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VITAMIN REQUIREMENTS OF FINFISH AND PRAWNS

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Vitamins are complex organic substances, usually of comparatively small molecular size (molecular weight usually less than 1000). They are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components (Cho et al., 1985). The importance of vitamins as essential constituents in the diets of animals came to light in the early part of this century and during the past five decades active and rapid progress in vitamin research was made almost in all the commercially important species. While contributions to vitamin nutrition of mammals and poultry are numerous (Mitchell, 1964), contributions from aquatic species are relatively less, and mostly came from studies with finfish (Cowey and Sargent, 1972; Halver, 1972, 1980; Mahajan and Agarwal, 1979; Millikin, 1982). In recent years, active research is in progress on the vitamin requirements of crustaceans, because of their growing commercial importance (Deshimaru and Kuroki, 1979; Guary et al., 1976; D' Abramo and Baum, 1981; Heinen, 1984).

The slow progress in nutrition research on aquatic organisms was partly due to the inherent problems posed by the aquatic medium. Leaching of vitamins from diets when introduced into the water is one of the major constraints. Another factor is the contribution from gut microbial flora

in certain species, which mask the actual requirements. It has also been observed that vitamins and their precursors, since already present in the raw materials, 'Blanket Applications' of vitamin premixes in multi-ingredient diets may result in some excesses (New, 1976). Conversely, as the vitamin requirements remain unknown, formulated diets may still be deficient in certain vitamins even after supplementation.

#### VITAMIN TEST DIET

McLaren et al. (1947) developed a vitamin test diet for fish containing crystalline vitamins, casein, dextrin and oils with crab meal or dried liver as the source for the antianaemic factor. Thus these pioneer fish nutritionists paved the way for vitamin requirement studies in aquatic species. Subsequently, Halver (1957) developed the vitamin-free casein purified diet, which is widely used as the standard diet for determining the qualitative and quantitative requirements of a number of species of finfish. The composition and preparation procedure for Halver's H-440 diet, which is recommended by the EIFAC of FAO/IUNS/ICES task force as the standard diet, is given in Table 1.

So far, four fat-soluble and 11 water soluble vitamins are known to be required by fish and crustaceans. Many of the water-soluble vitamins function either directly or in a modified form as coenzymes for one or more of enzymes. None of the fat-soluble vitamins is known to function as coenzyme. In contrast to higher vertebrates, vitamin deficiency symptoms, reported in finfish are non-specific (Table 2, Cho et al., 1985).

## WATER-SOLUBLE VITAMIN REQUIREMENTS

### VITAMIN C

Vitamin C, the antiscorvy agent participates actively in the metabolism of all species. - But fishes and crustaceans are unable to biosynthesize ascorbic acid (Halver, 1972, 1980; Magarelli and Colvin, 1978; Magarelli et al., 1979). This inability in these groups of animals is attributed to the genetic failure of enzyme synthesis or lack of expression of the same (Levin, 1976). An excellent review by Knox and Goswami (1961) highlights its specific role in intermediary metabolism. Some of the important functions of this vitamin are: as protector of enzymes and hormones from oxidation and inhibition; involvement in the functioning of neural stimuli; transmitter; in RNA synthesis; mild detergent action responsible for the dissolution of fat-cholesterol and cholesterol-phospholipid - calcium complexes; as endogenous protectors; growth regulators, reactants in enzyme systems such as in the hydroxylation of epinephrine and tryptophan and in the oxidation of tyrosine in collagen synthesis. In crustaceans, vitamin C has also been reported to influence the alkaline phosphatase activity during the synthesis of chitin and sclerotization of the epicuticle (Conklin, 1983). One of the important functions of ascorbic acid is in the collagen synthesis (Stone and Meister, 1957), which is an important aspect in muscle development of all animals including fish and crustaceans (Harper et al., 1967). Stone and Meister (1957) demonstrated the necessity of vitamin C for the hydroxylation of proline and lysine to form hydroxyproline an unusual amino acid that exclusively occur in collagen.

L-ascorbic acid is a biological reducing agent in hydrogen transport. It is involved in the detoxification of aromatic drugs and also acts in the production of adrenal steroids. Ascorbic acid is also involved in erythrocyte maturation.

Deficiency symptoms:

Deficiency of ascorbic acid molecules in the diets of animals results in metabolic disorders leading to diseases. Deficiency symptoms could be clearly delineated on deletion of the vitamin from diet. Deficiency leads to spinal deformities, e.g., scoliosis and lordosis in fish. X-rays of spinal deformities in the affected fish showed extreme dislocation of vertebrae, and atrophy of the spinal cord in the area of acute deformity. Gill cartilage was found to be distended and twisted; filamentous cartilage occurred in ascorbic acid deficient fish. In channel catfish a broken back syndrome appeared in those fed diets containing sub-optimal levels of vitamin C. Internal and external haemorrhage, fin erosion, dark-skin color and reduced formation of collagen are important symptoms observed in channel catfish. Agrawal et al. (1978) reported a protective effect of high dietary levels of ascorbic acid for snake-head fed an organochlorine insecticide.

Channel catfish had increased susceptibility to pathogenic bacterial infestation (Aeromonas liquefaciens) and occasional formation of hemivertebrae (Lovell, 1973). Lim and Lovell (1978) reported the following ascorbic acid deficiency symptoms in smaller channel catfish fingerlings (initial mean weight - 2.3 g): anaemia after 9 weeks, scoliosis, lordosis and dark pigmentation after 10 weeks; lower hematocrit values after 18 weeks.

Snake-heads fed with ascorbic acid deficient diets had elevated liver cholesterol content after 150 days, in addition to the occurrence of scoliosis, lordosis and decreased ascorbic acid concentrations in blood and kidney (Mahajan and Agrawal, 1979). Mahajan and Agrawal (1980) reported that snake-heads had reduced absorption of calcium

from-surrounding water by gills and skin, and lower muscle and bone calcium content when fed an ascorbic acid deficient-diet for 210 days. They attribute this to distortion of gill filaments from cartilage malformation.

In fish hyperplasia of jaw and snout have been reported. In Coho salmon, hypertrophy of the adrenal tissue and haemorrhage at the bases of fins have been observed. However on replacement of ascorbic acid in the ration growth becomes normal. Anaemia eventually develops in extremely deficient fish and scoliosis and lordosis do not repair but are walled off by new growth around the afflicted areas of the spine when ascorbic acid is once again added to the ration (Halver, 1980).

In fish examination of fragile support cartilage in the gill filaments under low magnification will detect early hypovitaminosis before clinically acute symptoms become noticeable. However, the best tissue for routine clinical analysis in fish tissue is anterior kidney of the fish.

Recently in Clarias betrachus ascorbic acid was reported to be essential. Deficiency resulted in scoliosis, external haemorrhaging, fin erosion, and dark skin colour at 12 weeks (Butlhev et al., 1985).

In crustaceans, very few studies have been carried out on ascorbic acid and the information available is restricted. Conklin (1983) reported that under vitamin C deficiency alkaline phosphatase activity was inhibited resulting in poor chitin synthesis and sclerotization of the epicuticle. In the prawns, Penaeus californiensis and P. stylirostris, malformation of collagen tissue culminating in the melanization of hemocytic lesions leading to death and designated as "Black Death Disease" occur (Lightner et al., 1979; Lightner, 1983). Studies conducted at the

CMFRI have shown that deficiency of ascorbic acid in the diet of P. indicus results in reduced food intake, poor conversion of food and protein, high incidence of post-molt deaths, dystrophy of muscle and hepatopancreas, blackening of gills etc.

In early juveniles of prawns (P. indicus) ascorbic acid between 0.4 and 0.8 g/kg diet has been suggested. While Guary et al. (1976) reported high survival rates with 2 g ascorbic acid/100 g diet, in the present study 0.4 g ascorbic acid/kg gave highest survival. Magarelli et al. (1979) reported maximum survival in P. californiensis at 1.2 g/100 g diet and in P. stylirostris 2.2 g/100 g diet. In P. indicus a concentration of 2 g and more ascorbic acid depressed growth. Ascorbic acid deficiency led to increased post-molt deaths. In these prawns, the calcium and phosphorous contents were relatively low and since ascorbic acid is essential for the uptake of calcium, it is suspected that ascorbic acid deficiency would have affected the calcium absorption and metabolism of the prawns.

#### Dietary requirements:

According to Halver (1980) an intake of 100 mg of vitamin C/kg of dry ration was sufficient for rainbow trout under normal conditions. However, the ascorbic requirements doubled or tripled by stress when severe abdominal or intramuscular wounds were inflicted. Young fish needed at least 500 mg of active ascorbate for tissue repair comparable with control fish receiving 1 g or more of ascorbate in the diet/kg of dry diet. Coho salmon, however required about half of these requirement for maximum severe wound repair. The requirement for ascorbic acid is related to stress, growth rate and size of the animal as well as to the other nutrients present in the diet. Halver (1980) suggests a compromise

value of 200 mg of ascorbic acid/kg diet for trout and salmon raised in freshwater systems between 10-15°C.

Large common carp can synthesize some ascorbate and the requirement for this species may be dependent on fish size and the environment in which they are reared. For carp and channel catfish 30-50 mg/kg diet.

Studies conducted at CMFRI has shown the essentiality of ascorbic acid for the fry of milkfish, Chanos chanos and the mullet Liza parsia fry for survival, proper growth and food intake.

The recommended levels of ascorbic acid for finfish and crustaceans are shown in Table. The requirement is in the range of 100-150 mg/kg diet for salmonids and 30-50 mg/kg diet for carp and channel catfish.

#### Sources and stability:

Ascorbic acid is widely distributed in nature with citrus fruits cabbage, liver, and kidney tissues are good sources for the vitamin. Fresh insects and fish tissues contain reasonable amounts of the vitamin. Synthetic ascorbic acid is also readily available. Ascorbic acid is added to feed as a dry dilution. Ethylcellulose or fat-coated products improve ascorbic acid stability in feeds. Ethylcellulose coated ascorbic acid show higher retention in crumbles (84%) than crystalline ascorbic acid (48%). - Room temperature for 3 months resulted in 40% loss in crumbles for the ethylcellulose-coated product (Frye, 1973). Ascorbate - 2 - sulfate is considered as a good source of ascorbic acid. Fish food should be protected from oxidising agents and kept sealed or frozen to prevent loss of the vitamins. Dipotassium ascorbic - 2 - sulfate (DAS) and dipotassium L-ascorbate - 2 - sulphate hydrate (AS) were found to prevent



deficiency symptoms. Rainbow trout require 160 mg DAS/kg dry diet to achieve normal growth (Halver et al., 1975) and 80 mg to avoid deficiency symptoms. Channel catfish fingerlings require about 25 mg as to avoid deficiency symptoms.

#### CHOLINE

It serves as a source of methyl groups; involved in a number of trans-methylations; as-phosphatidyl choline it has an important structural role in biomembranes; in methylated state as acetylcholine, functions as an important neurotransmitter; also functions as a lipotropic and anti-haemorrhagic factor.

#### Deficiency symptoms:

Poor growth, and food conversion and impaired fat metabolism. Halver (1957) reported increased gastric emptying time in salmon fed on choline deficient diets. In rainbow trout anaemia and kidney degeneration reported. In channel catfish haemorrhagic kidneys and intestine and enlarged livers have been reported. Japanese eels became anorexic after 4 weeks on a deficient diet (Arai et al., 1972).

In prawns, choline deficiency led to poor growth in P. japonicus (Kanazawa et al., 1976). In P. indicus poor growth and survival, poor food intake, aversion towards feed, hyposensitivity to shock, passive activity, dystrophy of muscle and hepatopancreas, postmolt deaths were observed. Lecithin (phospholipid) has a partial choline sparing action in P. indicus. If adequate levels of lecithin is included in the diets, choline can offset the requirements of choline (Gopal, 1986).

Sources and stability:

Rich sources of choline are what germ, beans, brain and heart tissue. Choline hydrochloride, the commercially available form, may inactivate -tocopherol and vitamin K when in direct contact with these vitamins. Choline is added to feeds as a 70 per cent choline chloride solution or as 25 to 60 per cent dry powder (Adams, 1978). Choline chloride is stable in multivitamin premixes but can decrease the stability of other vitamins in the premix (Frye, 1978). Choline is stable during processing and storage in pressure-pelleted extruded diets. Loss during water immersion of pellets is less than 10 per cent after 60 minutes (Goldblatt et al., 1979). Choline is hygroscopic, very soluble in water, and is stable to heat in acid but decomposes in alkaline solutions.

INOSITOL

Inositol is a water-soluble growth factor for which no co-enzyme function is known. The only known function of inositol is as component of the inositol phosphoglycerides that are found in many cells. It has lipotropic action by preventing accumulation of cholesterol in one type of fatty liver disease and is involved with choline in maintaining normal lipid metabolism. It is a growth promoting substance for micro-organisms.

Deficiency symptoms:

Poor growth, increased gastric emptying time, oedema, dark colour and distended stomach are symptoms observed in salmon, trout, carp and catfish held for long period. The major deficiency sign is inefficiency in digestion and food utilization and concomitant poor growth leading to a population of fish with distended abdomens. Inositol is not

normally required by channel catfish.- In Japanese eels and in red sea bream reduced growth was observed. In prawns, P. japonicus (Kanazawa et al., 1976) and P. indicus (Gopal, 1986) also reduced growth was observed.

Dietary requirements:

Inositol is added to feeds as a dry dilution (Adams, 1978). Rainbow trout 200-300 mg/kg, Chinook and Coho salmon 300-400 mg/kg; carp 200-300; sea break 300-500.

THIAMINE

Thiamine functions in all cells as the coenzyme co-carboxylase, thiamine pyrophosphate, which participates in the oxidative decarboxylation of pyruvic acid to acetate for entry into tricarboxylic acid (TCA) cycle. Thiamine pyrophosphate is also a co-enzyme of erythrocyte transketolase system by which direct oxidation of glucose occurs in the cytoplasm of cells (via) the pentose-phosphate-pathway. Erythrocyte levels of metabolites of this system have been used to indicate thiamine status in experimental animals, including fish. Thiamine is essential for good appetite, normal digestion, growth and fertility, normal functioning of the nervous tissue and the requirement is determined by the caloric density of the diet.

Deficiency symptoms:

Deficiency signs in salmonids include impaired carbohydrate metabolism, nervous disorders, poor appetite, poor growth and increased-sensitivity to shock. A trunk-winding symptom in eels has been reported, together with haemorrhage at the base of the fins. Skin congestion and subcutaneous haemorrhage occur in carp and eels fed thiamine deficient diets. Deficiency signs are evident in 6 to 8 weeks in

and about 8 weeks channel catfish; 8 to 10 weeks in Japanese eels/in common carp fed a high carbohydrate diet. In prawns P. japonicus poor growth was reported after 10 weeks (Deshimaru and Kuroki, 1979). In P. indicus poor growth, survival, poor food intake and hypersensitivity were observed (Gopal, 1986).

Dietary requirements:

-William and Spies (1938) based on the information available till that time reported that all species of animals require thiamine in their diets. Recent studies have shown that thiamine requirement of aquatic species is much higher than that of domesticated land animals (Hasting and Cowey, 1977), mainly due to leaching of the vitamin, from diets (New 1976; Infanger et al., 1980) into the surrounding water. Composition of dietary ingredients should be considered while determining thiamine requirements. In common carp the dietary requirement for thiamin has been correlated with the carbohydrate level of the diet (Aoe et al., 1969). Omnivorous fishes might be expected to have a higher dietary requirement for thiamine than carnivorous fishes, but this has not been found to be true (NRC, 1981).

Studies with crustaceans have shown that thiamine is essential in the diet of Kuruma prawn, P. japonicus (Deshimaru and Kuroki, 1979), the cladoceran, Moina macrocopa (Conklin and Provasoli, 1977), the lobster Homarus americanus (Conklin, 1980) the giant tiger prawn, Macrobrachium rosenbergii (Heinen, 1984) and the penaeid prawn Penaeus indicus (Gopal, 1986).

Infanger et al. (1980) observed that thiamine loss is maximum (68-100% in 2 hrs time) amongst all the vitamins from the diet, thus necessitating higher concentrations of vitamins in the diet of prawns.

Fat content of diet may affect not only calorie intake but also the thiamine requirement because cocarboxylase participates in the oxidation of that through  $\alpha$ -ketoglutarate. Therefore, fish on a high fat diet and low thiamine intake might take longer to develop deficiencies and will give an erroneous requirement (Halver, 1980).

Sources and stability:

Common sources of thiamine are dried-peas, beans, cereal bran. Thiamine is easily lost by holding diet ingredients too long in storage or by preparing the diet under slightly alkaline conditions or in the presence of sulphide. Wet or frozen diet pose a problem because moisture content increases the chance of enzymatic hydrolysis's and subsequent destruction of thiamine. Wet or moist diet preparations containing any fresh fish or shellfish tissue must be used immediately.

Thiamine is added to feeds as thiamine mononitrate or thiamine hydrochloride. Thiamine mononitrate is stable in vitamin premixes that do not contain trace minerals and choline chloride. Thiamine mononitrate premixes containing any of those substances and stored at room temperature can lose as much as 80 to 90 percent of their thiamine activity in 3 months (Frye, 1978). Thiamine losses in the pelleting or extrusion process range from 0 to 10% and during storage of feeds 11 to 12 percent (Slinger et al., 1979).

Thiaminases occurring in fresh water fish tissues and tissues of certain-shrimps and mussels render thiamine biologically unavailable. Thiaminase is inactivated by heating or pasteurization.

## RIBOFLAVIN

Riboflavin was the second water soluble vitamin to be discovered. It is synthesized by all plants and many microorganisms but not by animals. Lactoflavin, heptoflavin and ovelflavin were also shown to be identical with the pure riboflavin. Riboflavin is found in the tissue coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes function as prosthetic groups of oxidation-reduction enzymes that are needed for the degradation of pyruvate, fatty acids, and amino acids. Thus they function as coenzymes for many oxidases and reductases such as cytochrome c oxidase, D and L amino acid oxidases, Xanthine and aldehyde oxidase, SDH, glucose oxidase and fumaric dehydrogenase. Involved with pyridoxine in the conversion of tryptophan to nicotinic acid; and in the retinal pigment during light adaptation.

### Deficiency symptoms:

In salmonids poor appetite and poor diet efficiency are the first signs followed by photophobia mono- or bilateral cataracts; corneal vascularization, eye haemorrhage, incoordination, and general anaemia (Halver, 1980). Channel catfish fed riboflavin deficient diets for 8 weeks developed deficiency signs including anorexia, poor growth, short-body dwarfism (Murai and Andrews, 1978) and cataracts (Dupree, 1966).

In common carp Aoe et al. (1967) reported nervousness, photophobia and haemorrhages. Rainbow trout fed riboflavin-deficient diets developed bilateral corneal and lenticular lesions (Poston et al., 1977). Fin necrosis, snout erosion and spinal deformation (Poston et al., 1977). In P. indicus poor food intake, poor survival, incoordinated movements and

sensitive to shock have been observed.

Dietary requirements:

Riboflavin requirements of carp fingerlings seems to decline with increasing fish size. Common carp fingerlings with an initial mean weight 1.5 g required 20 mg riboflavin. But slightly larger ones required 10 mg, and those weighing 3.4 g required 5.7 mg riboflavin.

Rainbow trout of mean weight 7 g require 12.2 mg. Whereas larger rainbow trout require 3 mg riboflavin. Channel catfish fingerlings require 9 mg for maximum growth and 3 mg to prevent occurrence of short-body dwarfism (Murai and Andrews, 1978 b).

Sources and stability:

Milk, liver, kidney, heart, yeast, germinated grains, peanuts, soyabeans and eggs are good sources. Riboflavin is added to feed as a spray dried powder or dry-dilution product. It is stable in multivitamin premixes. However, processing loss is about 26 percent. Storage losses in pelleted feeds are slight. About 40% of the riboflavin may be lost when pellets are introduced in water (Goldblatt et al., 1979).

PYRIDOXINE

Pyridoxine (B6) is an essential vitamin, required by all animal species so far studied. This vitamin is widely distributed in almost all the natural products principally as complexes of proteins, such as pyridoxal phosphate (PLP) which is an active coenzyme participating in amino acid decarboxylation, transamination, racemization, desulphydration of methionine and cysteine, deamination of hydroxy amino acids and in a variety of miscellaneous transformations.

PLP also functions as a coenzyme for 22 or more transaminases, occurring in the body and in the decarboxylation of 5 hydroxytryptophan to produce serotonin. It is also involved in the synthesis of porphyrin,  $\alpha$ -amino levulinic acid and messenger RNA, which determines amino acids sequence in polypeptide synthesis. It is also essential for the metabolism of essential fatty acids and protein.

Deficiency symptoms:

Deficiency has been shown to affect the amino acids and protein metabolism in higher animals. A number of genetic diseases involving pyridoxine dependent enzyme systems have been reported. In fish epileptic type fits, general nervous disorders, hyperirritability, alteration in the control of melanophores and edema in the peritoneal cavity, rapid and gasping breathing with flexing of the opercles, and rapid occurrence of post-mortem rigor mortis, have been reported.

Channel catfish fed a pyridoxine-deficient diet for 6 to 8 weeks exhibited signs of deficiency, including anorexia, tetany, nervous disorders and greenish blue colouration (Andrews and Murai, 1979).

In rainbow trout normocytic normochromic anemia was reported indicating that pyridoxine has a function in the maintenance of normal erythropoiesis. Also lower aspartate amino-transferase activity in white muscle and reduced aspartate and alanine amino-transferase activity were reported.

Dietary requirements:

In fishes, the requirement of pyridoxine ranges from 2 mg to 20 mg/kg (Table). Though in crustaceans few works have been done, yet most nutritionists supplement their diets with pyridoxine ranging from 60-100 mg/kg dry diet.



The intimate association of pyridoxine with various phases of amino acids metabolism implies that the pyridoxine requirement of an animal should be greater on a high protein than on a low protein diet.- In carnivorous fish, the pyridoxine requirement has been shown to depend upon dietary protein level (Hardy et al., 1979).

-Among crustaceans, its essentiality and requirements have been reported for Artemia (Provasoli and Shiraishi, 1959), Moina (Conklin and Provasoli, 1977) M. rosenbergii (Heinen, 1984) and P. indicus (Gopal, 1986).

#### Sources and stability:

Good sources are yeast, whole cereals, egg yolk, liver and glandular tissues. Pyridoxine is added to animal feeds as pyridoxine hydrochloride in a dry dilution. It is stable in vitamin premixes. The loss of pyridoxine from feeds after 10 months of storage was 7-10 percent (Slinger et al., 1979).

#### PANTOTHENIC ACID

Pantothenic acid plays a stellar role in general metabolic pathways. It is a dipeptide derivative and its name (Panto-Greek Everywhere) implies its almost ubiquitous distribution. Pantothenic acid acts metabolically as a part of coenzyme (Co A) to transfer acyl groups in enzymatic reactions. It has significant role in fatty acid oxidation and synthesis, synthesis of cholesterol and phospholipid, in phosphate energy transfer and in the acetylation of organic compounds, acylation of acetate, succinate, benzoate, propionate and butyrate. Acetyl - Co A is also required in reactions in which the carbon skeletons of amino acids enter into energy yielding metabolic pathways. Since pantothenic acid is a part of acetyl Co A, it has been shown to be

required by all animal species studied so far, including the microorganisms (West et al., 1965).

Deficiency symptoms:

Symptoms associated with pantothenic acid deficiency are mostly non-specific and vary from species to species. Deficiency studies in higher vertebrates have shown retardation of growth, impairment of reproduction imbalance of salt-water metabolism and reduction of Co A content in tissues leading to poor utilization of pyruvate (Chow, 1964).

Aquatic species, especially fishes like salmon and trout, reared at 10-19°C water temperature, fed with pantothenic acid deficient diets were found to exhaust the vitamin stores rapidly in 8-12 weeks. The fish stop feeding and the gill filaments show proliferation of epithelial surface in addition to swelling and clubbing together of the filaments and lamellae (Phillips et al., 1945). The opercles become distended and the surface of the gills is often covered with an exudate. Fish become prostrate or sluggish. Necrosis, scarring and cellular atrophy of the tender gill elements occur and anaemia develops after prolonged deficiency. The same type of symptom has been observed in salmon, trout, eel, carp and catfish. In channel catfish erosion of skin, lower jaws, fins and barbels reported.

In Macrobrachium rosenbergii (Heinen, 1984) and Penaeus indicus (Gopal, 1986) unusual partial moulting of prawns has been reported.

Dietary requirements:

- The dietary pantothenic acid requirements are shown in Table. Murai and Andrews (1975) suggested that the relatively high dietary pantothenic acid requirements of

channel cat fish fry might be partially due to higher rates of micronutrient losses in small feed crumbles fed to fry compared with larger feed particles fed to fingerlings.

Heinen (1984) reported relatively higher growth in prawns (M. rosenbergii) fed with pantothenic acid deficient diet, than their control counter parts having 0.06% pantothenic acid and he presumed that the poor growth is due to the detrimental effect of excess vitamin dosage. In Penaeus indicus a dietary level of 50 to 75 mg/100 g diet seems to be required for proper growth, survival and utilization of the food and protein.

Sources and stability:

Good sources are cereal bran, yeast, liver, kidney, heart, spleen and lung. Fish flesh is a relatively rich source pantothenic acid is added to feed as either calcium-d-pantothenate (92 percent activity) or calcium dl-pantothenate (46% activity). Pure pantothenic acid is unstable hygroscopic and viscous. Calcium pantothenate is relatively stable in moist and dry diets. Processing losses during pelleting or extrusion can be as high as 10%.

NIACIN

The vitamin exists in its amide form nicotinamide, under its physiological active state, serving as co-enzyme for a variety of metabolic enzymes. The major function of niacin in NAD and NADP is hydrogen transport in intermediary metabolism. These co-enzymes serve as hydrogen acceptors from metabolic reactions activated by certain anaerobic dehydrogenases passing H-molecule to flavoproteins in glycolysis, Kreh's cycle and other metabolic cycles. Both NAD and NADP are involved in the synthesis of high energy phosphate bonds which furnish energy for certain steps in

glycolysis, in pyruvate metabolism, and in amino acid and protein metabolism.

In many animals the amino acid tryptophan can be converted to nicotinic acid, so deficiency signs can only be induced by restricting dietary tryptophan.

Deficiency symptoms:

Stores of niacin are more slowly exhausted under experimental conditions than are some of the other vitamins resulting in less defined and more slowly developing symptoms. Deficiency of niacin reduces the concentration of coenzymes in liver and muscle (Goldsmith, 1964). It has also been observed that niacin in the diet, increases the secretion of both free and total acids in the gastric juice (Goldsmith, 1964). Niacin deficiency symptoms are developed much slower in invertebrates and fishes than in higher vertebrates; the reason being that niacin is replenished through microbial population present in the intestinal region in many species, which produce the vitamin quantum just sufficient to meet the animal requirements (Halver, 1972). Niacin demands are also met through the conversion of the amino acid tryptophan present in the diet.

Loss of appetite, skin and fin lesions, deformed jaws, anaemia, exophthalmia, high mortality rates, poor feed conversion, appearance of lesions in colon, motion, oedema of the stomach and colon reported. In common-carp skin haemorrhages have been reported. In M. rosenbergii food efficiency was not markedly affected by niacin deficiency. In P. indicus poor survival and growth, poor food intake, black lesions in the body and gills were reported.

Dietary requirements:

The recommended levels of vitamin the diets are given in Table. Niacin requirement varies with the protein content in the diet. Besides, the type of carbohydrate, amount of dietary micro-nutrients like steroids, trace elements like chromium, zinc and molybdenum and a number of B vitamins significantly influence niacin requirements (Halver, 1980).

Sources and stability:

Niacin is found in most animal and plant tissues. Rich sources are yeast, liver, kidney, heart, legumes etc. In feeds it is added as either nicotinic acid or niacinamide as a dry dilution. Processing losses of niacin in extruded diets is about 20 percent. In aquatic systems, dietary losses are widely encountered due to leaching of the vitamin from diets. About 50% of the vitamin is lost from purified diets in 24 hrs due to leaching (Infanger et al., 1980). To compensate losses during diet preparation and due to leaching higher dosages are incorporated.

BIOTIN

Biotin is a monocarboxylic acid slightly soluble in water and alcohol and insoluble in organic solvents. It is required as an intermediate carrier of  $CO_2$  in several specific carboxylation and decarboxylation reactions, including the carboxylation of pyruvic acid to form oxaloacetic acid. Acetyl Co A carboxylase, pyruvate carboxylase and propionyl Co A carboxylase are enzymes requiring biotin. Biotin is also required for the metabolic pathways associated with the biosynthesis of long-chain fatty acids, purine, and the metabolism of odd-carbon fatty acids. It is also involved in the conversion of unsaturated fatty acids to the stable cis

form in the synthesis of biologically active fatty acids.

Deficiency symptoms:

In salmonids skin disorders, muscle atrophy, lesions in the colon, loss of appetite, spastic convulsions and fragmentation of erythrocytes have been reported (Halver, 1980). In brook-trout "blue slime patch disease" is caused specifically by biotin deficiency. Fish reared in 10° to 15°C water exhaust biotin stores in 8-12 weeks and the first signs are anorexia, poor food conversion-depressed liver acetyl Co A carboxylase and pyruvate carboxylase (Poston and Page, 1982). In channel catfish depigmentation has been noted (Robinson and Lovell, 1978). In brook trout abnormal synthesis of liver fatty acids and high liver glycogen content reported (Poston and McCartney, 1974). In Japanese eels reduced growth, abnormal swimming behaviour, and dark coloration have been reported after 8 weeks (Arai et al., 1972).

Dietary requirements:

Biotin is synthesized in channel catfish by intestinal microflora, but it has a role in blood glucose regulation and improves cell membrane function.

Deficiency symptoms:

Macrocytic normochromic anaemia; poor growth, anorexia, general anaemia, lethargy, fragile fins, dark skin pigmentation and infarction of spleen (Halver, 1980). In Coho salmon reduction in number of erythrocytes also reported (Smith and Halver, 1969). Dupree (1966) failed to demonstrate any major deficiency symptoms in channel catfish.

Common carp did not showed any major deficiency symptoms (Aoe et al., 1967). Folic acid is synthesized by the intestinal bacteria of common carp. John and Mahajan (1979), however, found a reduction in growth rate and

erythrocyte number in the Indian major carp, rohu, after 15 weeks on a folic acid deficient diet.

Dietary requirements:

Salmonids have been shown to require about 6-10 mg/kg dry feed (Table ). Lake trout fingerlings required a minimum of 0.1 mg biotin/kg dry diet for optimal growth rate. Dietary biotin concentrations of 8 mg/kg dry diet enhanced liver pyruvate decarboxylase activity in channel catfish fingerlings (Robinson and Lovell, 1978), whereas 6 mg biotin/kg dry diet increased acetyl Co A carboxylase in brook trout fingerlings. Common carp fingerlings-require 1 mg biotin/kg dry diet for maximal weight gain and biotin content in liver (Ogino et al., 1970).

Sources and stability:

Yeast, green vegetables, liver, kidney, glandular tissues, fish tissues and viscera. Insects contain xanthopterin which has folic acid activity. Folic acid is added to feeds as a dry dilution. Storage losses are as high as 43% in three months. Supplemental biotin in the diet may be required for maximum growth (Robinson and Lovell, 1978). The diets should be protected from strong oxidizing agents or conditions which promote oxidation of ingredients. In feeds it is added as d-biotin in a dry dilution. It is stable in multivitamin premixes. Processing losses account for about 15 percent.

FOLIC ACID

Folic acid is required for normal blood cell formation. It is involved as a coenzyme in one-carbon transfer mechanisms, particularly in the interconversion of serine and glycine, methionine - homocysteine synthesis, histidine synthesis and pyrimidine synthesis. The erythrocytes are the most

sensitive to folic acid deficiency. It is also involved in the conversion of megaloblastic bone marrow to normoblastic type. It has a role in blood glucose regulation and improves cell membrane function.

Deficiency symptoms:

Macrocytic normochromic anaemia, poor growth, anorexia, general anaemia, lethargy, fragile fins, dark skin pigmentation and infarction of spleen (Halver, 1980). In Coho salmon reduction in number of erythrocytes also reported (Smith and Halver, 1969). Dupree (1966) failed to demonstrate any major deficiency symptoms in channel catfish.

Common carp did not show any major deficiency symptoms (Aoe et al., 1967). Kashiwada et al. (1971) showed that folic acid is synthesized by the intestinal bacteria of common carp. John and Mahajan (1979), however, found a reduction in growth rate and erythrocyte numbers in the Indian major carp, rohu, after 15 weeks on a folic acid - deficient diet.

Dietary requirements:

Salmonids have been shown to require about 6-10 mg/kg dry feed. In channel catfish and common carp no requirement has been demonstrated.

Sources and stability:

Yeast, green vegetables, liver, kidney, glandular tissues, fish tissues and viscera. Insects contain xanthopterin which has folic acid activity. Folic acid is added to feed as a dry dilution. Storage losses are as high as 43% in three months.



## CYANOCOBALAMIN

Cyanocobalamin is a large molecule containing cobalt. Neither plants nor animals can synthesize this vitamin; but both depend upon certain microorganisms for the trace amounts required. Cyanocobalamin is required for normal maturation and development of erythrocytes, for the metabolism of odd carbon fatty acids and for the methylation of homocysteine to form methionine. A coenzyme incorporating the vitamin is involved in the reversible isomerization of methylmalonyl coenzyme A to succinyl coenzyme A and in the isomerization of methylaspartate to glutamate. It is also essential for normal cholesterol metabolism and in purine and pyrimidine biosynthesis.

### Deficiency symptoms:

In salmon pernicious anaemia characterised by fragmented erythrocytes has been reported. Poor appetite, poor growth and food conversion precedes anaemia in salmon (Halver, 1980). In channel catfish growth retardation occurred after 36 weeks (Dupree, 1966). Limsuwan and Lovell (1981) demonstrated that intestinal microorganisms synthesized about 1.4 ng of cyanocobalamin/g body weight per day in channel catfish. John and Mahajan (1979) reported occurrence of megaloblastic anaemia in the Indian major carp rohu fed diet deficient in folic acid cyanocobalamin or both the vitamins.

Salmonids require about 0.015 to 0.02 mg/kg dry diet.

### Sources and stability:

Fish meal, fish viscera, liver kidney, glandular tissues and slaughter house wastes. It is affected by storage temperature and in mild acid solutions easily destroyed by heating.

## FAT SOLUBLE VITAMINS

### VITAMIN A

Vitamin A occurs in two forms, vitamin A<sub>1</sub> (retinal) found in marine fishes, and vitamin A<sub>2</sub> (retinol 2) found in fresh water fishes (Lehninger, 1975). Interconversion of the two forms in living fish tissue has been demonstrated.

Vitamin A is essential for maintaining epithelial cells, preventing atrophy and keratinization of epithelial tissues. It promotes growth of new cells and aids in maintaining resistance to infection. It is also involved in calcium transport across some membranes, in reproduction and embryonic development, and in cellular and sub-cellular membrane integrity.

Coldwater fish can utilize  $\beta$ -carotene at 12.4°C to 14°C, but not at 9°C (Poston et al., 1977). Dupree (1970) reported that channel catfish could utilize  $\beta$ -carotene as a vitamin A source if it was provided in the diet at over 2000 IU/kg of feed.

#### Deficiency and hypervitaminosis symptoms:

Hypovitaminosis A is characterized by poor growth, poor vision, keratinization of epithelial tissue, xerophthalmia, night blindness, haemorrhage in the anterior chamber of the eye, haemorrhage of the base of the fins and abnormal bone formation.

Conversion efficiency of  $\beta$ -carotene to vitamin A has been examined for channel catfish and brook trout. Dupree (1966) found inefficient conversion of  $\beta$ -carotene to vitamin A in channel catfish. Poston et al. (1977) demonstrated indirectly that brook trout can convert

dietary  $\beta$ -carotene into vitamin A with conversion efficiency being greater at 12.4°C than at 9°C.

In rainbow trout, thickening of the corneal epithelium and degeneration of the retina have been reported. Channel catfish fed 0.4 ppm  $\beta$ -carotene developed signs of deficiency that included exophthalmia, edema and kidney haemorrhage (Dupree, 1966). Common carp showed anorexia, faded body colour, fin and skin haemorrhages, abnormal gill opercula etc. (Aoe et al., 1968).

Hypervitaminosis A results in enlargement of liver and spleen, abnormal growth, skin lesions, epithelial keratinization, hyperplasia of head cartilage and fusion of vertebrae. Very high liver oil vitamin A content and elevated serum alkaline phosphatase also reported.

Dietary requirement:

Rainbow trout require 2000-2500 I.U. Carp 100-2000 I.U.

Sources and stability:

Codliver oil is the best source, though many other fish oils contain relatively high levels of the vitamin. Whale liver oil contains kitol, which has no biological activity until heated to 200°C, when one molecule of kitol yields one molecule of vitamin A.

Vitamin A is added to animal feeds as acetate, palmitate or propionate esters to enhance vitamin A stability (Adams, 1978). Approximately 20% of vitamin is lost during extrusion of foods. At room temperature, storage losses is about 53%. Some fish species seem able to utilize  $\beta$ -carotene as a vitamin A source; whereas others are unable to split the  $\beta$ -carotene molecule and vitamin A must be added to the diet.

## VITAMIN D

Vitamin D<sub>2</sub> or ergocalciferol and vitamin D<sub>3</sub> cholecalciferol have vitamin D activity. Vitamin D<sub>3</sub> which is also called 7-dehydrocholesterol has the chemical formula C<sub>27</sub> H<sub>44</sub> O. It is formed in most animal tissues by the rupture of one of the ring bonds of 7-dehydrocholesterol by ultraviolet radiation.

Vitamin D is essential for maintaining calcium and inorganic phosphate homeostasis. It is also involved in alkaline phosphatase activity. Although fish can sequester calcium from water through the gill membrane, a need for dietary vitamin D has been demonstrated for fish (Barnett et al., 1979).

### Symptoms of deficiency or excess:

Qualitative requirements for cholecalciferol have been determined for channel catfish and rainbow trout. Lovell and Li (1978) demonstrated the essentiality of dietary cholecalciferol for channel catfish fingerlings for greater weight gain and bone mineralization. Vitamin D deficiency in diet induced reduced weight gain; lower body ash, lower body phosphorus and lower body calcium compared with controls. Barnett et al. (1979) established the essentiality of cholecalciferol for rainbow trout fingerlings using two dietary concentrations. Symptoms of cholecalciferol deficiency included decreased weight gain and feed efficiency, marked increase in plasma triiodothyronine (T<sub>3</sub>) levels, lethargy, anorexia, increased lipid content of white muscle and liver and clinical signs of tetany. In rainbow trout tetany of the white muscle fibers has been observed (George et al., 1979).

Hypervitaminosis in brook trout showed impaired growth, lethargy and dark colouration. High intake of vitamin D mobilizes phosphorous and calcium from the bone and tissue and may result in fragile bones.

#### Dietary requirements

Relative efficacy of dietary ergocalciferol compared with dietary cholecalciferol was examined in channel catfish fingerlings (Andrews et al., 1980) and rainbow trout fingerlings (Barnett et al., 1979). Based upon weight gain channel catfish fingerlings (2.3 g) were reported to require dietary cholecalciferol at greater concentrations than 1000 I.U./Kg dry diet; but less than 4000 I.U./Kg dry diet. But slightly larger channel catfish require dietary cholecalciferol at greater concentrations than 1000 IU/KG dry diet but less than 2000 IU/KG dry diet. In channel catfish hypervitaminosis occurred at 50,000 IU/Kg of ergo or cholecalciferol as evidenced by reduced weight gain and feed efficiency.

Leatherland et al. (1980) reported an inverse relationship between T3, A growth stimulating hormone and dietary vitamin-D concentration (cholecalciferol or ergocalciferol) in rainbow trout fingerlings. Barnett et al. (1979b) reported that rainbow trout fingerlings require between 1600 and 2400 IU of cholecalciferol/Kg dry diet and that cholecalciferol is three times more effective than ergocalciferol in promoting weight gain.

#### Sources and stability:

Fish liver oil is a rich source of vitamin D. Shark liver oil contains about 25 I.U./g of vitamin D; Codliver oil 100-500 I.U./g and albacore tuna liver oil 200000 I.U./g. One international unit (I.U.) is equal to 0.025 mg of crystalline vitamin D. In feeds it is added as a spray dried powder. Processing and storage losses are not clearly defined.

reported (Murai and Andrews, 1979). Addition of 25 mg/kg tocopherol plus 125 mg/kg ethoxyquin or 100 mg/kg

-tocopherol prevented symptoms associated with oxidized menhaden oil. In common carp feeds with oxidized silk-worm caused a disease "Sekoke disease" which is characterized by a marked loss of flesh on the back of the fish. This was prevented by supplementing diet with 500 mg/kg di- -tocopherol acetate (Hashimoto et al., 1966).

Adult female common carp (100 g) displayed reduced weight gain, lower gonadosomatic index, apparent muscular dystrophy, higher muscle water content, lower muscle protein content and lower concentrations of yolk granules (Watanabe and Takashima, 1977).

#### Dietary requirements:

Channel catfish fingerlings - 25 to 100 mg/kg Di- -tocopherol. Common carp adults 700 mg -tocopherol/kg dry diet. Common carp fingerlings (6.4 g) require about 300 mg/kg-dry diet. Larger rainbow trout fingerlings (10 g) require about 20 to 30 mg where as small fingerlings (0.9g) require 50 mg/kg.

Quantitative requirements depend upon interaction of several factors (1) Dietary concentration of polyunsaturated fatty acids (2) dietary selenium concentration (3) dietary concentrations of prooxidants and antioxidants (4) diet storage temperature and (5) length of diet storage.

#### Sources and stability:

Vegetable oils are rich sources. Synthetic -tocopherol in esterified acetate or phosphate form is commonly used as a diet supplement. Considerable losses of vitamin E occur during processing and storage particularly

## VITAMIN E

Vitamin E is composed of a class of compounds known as tocopherols. One of the most important tocopherols is  $\alpha$ -tocopherol. Tocopherols are stable to heat and acids, but are rapidly oxidized in the presence of nascent oxygen, peroxides or other oxidizing agents and ultraviolet light.

The tocopherols act as extracellular and intracellular antioxidants to maintain homeostasis of labile metabolites in the cell and tissue plasma. As physiological antioxidants, these protect oxidizable vitamins and labile unsaturated fatty acids. Tocopherols along with selenium and vitamin C provide normal reproductive activity and in the prevention of muscular dystrophy in yellowtail and carp. They also act as free radical traps to stop the chain reaction during peroxide formation and stabilize unsaturated carbon bonds of polyunsaturated fatty acids. This vitamin has been shown to be important in reproductive physiology of fishes.

### Deficiency and excess symptoms:

In chinook salmon, poor growth, exophthalmia, ascites, anaemia, clubbed gills, epicarditis and ceroid deposition in the spleen reported (Woodall *et al.*, 1964). In brook trout fingerlings reduced growth rates, increased mortality and lower microhematocrit values have been reported (Poston, 1965). In Atlantic salmon anemia, pale gills, anisocytosis, poikilocytosis, exudative diathesis, dermal depigmentation, muscular dystrophy and increased carcass fat and water content.

In channel catfish poor growth, reduced food conversion, exudative diathesis, muscular dystrophy, depigmentation, fatty livers, anemia and atrophy of pancreatic tissue

in hot climates (Adams, 1978). Vitamin E acetate is however, stable during feed preparation and storage.

#### VITAMIN K

Vitamin K is required for normal blood clotting mechanisms. Menadione or vitamin K3 is a synthetic-product used to supplement animal diets. Though fairly stable, it can be destroyed upon oxidation and exposure to ultraviolet radiation.

Vitamin K is involved-in hepatic synthesis of blood clotting proteins - prothrombin and proconvertin.

Dupree (1966) reported haemorrhages in channel catfish fed a vitamin K-deficient diet. However, Murai and Andrews (1977) were unable to demonstrate any requirement for 30 weeks. In salmon prothrombin time, was increased five times and prolonged deficiency states lead to anaemia and haemorrhagic areas in gills, eyes and vascular tissues (Halver, 1980).

#### Sources and stability:

Green and leafy vegetables, alfalfa leaves soybeans and animal liver. Vitamin K found in alfalfa is fairly stable. Synthetic components added to feeds are either menadione sodium bisulfite (50% K3), menadione sodium bisulfite complex (33% K3) (Adams, 1978). Basic pH, heat, moisture, and trace minerals increase the rate of destruction of menadione salts in pelleted feeds.



TABLE 1: Halver's Water-soluble vitamin test diet H-440 for fish

<u>Complete test diet</u>	(g)	<u>Mineral mix (mg)</u>	
Vitamin-free casein	38	USP XII No.2	: 100 g
Gelatin	12	Al Cl3	15
Corn oil	6	Zn SO4	300
Cod liver oil	3	Cu Cl	10
White dextrin	28	Mn SO4	80
-cellulose mixture	9	KI	15
-cellulose	8	CoCl2	100
vitamins	$\frac{1}{9}$		
		per 100 g of salt mixture	
Mineral mix	4		
Water	<u>200</u>	<u>USP XII No. 2</u>	(g)
	300	Calcium biphosphate	13.58
<u>Vitamin mix</u>	(mg)	Calcium lactate	32.70
Thiamine HCl	5	Ferric citrate	2.97
Riboflavin	20	Magnesium sulphate	13.20
Pyridoxine HCl	5	Potassium phosphate	
Choline chloride	500	- (dibasic)	23.98
Nicotinic acid	75	Sodium biphosphate	8.72
Calcium pantothenate	50	Sodium chloride	<u>4.35</u>
Inositol	200		99.50
Biotin	0.5		
Folic acid	1.5		
L-Ascorbic acid	100		
Vitamin B <sub>12</sub>	0.01		
Menadione (K)	4		
-Tocopherol acetate	40		
Dissolve -tocopherol in			
oil mix.			
Add vitamin B <sub>12</sub> in water			
during final mixing			

Contd..

Diet preparation:

- Dissolve gelatin in cold water
- Heat with stirring on water bath to 80°C.
- Remove from heat
- Add with stirring - dextrin, casein, minerals, oil and vitamins as temperature decreases. Mix well to 40°C. Pour into containers. Move to refrigerator to harden.
- Remove from trays and store in sealed containers in refrigerator until used.
- Consistency of diet adjusted by amount of water in final mix and length and strength of heating.