CoA Synthase activity in liver (Table 8) could suggest the alteration in the process of biosynthetic machinery in the liver during post hatching development. This increase in the cholesterol synthesis machinery might be the requisite for the production of lipoprotein by the action of Lecithin Cholesterol Acyl Transferase (LCAT) enzyme. Current evidence suggests that this cholesterol derives from the hepatic uptake of the VLDL remnants, which are produced by the action of various enzymes (Speake *et al.*, 1993). The maintenance of the steady state in serum HMG CoA reductase activity from 7 days onwards diminishes the probability of the influence of the dietary cholesterol in cholesterol metabolism pathway during post hatching development.

Age had a significant effect on the digestibility of dietary fat (Ortiz *et al.*, 1998). Thus, the apparent digestibility of crude fat, total fatty acids in terms of increased pancreatic lipase activity was higher in the older birds as compared to young birds (data not shown here). These are in accordance with the hypothesis that the increased fat digestion leads to higher absorption of fat in the form of triglycerol and phospholipid in various tissues (Wiseman and Salvador, 1989). This has been attributed to an inability of very young chicks to replace bile salts lost by excretion as readily as older birds with low lipase activity (Krogdahl and Sell, 1989).

4.2 Dietary Lipid And Post Hatching Development

Both linoleic (9,12 Octadeca dienoic acid) and linolenic acid (9,12,15 Octadeca trienoic acid) cannot be synthesized *de novo* by animals (Henderson and Tocher, 1987), but are very essential for animals for their growth and to be in physiological well being state. These two fatty acids undergo further elongation and desaturation to produce various PUFAs of both ω3 and ω6 series. PUFA further metabolize to produce large amounts of prostaglandins and thromboxanes of diene and triene series, which are the key regulatory factors to maintain the animals in well being state (Lands 1987, 2000).

Supplementation of broiler diet with small quantities of fat and oils is a long standing practice for improving consistency and palatability of mash (Summers and Leeson, 1979) which include increasing the energy density of broiler meat, stimulating growth, utilization of food and energy etc. In recent studies the fatty acid composition of broiler carcass has been customized for high concentration of PUFAs (both ω3 and ω6 fatty acids) through supplementing diets with the oils of different sources (Ackman *et al*, 1988; Phetteplace and Watkins, 1989; Yau *et al*, 1991). This suggests that the carcass fatty acid compositions depend on the origin of dietary fat.

In the present dissertation, a comparative study was undertaken to find out the effect of dietary fat (by supplementing the diet with extra fat of different origin) on the growth and well being state of *Gallus domesticus* during post hatching development. Three fat sources viz., Coconut oil, Sunflower oil and Fish oil were selected based on their fatty acid composition (Table A) and various doses (2.5%, 5% and 10%) were

supplemented along with the commercial diet. This supplementation of oils altered the fatty acid profiles of the diets (Table C) and the amount of crude fat content without altering the amount of crude protein and fiber content (Table B).

4.2.1 Dietary fat and growth of the birds

Dietary fat spare protein and amino acids from energy yielding processes and direct them towards the growth of the animal. The composition of dietary fatty acid in feed not only influences the composition of the lipid in avian eggs and meat (Leskanish and Noble, 1997; Schiavone *et al*, 2004) but also influences the utilization of fat for the energy yielding process (Mieczkowska *et al*, 2001). The energy content of food with the supplementation of fat enhances by the caloric factor 9.5 K cal per gram of added fat (Henkel *et al*, 1986). When chicks were supplemented with different doses (2.5%, 5% and 10%) of coconut oil, sunflower oil and fish oil along with the commercial feed for 15 days. The observed 19 – 84% increase (depend upon quality and quantity of added fat) in net weight gain (Figure A) along with partial increase in daily instantaneous growth rate (Gw) and partial decrease in Feed Conversion Ratio (Table 10a, 10b, 10c) once again indicates the sparing of protein and amino acids from energy yielding process and the direction of these protein and amino acids towards growth of the birds. However, with the prolonged treatment of coconut oil and sunflower oil for another 15 days, no significant increase in the net weight gain was detected up to 5% of oil supplementation. About 10% increase in net weight gain was detected with 10% coconut oil and sunflower oil supplementation (Tables 10ai and 10bi) during the extended 15 days feeding period. This indicates that during this period the supplied fat is not enough to meet required

energy demand to sustain the activity of the birds and hence dietary protein may be utilized for the energy yielding process. However, dietary supplementation of fish oil during extended period also enhances the net weight gain by 8-21% over the control birds of same age group (Table 10c).

No significant difference in the daily instantaneous growth rate was observed in the birds fed with various fats of different origin (up to 5% oil supplementation for 15 days and for all the doses up to 30 days of supplementation) which is in accordance with observations made by Atteh *et al.*, (1983); Sklan and Ayal (1989). However the feed conversion ratio (FCR) values were altered in the broiler chicks fed with various oils of different origin which indicates that the feed intake by a bird is greatly altered by the quality and quantity of oils in the feed. Rapid growth during post hatching developmental period with supplementation of ω3 fatty acids meets the nutritional requirement of α-linolenic acid and might instigate the gene transcription for growth promoting protein as in human infancy (Lapillone and Carlson, 2001). Thus, when the birds were supplemented with different doses of fish oil for 30 days, the increase in the net weight gain may be due to the expression of growth promoting gene of the *Gallus domesticus*.

(a) Increase in the relative concentration of saturated fatty acid and arachidonic acid along with the decrease in monounsaturated fatty acid and long chain PUFA in liver and muscle of coconut oil supplemented chicks; (b) increase in linoleic acid and arachidonic acid at the expense of saturated, monounsaturated and ω3 fatty acids in liver

and muscle of sunflower oil supplemented chicks and (c) the tremendous increase in ω3 fatty acids viz., linolenic, eicosapentaenoic and docosahexaenoic acids at the cost of linoleic acid and arachidonic acid along with the saturated and monounsaturated fatty acids in liver and muscle of fish oil supplemented birds (as summarized in tables 16a – 16d), are in accordance with the earlier observations of Byong *et al.,* (1987); Al Athari and Watkins (1988); Phetteplace and Watkins (1989); Hargis *et al.,* (1991); Cherian and Sim (1991); Manilla *et al.,* (1999); Mieczowska *et al.*, 2001; Schiavone *et al.,* (2004). Shift in saturated fatty acids and monounsaturated fatty acids towards the production of PUFAs of ω3 series and / or ω6 series in different tissues due to intake of higher amount of linoleic acid (for the sunflower oil supplemented diet) are in accordance with observation of Yau *et al* (1991). Dietary supplementation of the fat modulates the desaturation system of fatty acids in birds, which needs to be confirmed in future. With the increased doses of dietary fat, the accumulation of the protein (Figures C series), triacylglycerol (Figures D series), Cholesterol (Figures E series) and Phospholipid (Figures F series) in different tissues increased. Enhanced dietary supplementation of PUFA, particularly ω3 fatty acids helps the bird to reduce the accumulated fat (Figures D3 series) and channelize this fat towards the energy yielding processes. The reduction in the lipogenesis due to dietary ω3 fatty acids may be due to the inhibition of different lipogenic pathways (Kersten, 2001).

4.2.2 Dietary fat and well being state

The serum lipid profiles in the form of total cholesterol and total triglycerol concentration and in the form of HDL, LDL and VLDL Cholesterol concentration are the

key indicators to understand the health condition of animals. Increasing HDL cholesterol and lowering LDL and VLDL cholesterol concentration in serum prevents cardiovascular diseases (Lands, 1987). High content of ω3 polyunsaturated fatty acid in certain fish oil prevents the rat from cardiovascular diseases like thrombosis and artherosclerosis (Banerjee *et al.*, 1992). Dietary fatty acids influence the production of the polyunsaturated fatty acids (mainly arachidonic acid, eisosapentaenoic acid, docosahexaenoic acid) in various animal tissues as summarized in table 16a-16d which is in accordance with the reports of earlier workers like Manilla *et al.*, 1999; Mieczowska *et al.*, 2001; Schiavano *et al.*, 2004. PUFA in the cell is required for the production of chemical messengers that initiate or control wide range of physiological functions including cell growth and divisions, control of blood pressure, coagulation of blood, immunosensitive reaction, tissue inflammation etc (Lands, 2000). PUFA reduces the incidence of narcotizing enterocolitis by modulating platelet activating factor and endotoxin translocation (Caplan and Jilling, 2001). 40% -70% decrease in total counts of erythrocytes and leukocytes with coconut oil supplementation (Figure B1) and 66% - 72% decrease in the total count of erythrocytes with the supplementation of sunflower oil (Figure B2) without any change in the hemoglobin concentration indicate an anemia condition in a bird and defective immunoprotective mechanisms (Klinger *et al.*,1996; Sijben *et al.*, 2001). On the other hand, 20% – 25 % increase in the total count of leukocyte without altering the hemoglobin and erythrocyte count (Figure B3) with the supplementation of fish oil for 30 days confirms the earlier observation of Klinger *et al.*, (1996). Enhanced leukocyte count in the blood may be correlated with increased immunoprotective conditions with the supplementation of fish oil. The role of dietary

fatty acids mainly ω6 and ω3 individually or jointly plays a significant role in the action of different antigen to produce antibodies which ultimately affect the immuno response mechanism of a growing layer hen (Sijben *et al.*, 2001).

97% – 2.5 fold increase in the activity of liver alkaline phosphatase (Table 18a), 20% – 36% decrease in the liver glutamate oxaloacetate transaminase (GOT) activity (Table 20a), about 3.3 fold increase in liver lactate dehydrogenase (LDH) activity (Table 21a), along with about 25% increase in the activity of serum alkaline phosphatase (Figure H), with no change in glutamate pyruvate transaminase (GPT) activity with coconut oil supplementation, indicates the poor health status of the bird which might lead to necrosis of liver and cardiac tissues upon coconut oil supplementation for a longer period. 18% - 53% decrease in the alkaline phosphatase activity in both liver and serum (Figure H) and 50% decrease in liver GOT with 87 – 3.6 fold increase in serum GOT (Figure J) and increase in liver LDH activity (Figure K) due to sunflower oil supplementation indicates necrosis of cardiac tissue with sunflower oil supplementation. On the other hand, 15% – 30% decrease in liver and serum alkaline phosphatase activity (Table 18c), 20% - 60% decrease in liver and serum GOT activity (Table 20c) and 16% - 23% decrease in serum LDH activity (Table 21c) indicate the well being state of the bird without any necrosis of liver and cardiac tissue. The decreased activity of some liver and cardiac function enzymes may be correlated with shifting of metabolic pathways, which needs to be confirmed in future. Dietary supplementation with palm oil, lowered creatine concentration in serum and activity of GPT in broiler chicken. Dietary PUFA alter the

inositol phosphate metabolism and protein kinase C activity in order to regulate intracellular signaling system (Olurede and Longe, 2001).

About three-fold augmentation of LDL-cholesterol along with about 7%-25% increase in HDL and VLDL concentration due to supplementation of coconut oil for 30 days (Figure G1) is in accordance with the data of Castillo *et al.*, (2000). Saturated fatty acids which constitute about 60% of total fat in coconut oil raises plasma cholesterol by increasing LDL cholesterol concentration more than the HDL cholesterol (Hayes and Khosla, 1992). It is important to note that in neonatal chick 10% coconut oil supplementation to the diet for one week also produced a clear increase in the cholesterol levels along with the VLDL cholesterol (Castillo *et al*, 1998). Increase in CH: HDL ratio along with CH: TG ratio is detected due to the coconut oil supplementation (Table 17a). It is interesting to note that the CH: TG ratio did not alter significantly with increase in oil concentration from 2.5% → 10%. This indicates that the addition of coconut oil to the diet might lead to accumulation of liver glycogen rather than accumulating the fat in the tissue. Addition of 20% coconut oil in the neonatal diet of chick influences the glycogen biosynthesis in liver without affecting any protein deficiency as observed by Gill Villarino *et al.*, (1997). Around 50% increase (equivocal) in LDL Cholesterol concentration along with 15% decrease in VLDL cholesterol (equivocal) and 33% increase in HDL cholesterol with supplementation of 5% sunflower oil for 30 days leading to reduction of CH: HDL ratio and augmentation of CH: TG ratio (Table 17b) indicates the metabolization of cholesterol for the production of the lipoprotein by the action of LCAT enzyme. Around 35% decrease in LDL and VLDL cholesterol with

16% increase in HDL cholesterol which result in the decrease of CH: HDL ratio and increase of CH: TG ratio due to 10% fish oil supplementation for 30 days (Table 17c) clearly indicates that the bird does not have any severe health hazards. It is evident that even 5% supplementation of sunflower oil for a period of 30 days does not have any severe health hazards on birds but at the same time, 10% supplementation of fish oil might be more beneficial to the birds to maintain themselves in physiologically well being state. Daggy *et al* (1987) have already observed that long chain PUFA helps in lowering the production rate of VLDL Cholesterol in rooster.

It is reported that dietary fish oil reduces plasma TG levels in normal and hyper triglyceredemic individuals (Harris *et al.*, 1993) especially in VLDL fractions. The protective effects of Fish intake could be caused by n3 PUFA. It is proposed that n3 PUFA may alter the lipoprotein metabolism (Smidcth *et al*, 1993),

4.2.3 Dietary Lipid and Cholesterol Metabolism

HMG CoA Reductase [EC 1.1.1.34], an integral membrane protein of the endoplasmic reticulum catalyzes the rate limiting reaction in the biosynthesis of cholesterol. It has long been recognized that hypercholesterolemic animals (by feeding cholesterol enriched diet or excess fat in the diet of animals) markedly altered the rate of hepatic cholesterol biosynthesis. This is due primarily to altered HMG CoA Reductase and HMG CoA Synthase [EC 2.3.3.10] activity. Little or no increase in the HMG CoA Reductase activity with significant decrease in HMG CoA Synthase activity in liver (Table 22a and Figure L1) indicates that there is no increased cholesterol biosynthesis in

liver due to coconut oil supplementation. The increase in tissue level cholesterol (Figure E1) and triglycerol (Figure D1) concentration due to coconut oil supplementation might be from the dietary accumulation of more saturated fatty acids to increase the serum HMG CoA Reductase and HMG CoA Synthase activity. A diet rich in saturated fatty acid elevates the accumulation of cholesterol in various tissues (Ide *et al.*, 1978). The increased activity of HMG CoA Reducatse in liver and serum along with increased activity of HMG CoA Synthase in serum and decreased activity of the same in liver (Table 22b and figure L2) due to sunflower oil supplementation indicate the accumulation of cholesterol (Figure E2) and triglycerol (Figure D2) in various tissue of *Gallus domesticus* which may not only be due to the enhanced cholesterol biosynthesis machinery but also due to dietary accumulation of fat in the form of more ω6 fatty acids. Little or no increase in HMG CoA Reductase activity in liver and serum along with increased activity of HMG- CoA Synthase in liver and serum (Table 22c and Figure L3) due to fish oil supplementation confirm the depletion of tissue level cholesterol (Figure E3) and triglycerol (figure D3) at higher dose of fish oil supplementation for 30 days. This clearly indicates that no effect of dietary accumulation of ω3 fatty acids on cholesterol biosynthetic machinery towards the biosynthesis of cholesterol. Dietary PUFA seems to regulate $\triangle 6$ and/or $\triangle 5$ desaturase activity and impair arachidonic acid biosynthesis by feed back mechanism (Garg *et al.*, 1988). Castillo *et al.*, (1999) reported inhibition of HMG CoA Reductase activity by ω3 rich fish oil supplementation to the chick. Ness *et al* (1991) reported that a feed back regulation of hepatic CoA Reductase activity by dietary fats was not due to altered mRNA levels for cellular nucleic

acid binding protein, that is essential to bind sterol regulatory elements protein in the HMG CoA Reductase in Chinese hamster.

The available evidence indicates that ω3 PUFA have distinct physiological functions (Kobatake *et al.*, 1984; Willumsen *et al.*, 1993). PUFA is believed to be one of the major active components of fish oil to have effect on lipid metabolism (Mizuguchi *et al.*, 1993a). A highly purified ethyl ester of PUFA induced a clear inhibition of rat liver 3-hydroxy-3 methyl glutaryl CoA (HMG CoA) Reductase, the main regulatory enzyme of cholesterolgenesis (Miziguchi *et al.*, 1993b).

Whether these changes in the lipid metabolism, more particularly cholesterol metabolism by exogenous dietary ω3 PUFA is by inducing the transcription of genes encoding protein involved in lipid oxidation or by suppressing the expression of genes encoding protein involved in lipid synthesis (Jump and Clarke, 1999) is yet to be confirmed. Cellular cholesterol in animals is controlled by a family of transcription factors known as sterol regulatory element binding protein (SREBP), which exist in three isomeric forms. PUFA opposes cholesterol mediated induction of SREBP (Kim *et al.*, 2002). PUFA decrease the hepatic abundance of SREBP Ic, appears to be involved with the regulation of lipogenic gene transcription and SREBP Ia, which should be able to activate both lipogenic and cholesterolgenic genes (Osborne, 2000) by accelerating the rate of mRNA decay (Xu *et al.*, 2001). Supplementation of 10% fish oil in the diet for a period of 30 days might reduce the gene expression of SREBP result in no change in HMG CoA Reductase in liver and in serum and lowering of bad cholesterol (LDL

cholesterol and VLDL cholesterol) mRNA levels. Xu *et al* (2002) reported that the dietary PUFA increases the nuclear content of the third isomer of SREBP i.e., SREBP2 and the expression of the cholesterolgenic gene, HMG CoA Synthase, wheras they concomitantly suppress the hepatic abundance of SREBP and consequently the expression of lipogenic genes, challenge the contention that sterols and fatty acids up- and down-regulate the expression of cholesterolgenic and lipogenic genes by the same mechanism.

4.3 Marine Bacteria As α - Linolenic Supplement In The Diet

Although it is well established that both linoleic acid and α-linolenic acid are essential fatty acids for entire animal kingdom (Henderson and Tocher, 1987) and are to be consumed through diets. The natural distribution of these two essential fatty acids is not cosmopolitan. The availability of α - linolenic acid is very much restricted and more confined to the marine ecosystem rather than the terrestrial and freshwater ecosystem. This might be the reason for lower level of accumulation of ω3 PUFAs in terrestrial and freshwater animals and higher level of ω3 PUFAs in marine animals (Roy *et al.*, 1999). It was observed in our laboratory that marine sediments contain about 10% α - linolenic acid in comparison to 5% in brackish water sediments and 0.5% in fresh water sediments (unpublished data). Three bacterial strains (*Pseudomonas, Streptococcus* and *Staphylococcus*) were identified from coastal sediment samples containing about 15-20% α - linolenic acid when grown in sodium acetate medium (Pujari *et al.*, 2004).

The concept of using microorganisms in feed or enriching the feed with some specific microorganisms in fish is well established in Asian countries (Ringo and Oleson, 1999; Banerjee *et al.*, 2000; Soubenova and Puzyrevskaya, 2000; Al Azad *et al.*, 2002;). The use of living microbial supplementation in diet as additional ingredient for enriching the growth of an animal has been thrust area for nutritionist in recent past (Pradel, 1992; Gildberg *et al.*,1997; Manju and Dhevendaran, 1997). This probiotic has multiple effects on intestinal microflora and acts as health promoting microorganism (Yano *et al.*, 1994). Use of probiotics has become long tradition in animal husbandry (Starvie and Kornegay, 1995). Most frequently used probiotics are associated with lactic acid bacteria (Gildberg and Mikkelsen, 1998; Ringo and Gatesoupe, 1998; Ringo *et al.*, 2000). These bacteria often produce bacteriocins and other chemical compounds that might inhibit the growth of other pathogenic bacteria within the animal. Marine bacteria are known to produce wide range of compounds, which have potential application as bioactive compounds, probiotics and nutritional supplements (Prave *et al.*, 1987). These microorganisms are now been screened for the production of PUFAs as well as specific fatty acids (Bajpai and Bajpai, 1993; Yazawa, 1996; Watanabe *et al.*, 1997, 1996; Pujari *et al.*, 2004).

The bacterial strains identified and cultured in the laboratory might be the pathogenic strains and hence, the diet was supplemented with inactivated bacterial cells (rich in α - linolenic acid) instead of live microbial cells. Out of these 3 strains viz., *Pseudomonas, Streptococcus* and *Staphylococcus,* the *Streptococcus strain* was seemed to be more effective strain that could be used as a source of α - linolenic acid in a diet.

The observed 25% increase in growth in terms of net weight gain as observed with supplementation of B2 strain of bacteria in diet for a period of 30 days (Table 23 and figure M) indicates the bacteria as a growth promoting microorganisms. The increased growth might be due to the increase in crude fat and protein content in the experimental diet supplemented with bacteria or both (Table B). These observations once again confirm the involvement of dietary fat to prevent dietary protein to undergo energy yielding process and thus is in agreement with the findings of Atteh *et al.*, (1983); Sklan and Ayal, (1989); Henkel *et al.*, (1996); Mieczkowska *et al.*, (2001). Manju and Dhevendaran, (1997) reported that single cell protein (SCP) of microbial origin appears to be a 25% - 50% substitute for fishmeal for the growth of juvenile prawn.

The increased net weight gain of the bird with bacterial supplementation is reflected in the liver and intestinal protein concentration (Figure O); triglycerol concentration (Figure P), cholesterol concentration (Figure Q) and phospholipid concentration (Figure R) in all tissues, which, confirm once again the dietary role of α - linolenic acid in growth and lipid metabolism of the bird. Increase in the relative concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) along with decrease in oleic acid, linoleic acid and arachidonic acid in various tissues of chicks (Table 29a – 29d) due to bacterial supplementation in diet over a period of 30 days once again confirm the competition of α - linolenic acid with linoleic acid to bind with △5 and △6 desaturase enzyme sysytem for the production of long chain PUFAs. The similar observations were made on dietary supplementation of n3 fatty acid rich fish oil in chickens (Byong *et al* 1987; Al Athari and Watkins, 1988; Phetteplace and Watkins

1989; Hargis *et al.*, 1991; Cherian and Sim 1991; Manilla *et al.*, 1999; Mieczowska *et al.*, 2001; Schiavone *et al.*, 2004).

Dietary supplementation of bacteria as a source of α - linolenic acid did not significantly alter the haemoglobin concentration and total erythrocyte count in the blood. However, more than two fold increase was recorded in the total leukocyte count of the chicks (Table 24 and figure N). The enhanced leukocyte count in the blood may be correlated with increased immunoprotective conditions with supplementation of α - linolenic acid. Sijben *et al.*, (2001) reported that the dietary fatty acids of ω3 series plays a significant role in the immunoresponse mechaniosm of growing layer hen by controlling the actions of the different antigens. Decrease in the concentration of total cholesterol and triglycerol in the serum along with little increase in HDL cholesterol concentration without altering LDL or VLDL cholesterol due to dietary supplementation of B2 strain of bacteria (as a source of α - linolenic acid) resulted in reduction of CH:HDL ratio and increase in CH:TG ratio in *Gallus domesticus* (Table 30 and Figure S). These changes in serum lipid profiles indicate no health hazards in the bird with supplementation of bacteria in the diet. High content of α - linolenic acid in the diet converted into ω3 long chain PUFA (EPA and DHA) by *Gallus domesticus* mobilize cholesterol for the production of lipoprotein by the action of the LCAT enzymes. Involvement of PUFA towards the reduction of LDL cholesterol or VLDL cholesterol is been reported by Daggy *et al.*, (1987); Hargis *et al.*, (1993). It is proposed that n3 PUFA may alter the lipoprotein metabolism (Schmidt, 1993). Little increase in liver alkaline phosphatase and decrease in serum alkaline phosphatase activity (Figure T) with

decrease in liver GOT activity (Figure V); insignificant changes in GPT and LDH activity in liver and serum (Figures U and W) once again confirm the well being state of bird due to dietary supplementation of B2 strain of bacteria for 30 days. Little change in alkaline phosphatase activity in liver and serum and GOT activity in liver might be due to shifting of some metabolic pathways (which need to be confirmed in future) in *Gallus domesticus* due to supplementaiton of B2 bacterial strain over a period of 30 days. Olurede and Longe (2001) reported the change in the serum GPT activity in chicks due to dietary supplementation of palm oil. It is reported that dietary fatty acids alter the inositol phosphate metabolism and protein Kinase C activity to regulate intracellular signaling system (Olurede and Longe, 2001) and this might alter the functioning of desaturation system in the endoplasmic reticulum to convert linoleic acid and/or linolenic acid to their respective PUFAs.

Increase in HMG CoA Reductase activity in serum and HMG CoA Synthase activity in liver due to B2 bactrerial strain supplementation for 30 days along with increase in tissue cholesterol and triglycerol concentration (Table 35 and Figure X) confirm the involvement of dietary accumulation of α - linolenic acid towards the production of cholesterol. This also clearly indicates that no *de novo* biosynthesis of cholesterol takes place in the liver. A feedback regulation of hepatic CoA activity by the dietary fat was not due to altered mRNA levels of cellular nucleic acid binding protein which is essential to bind sterol regulatory element protein in Chinese hamster (Ness *et al.*, 1991). The change in cholesterol metabolism by exogenous dietary fatty acids as observed in present study might be due to induction of gene transcription encoding

protein for the lipid oxidation or by suppressing the gene expression for encoding protein for lipid synthesis (Jump and Clarke, 1991) are yet to be confirmed.

Summary and Conclusion

The main function of fats in the body is to provide energy. Fats are the richest source of energy to the body, but they are more expensive than carbohydrates. Apart from energy supply, fats also help in forming the structural materials of cells and tissues such as cell membranes and other organelle components. Fats can also be stored in the body for subsequent use. They fulfill important functions in our diet. It is a source of essential fatty acids i.e., ω3 and ω6 polyunsaturated fatty acids (PUFA). These two types of fatty acids (ω3 and ω6) maintain physiological homeostasis in animals. Each PUFA synthesize different eicosanoids (prostaglandin and thromboxane). PUFAs cannot be synthesized *de novo* by animals; therefore they must be obtained through diet. Linoleic acid (9,12-octadecadienoic acid, 18:2n6) and alpha- linolenic acid (9,12,15-octadecatrienoic acid, 18:3n3), the precursors of PUFAs, are known as essential fatty acids.

Fatty acid profiles of the tissue depend on dietary lipids. PUFAs were shown to reduce blood cholesterol and artherosclerosis in man and animal. In the present study broiler chick, *Gallus domesticus* was selected as experimental model to study the effect of various fats of different origin on lipid metabolism. The work has been aimed to lay emphasis on the quality and quantity of lipid in a diet. Since human beings are consumers of poultry birds, the flesh lipid quality would obviously help us to maintain our physiological homeostasis.

The main problem of the investigation was to increase the sustainable level of n3 and/or n6 PUFA so that the consumer of these animals get benefit by synthesizing

sufficient amount of metabolically active compounds required for being in a "well being state".

Based on the fatty acid compositions three oils were selected (coconut oil, sunflower oil and sardine fish oil). Coconut oil was having around 60% of the saturated fatty acids and 26% of monounsaturated fatty acids. Sunflower oil contained around 60% of linoleic acid (precursor of n6 series). In contrast, the fish (sardine) oil was having 25% of n3 fatty acids (including α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid). When different concentrations (2.5%, 5% and 10%) of these oils were mixed differently with the commercial feed, it did not alter significantly the proximate composition of the diet except the percent crude fat and to some extent the ash content. Exogenous addition of lipid to the commercial feed also altered the fatty acid and lipid profiles of the diet.

7 days old chicks were fed commercial feed supplemented with different doses (2.5%, 5%, and 10%) of the selected oils for 15 days and 30 days.

Our study with marine bacteria depicted significant conversion efficacy of ∝-linolenic acid when they were grown in sodium acetate medium. The diets were formulated by mixing the bacteria with commercial feed and the proximate composition as well as fatty acid profiles were estimated. These prepared diets were fed to different groups of birds.

When the chicks were fed with the commercial feed supplemented with coconut oil and sunflower oil, a significant increase in the level of total protein concentration was observed in all the tissues of the birds after 30 days of feeding. However, when the birds were fed commercial feed supplemented with fish oil, tissue total protein level was elevated 15^{th} day onwards.

When the commercial feed of chick was supplemented with different bacterial strains (with higher concentration of alpha linolenic acid), augmentation of total protein in the liver and pectoral muscle was noticed after 15 days of feeding.

There was a significant change in the lipid (triacylglycerol, cholesterol and phospholipid) content of the liver, pectoral muscle and intestine of the birds fed with coconut oil, sunflower oil and fish oil for 15days and 30 days. With the supplementation of bacterial strains the triacylglycerol and cholesterol concentrations were increased significantly after 15 and 30 days of feeding. The similar change was observed in phospholipid concentration except in birds supplemented with B2 strain of bacteria for 30 days.

The fatty acid profiles of the different tissues were also greatly affected with dietary supplementation of the oils/ bacteria.

There were marked changes in the serum lipid profiles of the chicks supplemented with different oils and bacteria. The significant increase in HDL cholesterol with significant decrease in reduction in LDL cholesterol in serum were prominent with the

supplementation of sunflower oil and fish oil and B2 strain of bacteria. It was evident from the activities of 3-HMG CoA reductase and 3 – HMG CoA synthase that the cholesterol metabolism was modified with the supplementation of different oils and bacteria.

Best results were obtained by supplementing 5% sunflower oil and 10% fish oil and B2 bacterial strain. These were further confirmed by checking the liver function test (by studying the activities of alkaline phosphatase and glutamate pyruvate transaminase) and cardiac functions tests (by studying the activities of glutamate oxaloacetate transaminase and lactate dehydrogenase) and the birds were found to maintain the activities of these enzymes.

From all these facts it is been concluded that varying the dietary levels of EFA and n3 PUFA, the fatty acid composition of n3 and n6 PUFA can be modified. The commercial feed contains about 6% of lipid (of which about 50% is the linoleic acid) required for the better performance of the poultry birds during the post hatching growth and development. However, from the present study it is recommended that the exogenous supplementation of sunflower oil (5%) or fish oil (10%) for better growth performance and to maintain the birds in a healthy state. Some bacteria (for e.g. strain B2) of marine source can meet the demand for the requirement of alpha linolenic acid in the birds.

The changes in the PUFA profiles of the tissues reflect on the production potentiality of different kinds of eicosanoids, which act in antagonistic fashions to

enhance the defense mechanism of poultry birds to give protection against various diseases. It also stimulates steroid biosynthesis and controls the different physiological functions, resulting into a healthy growth.

In conclusion, the amount and type of fat consumed is the focus of much interest on maintaining the good health. **It is not the quantity, rather the quality of the fat intake that determines the "well being state".**

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Indian Journal of Marine Sciences
Vol. 33(3), September 2004, pp. 243-247

Screening of bacteria from sediments of coastal ecosystem, as potential sources of alpha linolenic acid

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Received 31 July 2003, revised 24 June 2004

Marine bacteria, known to produce wide range of molecules that are beneficial to animals as well as to human beings, were screened for the presence of alpha linolenic acid (9,12,15-octadeca trienioc acid). The lipid and protein concentrations of predominant bacterial isolates, obtained from coastal marine sediment were determined. Out of twenty isolates, eight bacterial isolates with higher lipid – protein ratio (more than 0.5), were grown in mineral salt medium with sodium acetate as carbon source as well as in nutrient broth. Their lipid (triglyceride, sterol, fatty acid, glycolipid and phospholipid) and fatty acid (mainly C-18 series) profiles were analyzed. Only four bacterial isolates depicted significant conversion efficacy for alpha linolenic acid (more than 25%) when they were grown in sodium acetate media. Such bacteria can be used as supplement to enrich the animal feed with the required fatty acid.

[Key words: Linolenic acid, bacteria, sediment]

[IPC Code: Int. Cl.[7] C12P 1/04, C12Q 1/02]

Introduction

Polyunsaturated fatty acid act as precursor molecules for many biological compounds for e.g., prostaglandin, thromboxane etc., which help the organisms to remain in highly active and healthy condition[1]. However, animal systems do not synthesis PUFA directly from the dietary carbon source. The precursor molecules of PUFA viz. linoleic (9,12-octadeca dienoic) acid and alpha linolenic (9, 12,15-octadeca trienioc) acid are not been synthesized by animals, as they lack enzymes which could desaturase the oleic (9-octadeca monoenoic) acid. Hence, both linoleic and linolenic acid are known as essential fatty acids for animal system[2].

The concept of using microorganism in feed or enriching the feed with some specific microorganism in fish is well established in Asian countries. The use of living microbial supplementation in diet as an additional ingredient for enhancing growth of animal has been the thrust area of nutritionist in the recent past. These probiotics have multiple effects on intestinal micro flora and act as health promoting microorganisms [3].

Marine bacteria are known to produce wide range of compounds, which have potential applications as bioactive compounds, probiotics and nutritional supplements. These organisms are now being screened for the production of polyunsaturated fatty acids as well as specific fatty acids[4-6]. Since the natural distribution of alpha linolenic (9,12,15-octadeca trienoic) acid, over the linoleic (9,12-octadeca dienoic) acid, is very restricted, the present study was proposed to screen bacteria from marine ecosystems for potential production of alpha linolenic acid. We report here studies and characterization and lipid profiles of some bacterial isolates obtained from sediment samples from southwest coast of India.

Materials and Methods

Sediment samples were collected from Mangalore to Tuticorin (Table 1), south west coast of India at 50 m and 150 m contour depths during the cruise of *ORV Sagar Kanya* during October 2001. These samples were diluted with 0.85% saline and were plated on nutrient agar medium (NA). The predominant bacterial colonies were isolated, purified and stored on slants. The selected bacterial strains were grown in

mineral salt medium (MSM) containing 5% sodium acetate incubated on rotary shaker at room temperature for 48 hours. For comparison, same strains were grown in nutrient broth for 48 hours at room temperature. Cells were then harvested by centrifuging at 10,000 × *g* for 10 min. at 16°C, and washed with 0.85% saline. The bacterial pellet obtained was suspended in distilled water and sonicated. This suspension was used for extraction of bio-molecules. Lipid and protein were extracted from bacterial cell suspension by the method of Roy and Farkas [7]. The concentrated lipid was stored in 1 m*M* BHT (solution prepared in benzene).

Different lipid fractions were isolated by thin layer chromatography. The total sterol (by ferric chloride acid reagent), total triacylglycerol (using chromotropic acid reagent), total sugar containing lipid (by anthrone reagent), total phospholipid (using ammonium molybdate reagent) , total and free fatty acid (by titration) were estimated as per routine analytical procedures[8]. Total protein content of the bacterial isolates was estimated following the method of Lowry *et al.*[9].

Methyl esters of fatty acid from the total lipid were prepared by trans esterification in distilled methanol containing 5% HCl at 90°C for three and half-hours[10]. The purified methyl esters were analyzed by a gas chromatograph (Chemito – GC 8610) equipped with a flame ionization detector. A stainless steel column (2 m long and o.d. 3 mm) packed with 10% DEGS on 100-200 meshes was used. The column temperature was programmed[10]. The rate of nitrogen carrier flow was maintained at 17 ml/minute. Fatty acid methyl esters were identified by comparison with reference standards of known composition (obtained from M/s Sigma Chemicals Co., U.S.A.).

Results and Discussions

Only the predominant colonies were isolated and stored on NA slants. The physical parameter, with respect to temperature, salinity and oxygen, of the environment (water) from where the sediment samples were collected showed a wide variation in the dissolved oxygen content (Table 1) however, variation was not seen in the salinity. It was interesting to note that the sediment samples had high concentration of carbon, nitrate and phosphate in comparison to surrounding water (Table 1). The sediment samples collected during *ORV Sagar Kanya* cruise were plated on media and predominant organisms were isolated. The counts varied from 13×10^5 to 610×10^5 (Table 2). The correlation between nutrient concentration and number of bacterial colonies is well-documented[11]. The dynamics of sediments differ completely from water

Table 1—Physicochemical parameters of the environment (water) from where the sediment sample was collected during cruise

Sl. no.	Position		Contour depth(m)	Press. (paros)	Temp. (deg. C)	Salinity (‰)	Dissol. O_2 (ml/lit)	Nitrate (picog/l)	Carbon (µg/l)	Phosphate (picog g/l)
	Latitude	Longitude								
	Off Mangalore									
1	12 55.71N	74 25.09E	53	48	21.56	35.308	0.728	7.151	0.104	3.124
2	12 54.61N1	74 00.10E	152	150	16.97	35.126	1.148	7.032	0.135	4.025
	Off Calicut									
3	11 15.47N	75 09.74E	60	52	20.34	35.089	1.008	7.006	0.162	3.968
4	11 15.51N	74 55.63E	142	140	17.85	35.065	0.841	4.151	0.178	4.084
	Off Cochin									
5	09 58.12N	75 44.02E	63	52	21.68	35.077	0.868	7.564	0.104	3.711
6	09 56.34N	75 35.96E	150	141	16.41	35.083	0.561	4.657	0.150	4.388
	Off Trivandrum									
7	08 29.96N	76 34.58E	60	49	21.4	35.078	1.136	6.623	0.131	4.169
8	08 28.25N	76 25.64E	155	148	17.39	35.042	2.856	6.000	0.162	3.404
	Off Cape Comorin									
9	07 36.16N	77 37.15E	65	52	22.93	35.117	2.912	5.482	0.120	4.139
10	07 19.98N	77 33.82E	155	156	16.69	34.996	3.136	3.923	0.139	3.875
	Off Tuticorin									
11	08 37.62N	78 25.59E	60	54	26.4	35.2667	2.864	5.785	0.135	2.517
12	08 38.47N	78 28.08E	165	153	14.73	35.057	3.164	5.234	0.170	3.069

ecosystem as here large particulate matter settles and large number of adhered organisms play a role in the decomposition process[12]. The nutrients formed within the ecological niche thereby increase the productivity and nutritional levels. This is a direct effect on the total number of organisms as well as their physical and chemical composition.

Although large number of isolates were obtained from sediment samples, only few isolates had high concentration of total lipid and lipid protein ratio varied from 0.57 to 0.88 (Table 3). These isolates were selected for their lipid and fatty acid profiles.

Table 2—Nutrient analysis of sediment samples and number of bacterial colonoies

Sample no.	Depth (meter)	Nitrate (μg/l)	Carbon (mg/l))	Phosphate (μg/l))	Colonies (nos.×10^5/g sediment)
1A	53m	6.23	0.06	1.552	28.0
1B		9.89	0.073	3.174	13.0
2A	152m	7.12	0.045	1.422	14.0
2B		6.92	0.051	2.676	69.0
3A	60m	2.57	0.051	0.896	46.0
3B		1.28	0.075	0.588	78.0
4A	142m	4.74	0.08	1.294	130.0
4B		NS	NS	NS	NS
5A	63m	3.07	0.063	1.014	17.0
5B		NS	NS	NS	NS
6A	150m	15.03	0.042	2.796	143.0
6B		6.73	0.082	1.6	131.0
7A	60m	9.2	0.084	4.388	610.0
7B		2.67	0.056	1.462	145.0
8A	155m	3.76	0.062	1.482	29.0
8B		3.46	0.057	0.926	11.0
9A	65m	2.67	0.057	0.906	265.0
9B		NS	NS	NS	NS
10A	155m	2.87	0.057	0.586	173.0
10B		NS	NS	NS	NS
11A	60m	2.96	0.037	1.522	127.0
11B		5.54	0.096	1.732	64.0
12A	165m	5.54	0.097	3.294	17.0
12B		8.9	0.066	3.304	187.0

A—Top layer of the sediment
B—Bottom layer of the sediment
NS—No sample

Metabolism in bacteria depends upon carbon sources supplied as growth nutrients, which also help in directing the desired accumulation of the metabolites [13]. Induction of oxidation pathways for lipid is found to be regulated with simple carbon sources such as acetate, citrate etc. The bacteria grown on MSM containing 5% sodium acetate as carbon source showed better growth of the bacteria with increased lipid concentration, as compared to the bacteria grown on nutrient broth (Table 4). Acetyl-CoA being the common precursors of the different lipid molecules (viz. fatty acid and sterol), the excess acetate molecules converted into acetyl CoA is then directed towards the different biosynthetic routes of lipid molecules[14]. The augmentation of the total fatty acids in different bacterial isolates, grown in sodium acetate, leads to change in the relative fatty acid profile of the isolates (Table 5).

Table 3—Percent yield value of total protein and total lipid in bacterial isolates obtained from sediment samples during cruise. Mean values of four estimation and their standard error were tabulated here.

Isolate No.	Protein (mg/ 100 mg of wet cells)	Lipid (mg/ 100 mg of wet cells)	Lipid/Protein* ratio
1A	8.14 ± 1.23	6.27 ± 0.72	0.77
1B	10.12 ± 1.87	8.96 ± 0.69	0.88
2A	9.27 ±1.01	3.14 ± 0.41	0.34
2B	7.16 ± 1.06	4.94 ± 0.42	0.67
3A	9.17 ± 1.38	2.76 ± 0.25	0.30
3B	6.16 ± 0.76	4.39 ± 0.37	0.71
4A	8.13 ± 1.2	4.66 ± 0.47	0.57
5A	9.46 ± 1.46	4.13 ± 0.38	0.44
6A	9.37 ± 1.26	6.39 ± 0.93	0.68
6B	10.36 ± 1.73	2.17 ± 0.22	0.20
7A	10.14 ± 1.65	3.72 ± 0.52	0.37
7B	8.16 ± 0.99	3.29 ± 0.32	0.40
8A	8.12 ± 0.78	6.18 ± 0.81	0.76
8B	7.12 ± 1.67	2.17 ± 0.27	0.30
9A	7.05 ± 1.23	3.09 ± 0.32	0.44
10A	8.16 ± 1.46	4.94 ± 0.43	0.60
11A	7.17 ± 0.98	3.14 ± 0.21	0.44
11B	8.72 ± 0.92	3.78 ± 0.25	0.43
12A	8.76 ± 1.02	3.16 ± 0.21	0.36
12B	8.16 ± 1.34	2.82 ± 0.24	0.34

*Calculated from mean values only
Note: Isolate no. 1A, 10A are Gram negative and rest are gram positive
Isolate no. 1B, 2B, 3A, 4A, 5A, 6B, 7B, 8A, 10A, 11B, 12B are cocci in shape
Isolate no. 1A, 2A, 3B, 6A, 7A, 8B, 9A, 11A, 12 A are rod in shape

It was interesting to note that, about 15% to 70% enhanced accumulation of total C-18 chain fatty acids in most of the organisms (except culture no. 2B, 3B, 4A and 10A) when grown in sodium acetate media as compared to nutrient broth. A 2 to 5 fold augmentation in the conversion efficacy of alpha linolenic acid (linolenic acid/total C-18×100) was noticed in bacterial isolates 1B, 2B, 4A and 8A and about 30% reduction in this conversion efficacy was noticed in the isolates 1A, 3B, 6A when these were grown in sodium acetate medium as compared to grown in nutrient broth media (Table 5). The ability to synthesis specific unsaturated fatty acid totally depends upon the carbon substrate used by the isolates. It is interesting to note that none of these isolates showed any traceable (more than 0.1%) amount of gama linolenic (6, 9, 12-octadeca trienioc) acid. Moreover, the metabolic fate of alpha linolenic acid and gama linolenic acid are quite different[1,15].

In order to select the best bacterial isolates amongst these for supplementing the feed, it was important to characterize the organisms so as to know their

Table 4—Comparative table showing the lipid profiles (n mol/mg protein) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media . Mean values of four replicate sets of experiment were tabulated

Lipid Profile	Culture no.															
	1A		1B		2B		3B		4A		6A		8A		10A	
	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA
TG	15.0	68.0	17.9	87.3	6.2	54.3	9.9	40.0	28.1	54.3	5.3	9.8	16.2	30.1	18.4	37.0
ST	0.41	1.9	1.2	8.2	0.09	5.2	1.6	5.2	1.3	2.7	1.2	1.2*	1.5	2.9	1.6	7.67
FFA	2.0	9.8	4.6	24.3	1.0	10.6	3.8	7.7	8.6	9.7*	7.3	7.5*	3.0	6.2	3.9	4.8*
GL	0.14	1.9	0.9	1.9	0.17	5.8	0.8	5.7	2.7	2.9*	0.45	0.9	0.4	0.5*	3.2	4.8
PL	8.6	27.9	16.0	54.9	3.1	27.0	7.9	21.5	9.7	21.2	20.9	38.7	10.2	18.2	21.5	47.0
TFA	74.3	292.9	111.2	417.9	26.0	233.0	53.2	192.1	115.1	217.4	68.3	121.5	72.0	143.7	103.5	215.0

NOTE: TG : Triacylglycerides; ST: Total sterol; FFA: Free fatty acid; GL: Glycolipid; PL: Phospholipid; TFA: Total fatty acid (free+ esterified); NB: nutrient broth media; SA: sodium acetate media.
*The changes are not statistically significant when the same was compared with the isolates grown on nutrient broth media.

Table 5—Comparative table showing the fatty acid profiles (relative % composition) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media. Mean values of four different replicate sets of experiment were tabulated.

Fatty acid profile	Culture no.															
	1A		1B		2B		3B		4A		6A		8A		10A	
	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA
Unknown	13.30	10.46	5.19	5.21	-	-	3.96	4.96	11.06	4.00	5.23	4.81	-	-	4.18	5.76
C-12:0	14.84	15.77	3.26	4.05	4.85	9.02	9.67	4.36	0.67	5.50	8.20	5.81	11.28	2.42	6.96	8.71
C-14:0	2.28	4.19	1.61	0.58	2.53	6.85	3.20	2.63	0.60	8.06	5.93	2.02	-	3.92	2.47	6.96
C-16:0	12.76	6.39	7.30	7.03	7.91	7.20	13.19	13.32	5.23	13.51	15.11	9.54	14.12	11.75	27.21	25.45
C-16:1	1.32	0.18	6.98	0.61	2.76	1.27	0.52	1.06	1.59	-	0.59	0.20	-	1.15	-	1.25
C-16:2	1.41	0.45	15.68	2.93	3.06	2.64	1.18	0.43	6.95	6.25	1.78	1.26	-	2.69	1.87	1.95
Unknown	-	-	5.68	-	-	-	2.69	-	0.65	-	0.60	3.72	10.57	-	1.97	0.74
C-18:0	1.71	0.40	16.96	22.63	7.54	10.98	2.24	2.36	2.15	2.26	1.89	3.52	1.61	2.07	5.87	0.74
C-18:1(CIS)	21.15	20.39	7.62	9.54	20.19	20.74	18.40	16.30	28.25	23.04	14.85	11.52	27.87	20.24	11.57	10.19
C-18:1(TR)	2.65	0.15	4.16	2.06	8.04	0.45	10.37	10.59	6.45	3.00	2.48	6.34	3.59	0.53	3.87	6.30
C-18:2(W6)	4.16	18.69	8.59	12.35	10.93	10.07	5.44	13.72	4.13	6.09	15.32	25.32	3.35	6.62	13.31	15.80
C-18:2(W3)	2.15	0.15	-	-	2.75	1.45	2.64	-	-	-	-	-	2.72	2.52	-	-
C-18:3(W3)	4.82	2.46	2.65	21.95	8.03	15.54	9.02	5.96	3.82	12.99	6.12	5.58	3.80	20.84	7.73	6.92
C->18 others	17.45	20.32	14.32	11.06	21.14	13.79	17.48	24.31	28.45	15.30	21.90	20.36	21.09	25.25	12.99	9.23
Total C-18	36.64	42.24	39.98	68.53	57.48	59.23*	48.11	48.93*	44.80	47.38*	40.66	52.68	42.94	52.82	42.35	39.95*
Conversion efficacy of 18:3	13.15	5.82	6.63	32.03	13.97	26.24	18.75	12.18	8.50	27.42	15.05	10.67	8.850	39.45	18.25	17.30*

* The changes are not statistically significant when the same was compared with the isolates grown on nutrient broth media.