The process used to make experimental diets in the laboratory, exposes the diet ingredients to relatively mild conditions compared to those used in diet manufacturing. During the feeding trial fish were fed continuously and the loss of vitamin activity due to water leaching was minimized.

Only about 81% of L-ascorbic acid (AA) supplemented into the diet was retained during processing and storage as compared to about 98% of L-ascorbyl-2-monophosphate (AAP) and L-ascorbyl-2-sulfate [(AAS) Table 10]. Data in this study showed that about 18% of L-ascorbic acid was lost due to processing and subsequent storage. AA is much less stable during processing and storage (Hilton et al., 1977; Soliman et al., 1987) and during analysis (Shiau and Hsu, 1993). AA is extremely labile to oxidation which catalyze atmospheric oxidation into biologically inactive form, diketogluronic acid (Halver, 1989; Lovell and Lim, 1978; Hilton et al., 1979). In the present study approximately 2% of AAP and AAS lost during diet preparation and storage. Because L-ascorbic acid derivatives...
with sulfate and phosphate moieties at the unstable C-2 position in the lactone ring are highly resistant to oxidation and have shown vitamin C activity in fish and shrimp. The same result was also obtained by El Naggar and Lovell (1991) where, diet analysis indicated no appreciable loss of ascorbic acid from processing and storage in diets prepared with AAP or AAS, with recoveries of about 99% and 97% respectively. But only about 80% of AAS was recovered in diets supplemented with AA. The laboratory diet making process destroyed more AA activity than AAP and AAS and were more stable (Shiau and Hsu, 1993). The results of the present study may provide useful information with respect to the activity of the three sources of ascorbic acid for the preparation of fish diets. With labile forms of ascorbic acid such as L-ascorbic acid needs special caution in terms of the time allowed during process of diet preparation and storage.

Zeitoun et al (1976) have suggested the use of polynomial regression analysis as a means of approximating the relationship of weight gain and essential nutrient intake. As indicated by Zeitoun, the value corresponding to maximal gain estimated by quadratic regression is defined as the maximum concentration of dietary nutrients that produces optimal growth and beyond which growth is depressed.

The present study demonstrated that vitamin C is required in the diet of *H. fossilis* for normal growth and physiological conditions. More growth, survival rate and normal haemopoiesis were noticed in fish fed 100 mg AA and AAP and 1000 mg AAS / kg diet. L-ascorbic acid requirements for growth were 100-150 mg / kg diet for trout and salmon, 30-50 mg / kg diet for carp and 60 mg / kg diet for channel catfish (Halver, 1989). Minimum dietary vitamin C levels (L-ascorbyl-2-polyphosphate) required for normal survival of *Penaeus vannamei* were estimated
to be 120 mg AAP / kg diet for shrimp with an initial weight of 0.1 g and 41 mg AAP / kg diet for shrimp with an initial weight of 0.05 g. Dietary vitamin C levels of 120 and 90 mg AAP / kg diet were required for maintaining a normal whole body ascorbic acid content in shrimp with an initial weight of 0.1 and 0.5 g, respectively (He and Lawrence, 1993). Reported dietary vitamin C requirement for *Penaeus japonicus* ranged from 3000 to 10,000 mg / kg when L-ascorbic acid was used as the vitamin C source (Deshimaru and Kurioki, 1976; Guary et al., 1976) and 100 mg / kg when Mg-L-ascorbyl-2-phosphate was used (Shigueno and Itoh, 1988).

EL Naggar and Lovell (1991) observed in channel catfish fed diets containing different concentrations of three sources of ascorbic acid that AAS was approximately 30% as effective as AAP and AA in meeting the vitamin C requirement. It also indicates that L-ascorbyl-2-sulfate was only about 25% as effective as L-ascorbyl-2-monophosphate in meeting the vitamin C requirement in *Penaeus monodon* (Shian and Hsu, 1994). Among the vitamin C, AAP showed better performance followed by AA and AAS. This wide range in values could be explained by the unstable chemical nature of L-ascorbic acid which large amounts are added to shrimp and fish feeds to allow for processing losses and storage. The present study indicates that the dietary requirement of 100 mg AA and AAP / kg diet of *H. fossilis* was more or less similar to that of *P. vannamei* (90 and 120 mg AAP / kg diet), *P. japonicus* (100 mg AAP) and trout and salmon (100-150 mg AA / kg diet). But carp and channel catfish required L-ascorbic acid as dietary vitamin C requirement which was approximately one-half of the *H. fossilis* dietary requirement. Quadratic regression analysis of present study indicates that the minimum requirement of maximal growth is 537.61 mg AA / kg diet or the equivalent of 542.43 mg AAP / kg or the equivalent of 986.82 mg AAS / kg diet.
The present study has assumed that, blood, liver and/or anterior kidney concentration of ascorbic acid is representative of the body pool of ascorbic acid in *H. fossilis*. Major storage tissues for ascorbic acid in fish are blood, liver, kidney and skin. (Halver, 1972; Tucker and Halver, 1984; Guerin, 1986). Tissue concentrations are influenced by level of ascorbic acid in the diet (Hilton et al., 1978; Tucker and Halver, 1984). In the present study, blood, liver and kidney concentrations of ascorbic acid changed in accordance with dietary concentration of ascorbic acid, thus each value should represent the size of the body pool of ascorbic acid. Guerin (1986) found similar changes in ascorbic acid concentration in serum, liver, anterior kidney and skin in channel catfish as the dietary level of ascorbic acid changed indicating that a shift in size of body pool of ascorbic acid is reflected simultaneously by changes in each of these tissues. Based on the results obtained in present study, APP is markedly more effective in maintaining tissue concentrations of ascorbic acid in *H. fossilis* than AA; whereas, AAS is relatively ineffective at the doses fed. Similar studies in channel catfish (Wilson et al., 1989) and rainbow trout (Gront et al., 1989) also showed that phosphorylated ascorbic acid (L-ascorbyl-2-polyphosphate) provided higher levels of tissue ascorbic acid than AA. El Naggar and Lovell (1991) showed that AAP is effectively utilized by channel catfish as vitamin C source for growth and is more effective than AA for maintaining the body pool of ascorbic acid whereas, AAS has much less vitamin C activity for growth and is relatively ineffective in maintaining body pool of ascorbic acid pool. The same result was also obtained for *P. vannamei* (He and Lawrence, 1993) and *P. monodon* (Shiau and Hsu, 1993).

Reason that AAP provides for more effective in growth and higher levels of tissue storage of ascorbic acid is that the phosphate moiety protects ascorbic acid from oxidation in the digestive tract (Wilson et al., 1989) or enhances its absorption
form the intestine (Lovell and El Naggar, 1989). Because at neutral to alkaline pH the C-2 position in the lactone ring of L-ascorbic acid is highly reactive and susceptible to oxidation and rapid nucleophilic attack substitution reaction (Tolbert et al., 1975). L-ascorbic acid derivatives with sulfate and phosphate moieties at the unstable C-2 position in the lactone ring are highly resistant to oxidation and have vitamin C activity in fish and shrimp. The remarkably low tissue levels of ascorbic acid in fish fed AAS is assumed to be the low rate of absorption from the digestive tract. Meier and Garbaudan (1990) found that negligible amounts of ascorbic acid in blood of rainbow trout following stomach gavage of AAS, but high blood levels of ascorbic acid followed gavage of AAP or AA. Dabrowski and Kock (1989) reported that absorption of ascorbic acid from AAS is negligible because of the absence of sulfatase in the intestinal epithelium. Murai et al (1978) had also suggested that the rate of enzymatic hydrolysis of AAS to AA or rapid excretion of AAS may have been the limiting factor.

In the present study weight gain was normal and no deficiency signs were observed in several of the treatments when liver ascorbic acid levels were more than 20 μg / g tissues. *H. fossilis* is unable to synthesize ascorbic acid because no ascorbic acid was found in tissues of control fish. Wilson and Poe (1973) explained channel catfish and most other fish, are incapable of synthesizing ascorbic acid and require a dietary source to maintain a body pool of that vitamin. No detectable ascorbic acid were found in the tissues of fish fed control diets (El Naggar and Lovell, 1991). Apparently fish will maintain normal growth and health regardless of immediate dietary intake of ascorbic acid as long as the body pool of ascorbic acid is maintained above a minimum critical level. When body stores of ascorbic acid in fish reach a critically low level, deficiency signs occur (Lim and Lovell, 1978; Hilton et al., 1978); whereas, uncommonly high tissue
concentrations of ascorbic acid have been associated with an increase in fish tolerance to environmental pollution (Eichbaum et al., 1977; Agrawal et al., 1978; Mayer et al., 1978) and increased resistance to bacterial infections (Halver, 1972; Durve and Lovell, 1982; Lim and Lovell, 1985; Navarre and Halver, 1989). In the present study, *H. fossilis* developed serve deficiency signs when the liver ascorbic acid level recorded less than 20 μg / g tissues. Lightner et al (1979) reported that tissue levels of approximately 30 μg ascorbic acid /g of whole shrimp tissue appeared to be sufficient to prevent black disease, while tissue levels of 20 μg / g or less tended to redispose the shrimp to the disease. Shaiu and Jan (1992) reported that, tissue levels below 23.2 μg / g was insufficient for shrimp (*P. monodon*) to grow maximally. He and Lawrence reported that 10 μg / g liver ascorbic acid was required for maintaining a normal whole body ascorbic acid content in shrimp (*P. vannamei*) with in initial weight of 0.1 and 0.5g. Previous reports (Hilton et al., 1978, Lim and Lovell, 1978) indicated that 20 and 30 μg of ascorbic acid / g liver was associated with ascorbic acid deficiency in rainbow trout and channel catfish. Possibly fish size could be a factor, or, with a longer feeding period (beyond 14 wk), the fish with low liver levels of ascorbic acid with normal growth would have become scorbutic (El Naggar and Lovell, 1991).

McCay and Tunision (1934) reported vitamin deficiency leads to scoliosis in brook trout. McLaren observed haemorrhages in trout fed low ascorbic acid diet. An anaemia, scoliosis and lordosis eventually developed in extremely deficient fish when fish fed vitamin deficient diets (Kitamura et al., 1965) In the present study fish fed diets devoid of AA and AAP and AAS developed anaemia, anorexia, ascites, scoliosis, prostration, lordosis, lethargy, hypersensitivity, erosion in fins, exophthalmia, cataract and degeneration of gills during the 9th week of the experimental period. Fish fed 50 mg AAS / kg diet showed anaemia,
erosion in fins and haemorrhage in fins. At the end of the experiment, postmortem
of the fish showed, haemorrhages in intestine, kidney, liver and muscle. The
deficiency symptoms are intense in fish fed control diet. Halver (1969) reported
that the deficiency signs on fish related to hyperplasia of collagen and cartilage,
then lordosis, scoliosis internal haemorrhage, reabsorbed opercles and abnormal
support cartilage in gills, spins and fins. In the present study haemorrhages and
erosion in fins led to death. It is clear that ascorbic acid is necessary for the
production and maintenance of intercellular substances which involved in wound
repair (Pinkerton, 1977; Lim and Lovell, 1978; Lightner et al., 1977; Jaancey et
reported that ascorbic acid is involved in the formation of chondrin sulfate
fractions and intercellular ground substances and is capable of forming sulfate
derivatives. The same symptoms have been observed in trout, salmon, yellowtail,
carp, cuppies (Poecilla reticulasia), catfish, snakehead, tilapia, minnows, mullets
and other fishes and char when fish fed vitamin C deficient diet (Kitamura et al.,
1965; Poston, 1967; Lovell, 1973; Stickney et al., 1984; Sakaguchi et al., 1969;
Yone and Fujii, 1974; Mahajan and Agrawe 1979; Halver, 1989; Tucker and
Halver, 1986).

Fish fed vitamin C deficient diet showed reduction in haemopoiesis. The
differential blood counts showed that changes in the concentrations of WBC.
Johnson et al (1971) reported that ascorbic acid is involved in maturation of
erthrocytes and for maintenance of normal haematology. In the present study fish
fed diet devoid of vitamin C showed greater blood cell anomalies. Erythrocyte
fragmentation, poikilocytosis, anisocytosis, cytoplasmic clearing and smudge cells
were often observed in fish fed control diet. Results from this study conform that
the estimated value of 542.43 mg AAP / kg diet is effectively utilized by H. fossilis
as a vitamin C source for maximum growth and is more effective than AA for maintaining the body pool of ascorbic acid, whereas, AAS has much less vitamin activity for growth and is relatively ineffective for maintaining tissue storage of ascorbic acid pool.

The present folic acid experiment explained that folic acid is required in the diet of *H. fossilis* for normal growth and physiological conditions. More growth, survival rate, and normal haemopoiesis were recorded in fish fed 4 mg folic acid / kg diet. Quadratic analysis indicates that the optimum dietary requirement for maximal growth is 5.72 mg / kg diet. Halver (1989) explained 6-10 mg folic acid / kg diet is necessary for trout and salmon. Channel catfish fed 4.0 mg / kg of folic acid showed higher growth and peak responses in haemopoiesis, this result is as same with the present study, however, broken line analysis showed that the dietary requirements of folic acid for optimum weight gain, haematocrit, erythrocyte and leukocyte numbers in channel catfish were 1.01, 1.17, 1.12 and 1.15 mg / kg, respectively (Duncan et al., 1993). Duncan and Lovell (1993) observed in channel catfish at the lower concentration of ascorbic acid, 4 mg / kg of folic acid was required to reduce mortality, but at the higher concentration of ascorbic acid, only 0.4 mg / kg folic acid was needed to reduce mortality. Because in the high concentration of ascorbic acid, folic acid is transformed into the active 5-formyl-5,6,7,8-tetrahydropteroyl glutamic acid (Halver, 1989). Vitamin C also have a significant interaction with folic acid on the immune response (Duncan and Lovell, 1993). In the present study, 5.72 mg / kg of folic acid is required for maximum growth of when the experimental diets were supplemented with 1000 mg / kg of vitamin C.
In salmonoid, diets folate deficiency results in reduced growth anaemia and a disruption in normal haemopoiesis (Philips, 1963; Smith and Halver, 1969; Wolf, 1951). Duncan et al (1993) and John and Mahajan (1979) observed the same results in channel catfish and rohu fed low folate diet. Dupree (1966b), reported no folate deficiency signs except increased mortality in channel catfish because, in the Dupree’s study the intestinal bacteria synthesis could have provided sufficient folate for the fish. In the present study intestinal microorganisms are a significant source of folate for *H. fossilis*, as indicated by marked reduction in growth survival, and haemopoiesis observed when sulfonamide was included in the diet when compared to fish fed the low folate diet without sulfonamide. The same result was also obtained by Duncan et al (1993). Limsuwan and Lovell (1981) demonstrated that significant amounts of vitamin B\(_{12}\) are synthesized in the intestine and subsequently absorbed in to the body by channel catfish. Kashiwada et al (1971) isolated folate synthesizing bacteria form intestine of common carp and concluded that this explain why some fish, such as common carp do not require a dietary source of folate (Aoe et al., 1967). The present study shows that anaemia and abnormal erythrocytes in folate deficient *H. fossilis* were similar to those found in salmonids (Smith, 1968; Smith and Halver, 1969) and channel catfish (Duncan et al., 1993). A marked reduction in haematocrit and total number of RBC occurred in *H. fossilis*, coho salmon and channel catfish fed low folate diets. An increase in MCV of RBC and occurrence of macrocytic erythrocytes were in *H. fossilis*, coho salmon and channel catfish fed low folate diets. Folate deprived fish of these species demonstrated poikilocytic, binuclear and macrocytic RBC. Coho salmon appears more sensitive than catfish to folate deficiency, because a significant reduction in growth rate occurred at 8 wk (Smith, 1968) whereas, a significant reduction in growth rate occurred at 11 wk in both channel catfish (Duncan et al., 1993) and *H. fossilis*. Macrocytic normochromic anaemia was observed in several experimental animals, including fish, fed diets
devoid of folic acid (Phillips et al., 1963; Aoe et al; 1967; Smith, 1968; Smith and Halver, 1969). Other signs have been observed poor growth, anorexia, general anaemia, lethargy, fragile fins, dark skin pigmentation and infraction of spleen (Arai et al., 1972; Jhon and Mahajan, 1979).

Relatively little research has been done with fish on the effects of folate on leukocytes. In the present study, *H. fossilis* without a supplement of folic acid shows leukopenia and lymphocytopenia. Feeding sulfonamide further reduced the concentration of lymphocytes and caused a decrease in thrombocytes and an increase in neutrophils. The low levels of lymphocytes would lead to a decreased immune response, which helps explain the increased mortality of sulfonamide fed fish compared with control fish. The same result was also observed in channel catfish fed sulfonamide supplemented diet (Duncan et al., 1993). *H. fossilis* shows similar changes in WBC number and composition as do monkeys and chicks deprived of folate. Rhesus monkeys developed anaemia and a marked leukopenia (Day, 1944) and these symptoms which were also demonstrated in the present study and also channel catfish (Duncan et al., 1993). Chicks fed a low folate diet developed macrocytic anaemia and reduced growth rate along with thrombocytopenia and an increased proportion of heterophils relative to lymphocytes and leukopenia (Maxwell et al., 1988). Human with folate deficiency and tropical spur commonly exhibit macrocytic anaemia, thrombocytopenia and leukopenia (Perez-Santiago and Butterworth, 1957). This study indicates that dietary deficiency of folic acid will cause a decrease in erythrocyte number in *H fossilis* as described in channel catfish (Duncan et al., 1993), but apparently does not produce the server reduction in erythrocytes observed in the idiopathic anaemia as described by Butterworth et al (1986). Morphological characteristic of blood cells in the study such as macrocytes, spectacle cell and the increased number of haemocytoblasts were similar to the
The minimum diet required to support maximal growth and survival of Chironomus salmon in sea water is 5.7 mg/kg diet. Dietary vitamin E levels greater than 5 mg/kg diet enhanced the growth, survival in the laboratory, and hatching success of Chironomus salmon. However, the optimal dietary vitamin E level was not determined. Results from the present study confirm previous findings that vitamin C is required when the amount of vitamin C fed is less than the amount used in the body. The minimum requirement of folate acid seems to be 5.7 mg/kg diet, which is higher than the amount required when the amount of vitamin C fed is less than the amount used in the body. The minimum requirement of folate acid seems to be 5.7 mg/kg diet.
growth of salmon and channel catfish are 30 mg / kg diet and 80-100 mg / kg diet for carp (Halver, 1989). The exact requirement of fish for α-tocopherol, may depend on the amount an type of polyunsaturated fatty acids in the oil components of the ration (Woodall et al., 1964; Watanabe et al., 1970). The tocopherol acts as free radical traps to stop the chain reaction during peroxide formation an stabilizes unsaturated carbon bonds or polyunsaturated fatty acids and other long-chain labile compounds (Dam and Granados, 1945; Dam et al., 1952; Tapple and Zalkin, 1960). Several nonspecific cell degenerative conditions have been described in several species of fish fed large quantities of polyunsaturated fatty acids with inadequate tocopherol in the diet. Polyunsaturated labile fish oil may invoke on increased requirement for intracellular antioxidants (Hashimoto et al., 1966). An interrelationship between vitamins E, C and A is involved in the protection of the labile vitamin A molecule (Dam and Granados, 1945; Dam et al., 1952). Therefore, it is essential to prepare, store, and feed fish diets containing labile fish oils (Sinnhuber, 1969; Stansby, 1967) and it is also required to prevent loss of tocopherol content and subsequent rapid destruction of vitamins E, C and A. In the present study fish fed lacking of vitamin E diet with adequate amount of polyunsaturated fatty acid showed several deficiency symptoms. Polyunsaturated labile fatty oils may invoke an increased requirement for intracellular antioxidants (Hashimoto et al., 1966). If dietary oil levels and the degree of unsaturated fatty acid in the oil are increased, more vitamin E may be required for adequate antioxidant activity (Cowey et al., 1981). The tocopherols act as inter-and intracellular antioxidants to maintain haemostasis of labile metabolites in the cell and tissue plasma. As physiological antioxidants, these usually protect oxidizable vitamins and labile unsaturated fatty acids. Vitamin E functions together with selenium and ascorbic acid in the enzyme glutathione peroxidase or superoxide dismutase to stop the chain reactions of polyunsaturated fatty acids peroxidation (Lehninger, 1977).
Vitamin E involved in erythrocyte haemolysis in several animals (Gyorgy and Rose, 1948; Horwitt et al., 1963; Woodall et al., 1964), and steatites in mink pigs and farm animals (Dam and Sondergaard, 1964). The tocopherols prevent exudative diathesis in chicks, white muscle disease in fish (Poston et al., 1976) and dietary liver necrosis in rats. Vitamers E are involved with selenium and with vitamin C for normal reproductive activity and are involved in prevention of nutritional muscular dystrophy in the chick, the yellow tail (Sakaguchi and Hamaguchi, 1969) and carp (Hashimoto et al., 1966). The present experiment showed that the gross vitamin E deficiency signs, such as dermal depigmentation, erratic swimming behavior (Castell et al., 1972) and ascites (Woodall et al., 1964) were observed in fish fed unsupplemented vitamin E diets. However, certain other parameters measured in this study indicate that a dietary vitamin E level of 50 mg/kg diet may be sufficient for maintaining the health of fish. Reduction in haematocrit values in fish fed diet deficient in vitamin E was reported by Woodall et al (1964); Poston et al (1976); Smith (1979); Cowey et al (1984) and Bell et al (1985, 1986a, 1987). The same results have also been observed in the present study. Although the fish fed no vitamin E diets in the present study are considered anaemic, anaemia characterized by decreased number of matured erythrocytes and increased number of polychromatocytes (immature erythrocytes). It has been reported in rainbow trout fed a diet containing oxidized, vitamin E deficient fish oil (Moccia et al., 1984). Erythrocyte fragility has been observed in similarly debilitated atlantic salmon (Bell et al., 1987). Deficiency signs for fish fed normal amounts of polyunsaturated fatty acids developed erythrocyte fragility followed by anaemia, ascites, xerophthalmia, poor growth, poor food conversion, epicarditis, and ceroid deposits in spleen and liver. Muscle dystrophy and xerophthalmia have been described in yellowtail and carp (Sakaguchi and Hamaguchi, 1969; Hashimoto et al., 1966). Impaired erythropoiesis, fragmentation of erythrocytes, marked susceptibility to stress of handling, ascites and exudative diathesis have been reported in deficient salmon and trout. Similar signs of deficiency have been reported in warm-water and marine
species fed diets low in vitamers E and high in polyunsaturated fatty acid oils. Several non specific cell degenerative conditions have been described in different species of fish species fed large quantities of polyunsaturated fatty acids with inadequate tocopherol in the ration (Halver, 1989). In the present study microscopic examination of blood smears in fish fed neither vitamin E nor selenium diet show that erythrocyte fragility, and more immature erythrocytes among total RBC.

Selenium is found to be an essential element for all species studies (Underwood, 1977) including atlantic salmon (Poston et al., 1976; Bell et al., 1987). In atlantic salmon signs of selenium deficiency have been described as growth retardation, reduce packed cell volume, reduce selenium concentration in tissues and reduced glutathione peroxidase (GSH-Px) activity (Bell et al., 1987). Selenium is a component of the enzyme glutathione peroxidase which destroys lipid peroxidase (Noguchi et al., 1973). GSH-PX (EC 1.11.1.9) is part of the cellular defense system against oxygen induced damage, together with the antioxidant vitamins (i.e. vitamins C and E) and other enzymes such as superoxide dismutase, catalase and the glutathione transferase. In the present study, fish fed selenium deficiency diets show that poor growth and it is conformed that H. fossilis requires selenium and vitamin E for normal growth and cellular physiology. Lack of selenium leads to a decreased ability to oxidize and adequately catabolize hydrogen peroxide and results in peroxidative damage to cellular membrane and organelles (Koller and Exon, 1986) When selenium is deficient in salmonids, GSH peroxidase levels in the plasma and liver drop proportionally (Poston et al., 1976; Hilton et al., 1980; Bell et al., 1986a). Sheffy and Schultz (1979) showed that dietary vitamin E and selenium can exert immunostimulatory effects in a variety of animal species when the nutrients are administered in excess of established dietary requirements. Oh et al (1982) found that supplementing rat diets with vitamin E and selenium increased the survival of phagocytes during phagocytosis and that both nutrients were required to protect cells from peroxidative damage. Blazer and Woke
(1984a, 1984b) demonstrated that humoral and cellular immunity were impaired when rainbow trout were fed low levels of vitamin E. In the present study high mortality was observed in fish fed vitamin E deficient diet may be due to poor cellular immunity. Vitamin E and selenium deficiencies may aggravate each other, therefore by speeding the production of lipid peroxides and retarding their removal. Along with selenium vitamin E protect phagocytic macrophages from peroixdation during phagocytosis.

Hepatocytic vaculation and ceroid accumulation, as observed in livers of vitamin E deficient rainbow trout (Moccia et al., 1984), might result in increased relative liver weight. The absence of histologically detectable ceroid in liver and spleen from representative samples in fish is a good clue to the presence of adequate amounts of physiological antioxidants in the fish (Wood and Yasutake, 1956). Cowey et al (1981) found ascorbic acid stimulated lipid peroxidation in liver mitochondria and microsomes of trout reflected the _α_-tocopherol status of the animal. Peroxide haemolysis of fish erythrocytes may determine vitamin E deficiency (Hung et al., 1981). In the present study no histopathological analysis and hepatosomatic index of fish were conducted. However at the end of the experiment the postmortem report of fish fed unsupplemented vitamin E diets showed fatty and ceroid livers. The release of thiobarbituric-acid-reactive substances (TBARS) or related products from lipid peroxidation, as measured in livers of vitamin E deficient Atlantic salmon (Poston et al., 1976) and rats (Leibovitz et al., 1990), might also caused a fatty and ceroid livers. By measuring hepatic TBARS and by conducting histochemical analysis one may be able to determine if a correlation exits between hepatic TBARS and hepatic somatic index. Results from the present study conform that the estimated value of 56.93 mg _α_-tocopherol / kg diet with 3 mg Se / kg diet is effectively utilized by _H. fossilis_ as a dietary vitamin E source for maximal growth, normal haemopoiesis and free from deficiency signs.
The essentiality of vitamin K for normal growth and physiology of *H. fossilis* was clearly demonstrated in the present study. The minimum requirement of vitamin K by *H. fossilis* for maximal growth is 10 mg / kg diet. However, dietary vitamin K requirement for maximal growth determined by quadratic regression equation is 140.89 mg / kg diet. The vitamin K requirements for growth has been reported in only a few aquatic animals. For example 10 mg menadione / kg diet has been reported as requirement for salmon and trout (Halver, 1989). There were no dietary vitamin K requirements demonstrated under experimental conditions for channel catfish (Murai and Andrews, 1977) or common carp (NRC, 1983). Only few previous studies of the vitamin K requirements of penaeid shrimp have been reported. He et al., (1992) were unable to demonstrate a vitamin K requirement for *P. vannamei*. Shiau and Liu (1994a,1994b) reported that 30-40 mg vitamin K / kg of diet was adequate for *P. monodon* and 160 mg vitamin K / kg diet was adequate for *P. chinesis*. Vitamin K status have been reported to related to age since it is much easier to develop a vitamin K deficiency in older rats, than in young rats (Suttie, 1991). Species differences may also play an important role in affecting the requirements (Will et al., 1992).

Vitamin K is a cofactor for carboxylation of glutamyl residues to γ-carboxy glutamic acid precursors of blood-clotting proteins (Suttie, 1980). The primary role of vitamin K is to maintain a fast normal blood-clotting rate, which is so important to fish living in a water environment. Vitamin K was shown to be a substrate for a hepatic enzyme that converts specific glutamyl residues in intracellular precursors of vitamin K-dependent proteins to γ-carboxyglutamyl (Gla) residues in the mature protein of vertebrates (Furie and Furie, 1990). Vitamin K deficiency results in an increase in the intracellular concentration of precursors of vitamin K dependent proteins (Suttie, 1973) and also results in an increase in the activity of the vitamin K dependent carboxilase enzyme (Shah and Suttie, 1978; Kingberg and Suttie, 1989), further indicated that direct measurements of alternation in liver vitamin K dependent...
carboxylase activity and vitamin K dependent precursor are sensitive method of quantifying vitamin K requirements in rats. The same method was followed by Shaiu and Liu (1994a) for estimating the vitamin K requirement of *P. monodon*.

Osteocalcin is an abundant calcium-binding protein of bone containing three residue of vitamin K-dependent γ-carboxyglutamic acid (Gla) among its amino acids. The Gla side chains participate directly in the binding of calcium ions and the absorption of osteocalcin to hydroxyapatite surfaces *invivo* and *invitro* (Hauschka and Carr, 1982). The formation of the Gal-containing osteocalcin was vitamin K dependent, and studies of vitamin K deficiency demonstrated that Gla formation in developing chick embryo or in young growing chicks was depressed (Hauschka and Reid, 1978a). The interaction of osteocalcin with calcium ions has been studied by Hauschka and Carr (1982). The important of α-carboxyglutamic acid for calcium binding and its essentiality for biological activity of vitamin K-dependent protein have been demonstrated in vertebrates (Hauschka and Carr, 1982; Suttie, 1991; Will et al., 1992), King (1978) reported that matrix protein-bound γ-carboxyl glutamic acid is not obligatory (the amount is < 0.1 nmol /g tissue) for the calcification process in invertebrates. In the present study, increased dietary vitamin K results in elevated levels of calcium deposition in *H. fossilis*.

Vitamin K is involved in the synthesis of messenger RNA of blood-clotting proteins—prothrombin, plasma thromboplastin, proconvertin, and at least one other factor. Vitamin K is involved with vitamin A and E and ascorbic acid for homeostasis of physiologically active vitamins A and E (Damand Sondergaard, 1964). Prothrombin time in salmon fed diets devoid of vitamin K was increased three to five times and during prolonged deficiency states, anaemia and haemorrhagic areas appeared in the gills, eyes and vascular tissues. Increased blood-clotting time has also been reported for other fish reared on diets with low vitamin K content (Dupree, 1966a; Poston,
In the present study, slow blood clotting, haemorage in fins and tetany were observed in fish fed unsupplemented vitamin K diet. Vitamin K deficiency results in anaemia and prolonged coagulation time in fish (NRC, 1973; Halver, 1989). A diet deficiency in vitamin K results in slightly lower hepatosomatic index and anaemia in rainbow trout (Kitamura et al., 1967) and skin haemorrhages in channel catfish (NRC, 1983). It has been demonstrated that menadione is highly effective in preventing a molinate-induced anaemia in common carp (Kawatsu and Ikeda, 1988; Kawatsu et al., 1989). Prothrombin time in salmon fed diets devoid of vitamers K were increased three to five times and during prolonged deficiency states, anaemia and haemorrhagic areas appeared in the gills, eyes, and vascular tissues. In the present study fish fed control diet show that haemorrhages in fins and barbells. Results from this study conform that the estimated value of 140.89 mg menadione / kg diet is required by *H. fossilis* as a vitamin K source for maximal growth and normal health.