Fish Feeding: 6-Vitamins and Minerals

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Part 1:Vitamins

Vitamins Definition :

1) Natural micronutrient complex organic substances.

2) Having specific biochemical functions in the human body (essential for health maintenance).

3) Obtained from animals, plants, and microorganisms.

4) Required in very tiny (mcgs) and balanced amounts.

5) Not made in the body (or not in sufficient quantity)

Vitamins

- They are necessary dietary factors, not produced by the body (except vitamin D3).

- They function as co-factor (regulator) of metabolic reactions in the body. Regulate metabolism. Help convert energy in fat, carbohydrate, and protein into ATP. Promote growth and reproduction.
- These compounds were initially termed vitamines, from ‘vital amines’, because it was thought that they contained amino-nitrogen.
- It is now known that only a few of them contain amino-nitrogen, but the adjusted group name vitamins is still used.

- Some of the vitamins can be synthesized in small amounts by the microflora of the gastrointestinal tract.
- The vitamins are a mixed group of compounds that are not closely related to each other chemically.

Classification of Vitamins

Vitamins are classified by their biological and chemical activity.

The vitamins are a mixed group of compounds that are not closely related to each other chemically, and it has become practice to divide the vitamins into two groups on the basis of their solubility characteristics: lipid-soluble vitamins and water-soluble vitamins. The lipid-soluble vitamins are usually found, and extracted from feeds, in association with lipids. They are absorbed from the gastrointestinal tract along with lipids, are not normally excreted and tend to be stored in the body. In contrast, the water-soluble vitamins are not normally stored in the body in appreciable amounts, and any excess is excreted.

Eleven water-soluble and four lipid-soluble vitamins are known to be required by fish. Eight of the water-soluble vitamins, the B complex, have coenzyme functions and are required in small quantities. The other water-soluble vitamins ascorbic acid (vitamin C), myo-inositol and choline are required in larger amounts, and these compounds are sometimes referred to as the macrovitamins.

The lipid-soluble vitamins are vitamins A, D, E and K. based upon chemical structure, function or source.

1- Fat soluble vitamins:

1) Vit. A
(Retinol, Retinal, Retinoic acid)

2) Vit. D
(Vit. D3: Cholecalciferol - Vit. D2: Ergocalciferol)

3) Vit. E
(α-Tocopherol)

4) Vit. K
(Vit. K1: Phylloquinones - Vit. K2: Menaquinones)

2-Water soluble vitamins:

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(1) **Vit. B group**

- B1 (Thiamin)
- B2 (Riboflavin)
- B3 (Niacin)
- B5 (Pantothenic acid)
- B6 (Pyridoxine)
- B9 (Folic acid)
- B12 (Cyanocobalamin)

(2) **Vit. C :** (l-Ascorbic acid)

(3) **Vit. H:** (Biotin)

WATER-SOLUBLE VITAMINS

1.Thiamine
2.Riboflavin
3.Pyridoxine
4.Pantothenic Acid
5.Niacin
6.Biotin
7.Folic Acid (Folacin)
8.Vitamin B12
9.Ascorbic Acid
10.Inositol
11.Choline
12.p-Aminobenzoic Acid
13.Lipoic Acid

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- Ascorbic acid(Vitamin C)
- Thiamin
- Riboflavin
- Pyridoxine
- Pantothenic acid
- Niacin
- Biotin
- Folic acid
- Cyanocobalamin
- Myo-inositol
- Choline
- Lipoic acid

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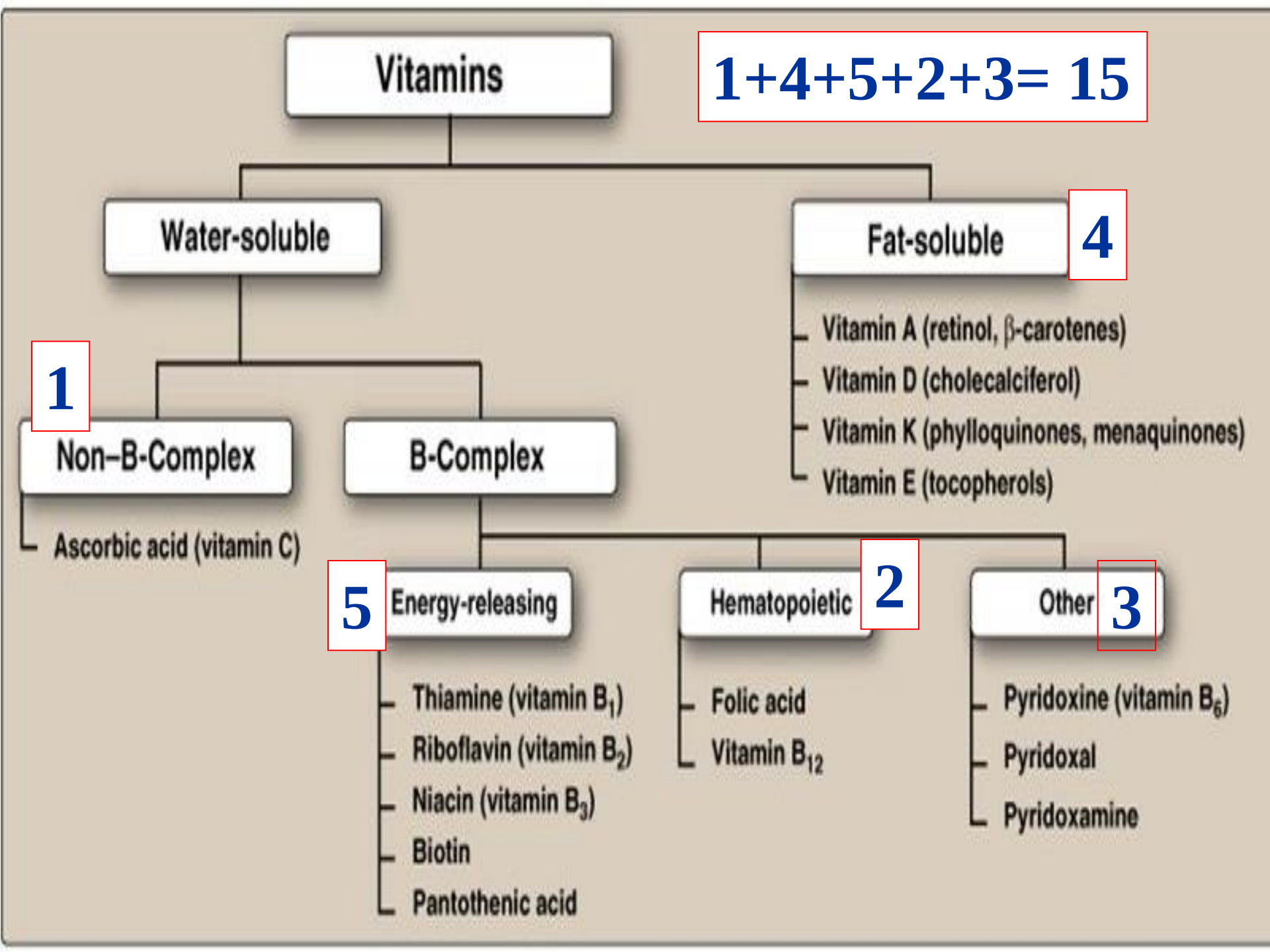

Vitamins
1+4+5+2+3= 15
Water-soluble
Fat-soluble
4
Vitamin A (retinol, β-carotenes)
Vitamin D (cholecalciferol)
Vitamin K (phylloquinones, menaquinones)
Vitamin E (tocopherols)
1
Non–B-Complex
B-Complex
Ascorbic acid (vitamin C)
5
Energy-releasing
Hematopoietic
2
Other
3
Thiamine (vitamin B1)
Riboflavin (vitamin B2)
Niacin (vitamin B3)
Biotin
Pantothenic acid
Folic acid
Vitamin B12
Pyridoxine (vitamin B6)
Pyridoxal
Pyridoxamine

Role of Vitamins in Metabolism

- Vitamins have catalytic functions (co-factors) in the metabolic reactions and do not act as building substances (that is why the daily requirement is very small).

- Since each vitamin has very specific function in metabolism, therefore, its deficiency will adversely affect one or more biochemical reactions in certain organs and very characteristic deficiency symptoms will appeared.

Cases of Vitamin Deficiency and Toxicity

(1). **Avitaminosis**: It is any disease caused by chronic or long- term vitamin deficiency or caused by a defect in metabolic conversion, such as tryptophan to niacin. It leads to well defined symptoms e.g.

- Xerophthalmia due to Vitamin A deficiency.
- Rickets due to Vitamin D deficiency.
- Pellagra due to Vitamin B3 deficiency.
- Beriberi due to Vitamin B1 deficiency.
- Scurvy due to Vitamin C deficiency.

(2). **Hypovitaminosis**: Resulted from inadequate supply of one or more vitamins. It appears in the form of well defined symptoms as skin changes, reduced vitality and low resistance to infections.

3. Hypervitamninosis:

A case which develops only upon prolonged use of excessive amount of vitamins.

EXAMPLES:

Hypervitaminosis A

This occurs after large over dosage of the vitamin.

Symptoms include:

- Headache
- Abdominal pain
- Laziness
- Visual changes
- Impaired consciousness

Hypervitaminosis D

Usually this is caused by excessive ingestion or over prescription of prescribed medications such as calcium with vit. D.

Symptoms include:

- Poly-uria
- Unsettled stomach
- Constipation
- Hypertension

Toxicity

- Water-soluble vitamins can reach toxic levels with supplement use.
- Fat-soluble vitamins are likely to reach toxic levels with supplement use.

Dietary vitamin requirements

Dietary vitamin requirements will depend upon a number of important factors:

1. The feeding behavior of the fish or shrimp species. Shrimp which consume their food slowly over a period of hours require higher dietary vitamin levels so as to counteract the progressive loss of water-soluble vitamins through leaching.
2. The vitamin synthesizing capacity of the gut microflora. Gut microflora is capable of synthesizing most B vitamins, pantothenic acid, biotin, choline, inositol and vitamin K, which in turn may become available to the animal, thereby reducing the dietary requirement. This may be particularly true for pond reared herbivorous or omnivorous fish and shrimp species.
3. The intended culture system to be used (ie. intensive, semi-intensive or extensive) and availability of natural food organisms within the water body.No beneficial effect of dietary vitamin supplementation was observed with fish either in fertilized ponds or cages (within the pond) at stocking densities of 2/m2 and 100/m3 respectively . Here, the important factor is the natural fertility of the water body and the total biomass of the fish or shrimp species stocked; the importance of dietary vitamin supplementation increasing with increasing stocking density and decreasing natural food availability per animal stocked. Natural pond food organisms therefore represent a potential source of dietary vitamins for pond cultured aquaculture species.

4. The size and growth rate of the fish or shrimp species cultured (i.e.. daily vitamin requirement per unit of body weight decreasing with increasing animal size and decreasing growth rate).
5. The nutrient content of the diet used. For example, the dietary requirement for tocopherol, thiamine and pyridoxine has been shown to increase with increasing dietary concentrations of polyunsaturated fatty acids, carbohydrate and protein, respectively.
6. The manufacturing process to be used for the production of the ration. For example, so as to counteract the destruction of the heat labile vitamins during feed manufacture, dry heat or steam pelleted feeds require higher dietary vitamin fortification than cold or wet pelleting processes.
7. The physico -chemical characteristics of the water body and physiological condition of the fish or shrimp species cultured. For example, the negative effects of pollution, disease, body wounds, and stress on fish have been found to be reduced in-part by dietary supplementation with ascorbic acid over and above that normally required by a healthy ‘non-stressed’ animal

Dietary vitamin requirements for rainbow trout, channel catfish and common carp (mg/kg dry diet unless specified)

Vitamin	Rainbow trout	Channel catfish	Common carp
Vitamin A (IU)	2000-15000	5500	1000-20000
Vitamin D (IU)	2400	500-4000	NR
Vitamin E	30-50	50-100	80-300
Vitamin K	10	10	NR
Thiamine	1-12	1-20	NR
Riboflavin	3-30	9-20	4-10
Pyridoxine	1-15	3-20	4
Pantothenic acid	10-50	10-50	25
Niacin	1-150	14	29
Folic acid	5-10	NR or 5	NR
Vitamin B_{12}	0.02	0.02	NR
Choline	50-3000	400	500-4000
Inositol	200-500	NR	200-440
Biotin*	0.8	0.1	1.1
Ascorbic acid	100-500	NR or 100	R

NR = Not required;
R = Required

VITAMIN MIX

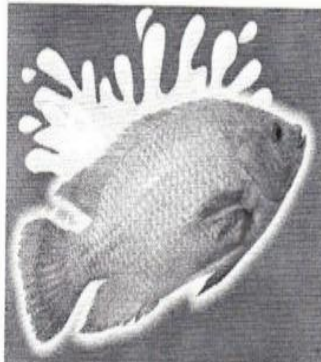
Water Soluble
Dosage 1 lb/100 lbs food
Guarnteed Analysis/pound (453g)

Vitamin A, USP Units	325,000
Vitamin D3, USP Units	65.000
Vitamin E, IU Units	32,500
Vitamin K mgs	793.65
Vitamin B12,	10.08
Riboflavin, mgs	3.250
p-Panthothenic acid, mgs	15,600
Niacin, mgs	19.500
Choline, mgs	2,600
Thiamine, mgs	2,600
Pyridoxine, mgs	2,600
Folic acid, mgs	780
Ascorbic acid, mgs	87,100
Biotin, mgs	40
BHT, mgs	200
Inositol, mgs	13,000

PRODUCT GRADE NOT INTENDED FOR HUMAN CONSUMPTION

Formulated and Packaged By
Florida Aqua Farms Inc.
Dade City, Florida

Formulated & Packaged by FAFUSA



PREMIX

Prevents for TILAPIA Fish

Composition : Each Kg. contains

Vitamin A	: 200,0000 IU	Cobalt Sulphate	: 80 mg
Vitamin D_3	: 400,000 IU	Sodium Selinate	: 20 mg
Vitamin E	: 5,000 mg	Potassium Iodide	: 240 mg
Vitamin C (Coated)	: 20,000 mg	Calcium D Panthenate	: 2,000 mg
Vitamin B_1	: 800 mg	Biotin	: 150 mg
Vitamin B_2	: 1,000 mg	Ferrous Sulphate	: 28,000 mg
Vitamin B_6	: 2,400 mg	Copper Sulphate	: 24,000 mg
Vitamin B_{12}	: 40 mg	Zinc Sulphate	: 24,000 mg
L-Lysine	: 3,000 mg	Manganese Sulphate	: 6,800 mg
DL Methionine	: 2,000 mg	Inositol	: 5,000 mg
Choline Chloride	: 5,000 mg	Calcium Crbonate	
Niacinamide	: 10,000 mg	as carrier	: up to 1,000 grams
Magnesium Sulphate	: 24,000 mg		

Dosage : 1-2 kg. / ton of Tilapia fish feed

1 kg.

Not for Human use / Medicinal use
Keep in a cool dry place
Fish feed supplement only


Part 2: Minerals

Minerals

- Minerals are inorganic elements found in the body
- Not all of them are essential and probably are there simply because of ingestion of feed
- Dietary requirement has been demonstrated for at least 22 in one or more species
- Those required in large quantities are known as **macro or major minerals**
- Those required in trace quantities are known as **trace minerals or elements**
- **Toxic**

Preliminary Concepts

- Major (Macroelements) : calcium, phosphorus, magnesium, sodium, potassium, chlorine and sulfur

More than 100 mg/day

- Trace (Microelements) : iron, iodine, manganese, copper, cobalt, zinc, selenium, molybdenum, fluorine, aluminum, nickel, vanadium, silicon, tin and chromium

Less than 100 mg/day

Dietary mineral requirements of various freshwater fish species g/kg or mg/kg dry diet)

Mineral	Rainbow trout	Japanese eel	Channel catfish	Common carp	Tilapia
Microelements					
Calcium (g/kg)	0.3-3.0	0.3-3.0	4.5	0.3-3.0	7.0
Phosphorus (g/kg)	=6.0	=6.0	4.2-4.5	=6.0	4.5-6.0
Magnesium (g/kg)	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7
Sodium (g/kg)	Max 1.6		0.026*		
Microelements					
Iron (mg/kg)	R	170	30	200	
Copper (mg/kg)	3		5	3	
Manganese (mg/kg)	12-13		2-3	12-13	
Zinc (mg/kg)	15-30		200	15-30	
Cobalt (mg/kg)	0.05				
Selenium (mg/kg)	0.2-0.4	0.3-0.5	0.25	R	R
Iodine (mg/kg)	0.6-2.8				

R = Required

Minerals

The elements required for the metabolic processes in fish can be classified into three groups:

(a) Constructional: Calcium, phosphorus, fluorine and magnesium are all important for the construction of the bones; sodium and chlorine are the main electrolytes of blood plasma and the extracellular fluid, while sulphur, potassium and phosphorus are the main electrolytes of the intracellular fluid. These elements are necessary, therefore, for the production of the above mentioned tissues.

(b) Respiratory: Iron and copper are important elements in haemoglobin and, therefore, also in the transfer of oxygen in blood.

(c) Metabolic: Many mineral elements, including some of those already mentioned above, take part in the metabolic processes. Usually they are required in much smaller amounts than for the previous two functions, and some only in trace quantities.

General Functions of Minerals

- Provide rigidity and strength to skeletal structures, exoskeletons
- primary components of bones and teeth
- constituents of organic compounds such as proteins and lipids as structural constituents of soft tissues.
- enzyme activators (**coenzymes**)
- osmoregulation, acid/base equillibria
- effect irritability of muscles and nerves
- essential components of many vitamins, hormones, and respiratory pigments

Difficulties in Studying Mineral Requirements of Fish

Inorganic elements are difficult to study, particularly trace elements.

1- The exchange of ions from the aquatic environment across gills and skin of fish complicates the determination of the quantitative dietary requirements.

2- Many trace elements are required in such small amounts that it is difficult to formulate purified diets low in mineral and maintain water sufficiently free of the test element.

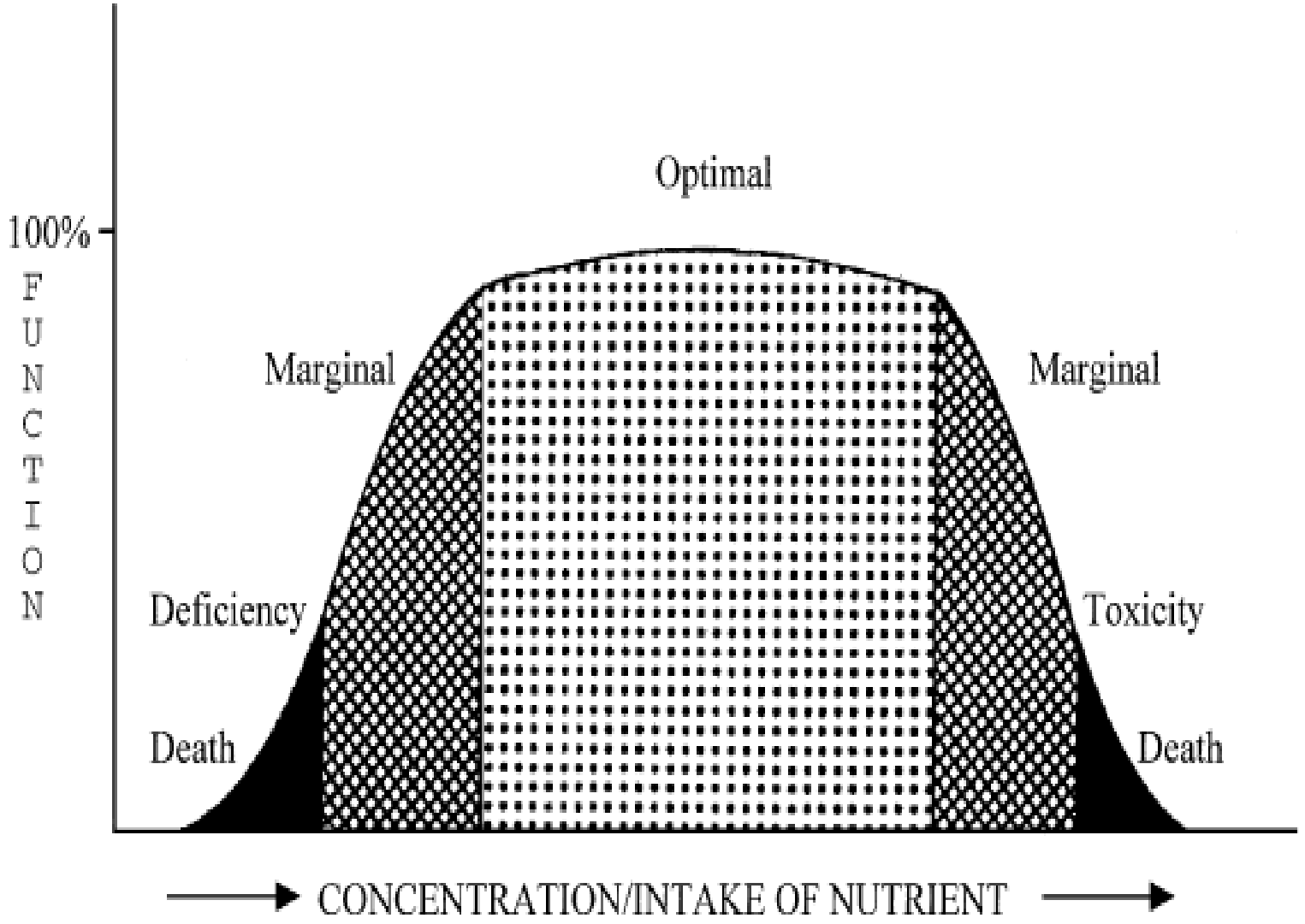
3- Despite advances in instrumental analysis of trace elements making lower detection limits possible, there are still many problems associated with their accurate measurement in fish tissue to be overcome.

4-A critical factor in the determination of ultratrace elements, such as manganese, vanadium, and chromium, is the need for meticulous sample preparation.

5-Techniques that involve the use of high purity reagents, acid-cleaned glassware, and clean-room facilities should be employed

Biological dose–response curve.

Dependence of animal function on intake of an essential nutrient



Requirements by Fish

- Fish require a supply of essential minerals for healthy growth. In the wild these are obtained from the surrounding water and the tissues of prey items.
- Similar to warm blooded animals for tissue formation and various metabolic functions
- can absorb dissolved minerals from the water across gill membrane/exoskeleton
- also via drinking (for drinking species)
- most Ca required comes from water
- for marine species, seawater provides most iron, magnesium, cobalt, potassium, sodium and zinc
- phosphorus not typically available in water

Table 8. Dietary mineral requirements of various freshwater fish species g/kg or mg/kg dry diet)

Mineral	Rainbow trout	Japanese eel	Channel catfish	Common carp	Tilapia
Microelements					
Calcium (g/kg)	0.3-3.0	0.3-3.0	4.5	0.3-3.0	7.0
Phosphorus (g/kg)	=6.0	=6.0	4.2-4.5	=6.0	4.5-6.0
Magnesium (g/kg)	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7
Sodium (g/kg)	Max 1.6		0.026*		
Microelements					
Iron (mg/kg)	R	170	30	200	
Copper (mg/kg)	3		5	3	
Manganese (mg/kg)	12-13		2-3	12-13	
Zinc (mg/kg)	15-30		200	15-30	
Cobalt (mg/kg)	0.05				
Selenium (mg/kg)	0.2-0.4	0.3-0.5	0.25	R	R
Iodine (mg/kg)	0.6-2.8				

R = Required

(Fish Premix)

0.5% VITAMINS AND TRACE MINERALS PREMIX FOR WARM WATER FISH

DIRECTION FOR USE

5kg per 1 tonne of final Warm Water Fish Feed

Each 5kg Contains

Vit A	20,000,000	I.U	Choline Chloride	400	gr
Vit D_3	4,000,000	I.U	Manganese	30	gr
Vit E	200,000	I.U	Zinc	40	gr
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	gr
Vit B_1	10,000	mgr	Copper	4	gr
Vit B_2	30,000	mgr	Iodine	5	gr
Vit B_6	19,000	mgr	Selenium	0.2	mgr
Vit B_{12}	1,000	mgr	Cobalt	0.2	mgr
Niacin	200,000	mgr	Calcium	600	gr
Folic Acid	5,000	mgr	Lysine	100,000	mgr
Panth Acid	50,000	mgr	Phosphorus	4,000	gr
Biotin	400	mgr	Methionine	100	gr
Antioxidant	125	gr			

Fish Premix

ZDHF PHARMACEUTICALS

Good Manufacturing Practice GMP Certified Quality Product

Calcium and Phosphorus

- Calcium and phosphorus are usually discussed together because they occur in the body combined with each other for the most part and because an inadequate supply of either limits the nutritive value of both.
- Almost the entire store of calcium (99 percent) and most of the phosphorus (80 percent) in the fish's body are present in bones, teeth and scales.
- composition consists of calcium and phosphorus in the ratio of approximately 2:1.

Calcium and Phosphorus

- Ca and P are two of the major inorganic constituents of feeds
- Ca: essential for blood clotting, muscle function, proper nerve pulse transmission, osmoregulation
- P: component of ADP, ATP, P-lipids, DNA, RNA
- Phosphates serve as pH buffer systems

Calcium and Phosphorus

- Dietary Ca is primarily absorbed from the inestine by active transport
- in vertebrates, blood levels of Ca and P are regulated by the vitamin/hormone **cholecalciferol (D_3)**
- absorption depends upon whether the mineral is soluble at the pH of the gut
- Ca, for example, can be put in the diet as Ca-lactate, Ca-PO_4 tribasic, or $CaCO_3$
- digestibility of above: 58%, 37%, 27%, respectively

Phosphorus Availability

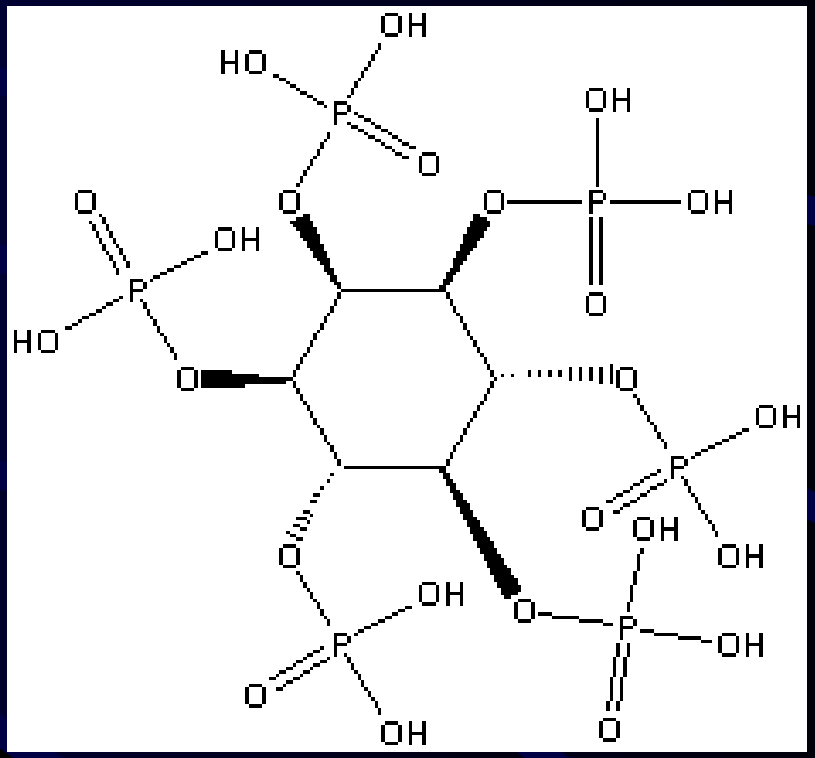
- The main question regards whether the mineral is soluble in water
- monobasic sources (sodium phosphate) are highly digestible (90-95%)
- availability of di- and tri-basic phosphorus sources varies with species, but is generally around 45-65%
- monobasic sources are more expensive

Calcium and Phosphorus

- Besides the form in which it is included in the diet, availability of Ca and P can depend upon:
- 1) level of lactose intake
- 2) dietary form of Vitamin D
- 3) iron, aluminum, manganese, potassium and magnesium intake
- 4) level of fat intake
- 5) level of dietary phytate (phytic acid)
- obviously, many interactions

Phosphorus Availability

- The major source of P in natural grains (67%) is a compound known as **phytate phosphorus**
- this form of P is poorly available
- the presence of phytate inhibits the availability of dietary Ca and other sources of P
- forms insoluble complexes in the digestive system



phytic acid

Phosphorus Availability

- Question: how do we make P more available? Why should we?
- Answer: possible that addition of phytase to feeds could make grain-based P more available (also could work for Zn)
- Recent studies with mammalian systems have shown that phytate inhibits uptake of iron

Calcium and Phosphorus

- Since levels of P are low in most natural waters, there is a dietary requirement
- Supplementation of dietary Ca inhibits P availability
- Thus, dietary ratios of less than 2:1 Ca:P are recommended

the dietary requirement of a fish species for a particular element will depend to a large extent upon the concentration of that element in the water body. At present there is little information concerning the contribution of waterborne elements to the total mineral balance of fish

Element/species	Deficiency signs [1]
PHOSPHORUS	
Common carp (C. carpio)	Reduced growth, poor feed efficiency (1,2); bone demineralization, skeletal deformity, abnormal calcification of ribs and soft rays of pectoral fin (1); cranial deformity (1,3); increased visceral fat (4)
CALCIUM	
Channel catfish (I. punctatus)	Reduced growth, low carcass ash, Ca and P content (fed vitamin D deficienct diets, 6)
MAGNESIUM	
Common carp (C. carpio)	Reduced growth (11, 18); sluggishness, anorexia, convulsions, high mortality (11); cataracts (18)
IRON	Hypochromic microcytic anaemia (C. carpio - 26; C. major - 27; Salvelinus fontinalis - 28; A. japonica - 20; I. punctatus - 42; reduced growth and feed efficiency (42)
ZINC	
Common carp (C. carpio)	Reduced growth (18, 30); cataracts (18); anorexia, high mortality, erosion of fins and skin, elevated tissue concentrations of Fe and Cu in intestine and hepatopancreas (30)
MANGANESE	
Common carp (C. carpio)	Reduced growth (34, 18); short body dwarfism, cataracts (18)
COPPER	
Common carp (C. carpio)	Reduced growth (34, 18); cataracts (18)
SELENIUM	
Common carp (C. carpio)	Reduced growth (18, 37); cataracts (18); anaemia (37)
IODINE	
Salmonids	Thyroid hyperplasia/goitre (39, 40)

Vitamin A: retinol

- Can only be found intact in animal sources
- in its natural form, it is alcohol known as retinol
- also isolated from various lipids and beta carotene
  - 1 beta carotene (plants) ~ 2 retinols (body)
- stored in the liver
- retinol + opsin (protein) = rhodopsin (vision)
- deficiency = improper growth, exophthalmia
- feeds contain non-oxidizable form, proper storage
- requirement level = 1,000 I.U. (international units)
- sources: fish oils

Vitamin D_3: cholecalciferol

- Vitamin D found as ergocalciferol (D_2) and cholecalciferol (D_3)
- most land animals can use both, except chickens (only D_3)
- fish appear to use only D_3
- both activated in plants/animal skin by UV radiation
- D_3 primarily used as precursor for calcium regulation

Vitamin E: tocopherol

- Active form is alpha tocopherol
- good antioxidant: most feed antioxidants have vit E activity, but only 1/6 that of α-tocopherol
- antioxidants used to prevent oxidation (spoilage) of lipids (HUFAs & PUFAs)
- requirement is tied to selenium deficiency (Se is cofactor in glutathione peroxidase)
- deficiency in fish = muscular dystrophy, reduced fertility
- increased dietary requirement in absence of PUFA's
- requirement: 50-100 mg/kg for fish/shrimp
- sources: alfalfa meal, fish meal, rice bran, wheat middlings, barley grains

Vitamin K: menadione

- Originally identified as a “fat-soluble factor” required for normal blood clotting
- menadione is the most active form
- actually works by activating blood-clotting proteins
- requirement: shrimp (none), fish (unknown)
- dietary sources: alfalfa meal, liver meal

Water Solubles: thiamine (B_1)

- Function: metabolism of COH
- sources: brewers yeast, wheat middlings, rice bran, rice polishings, wheat bran, soybean meal
- deficiency: central nervous system failure
- requirement: 2.5 mg/kg (tilapia), 10-15 mg/kg (salmon)
- requirement: 40-50 mg/kg (shrimp)

Riboflavin: B_2

- Function: metabolic degradation of proteins, COH, lipids
- sources: plants, bacteria, yeast, fish solubles
- deficiency: cataracts (fish), vision, crooked limbs
- requirements: 9 mg/kg (channel catfish), 5 mg/kg (tilapia)
- requirements: 50 mg/kg (shrimp)

Niacin

- Function: transport of hydrogen ions as NADP, NADPH; electron transport, fatty acid, cholestrol synthesis
- forms: niacin, nicotinic acid, nicotinamide
- sources: rice polishings, yeast, rice bran
- deficiencies: pellagra (dermatitis), anemia (fish), skin lesions (fish), sunburning (fish)

- Can fish convert tryptophan to niacin? (Data inconsistent.)

- requirements: 14-28 mg/kg (carp, catfish)
- requirements: 400 mg/kg (shrimp)

Folic Acid

- Recently shown as very important for pregnant females to avoid birth defects
- function: synthesis of purines, pyrimidines, nucleic acids
- sources: yeast, alfalfa meal, full-fat soybeans
- deficiencies: anemia, large erythrocytes, pale gills (fish)
- requirements: 1-4 mg/kg (fish, shrimp)

Cyanocobalmine

- Last of 15 vitamins to be identified
- chemically complex, cobalt nucleus
- function: coenzyme in metabolic reactions, maturation of erythrocytes, uracil->thymine
- deficiency: pernicious anemia, nerve disorders
- requirement: very low 0.015 mg/kg or not at all

Ascorbic Acid: C

- Both finfish/shellfish very sensitive to this vitamin, especially as juveniles
- function: antioxidant, stress reducer, bone calcification, iron metab, tyrosine metab, blood clotting
- deficiency: scoliosis (lateral), lordosis (vertical), fin erosion, black death (shrimp)
- toxicity: toxic at over 150-200 mg/kg (shrimp)
- sources: synthesized from glucose, usually added as chemical form
- requirement: 100 mg/kg varies w/age, metabolism