Discussion
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<td>VLDL</td>
<td>Very low density lipoprotein</td>
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<td>GPT</td>
<td>Glutamate pyruvate transaminase</td>
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<td>GOT</td>
<td>Glutamate oxaloacetate transaminase</td>
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<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<tr>
<td>FCR</td>
<td>Feed conversion ratio</td>
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<td>Gw</td>
<td>Daily instantaneous growth rate</td>
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<td>N</td>
<td>Normality</td>
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<td>CH</td>
<td>Cholesterol</td>
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<td>TG</td>
<td>Triglycerol</td>
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<td>HMG CoA</td>
<td>Hydroxy methyl glutaryl Coenzyme A</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>LCAT</td>
<td>Lecithine cholesterol acyl transferase</td>
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<td>SCP</td>
<td>Single cell protein</td>
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<td>SREBP</td>
<td>Serum regulatory element binding protein</td>
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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., 1998) 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 28th 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, eicosapentaenoic 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 immune 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 (Smidch 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 Reductase 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 Δ6 and/or Δ5 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 cholesterologenesis (Mizuguchi 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 1c, appears to be involved with the regulation of lipogenic gene transcription and SREBP 1a, which should be able to activate both lipogenic and cholesterologenic 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 diet 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 Dhevendran, 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 Δ5 and Δ6 desaturase enzyme system 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 251
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 mechanism 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 \( \alpha \) - 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.