Vitamin Requirements of Tilapia – A Review

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Abstract

Vitamins are organic substances that are essential for growth, health, reproduction and maintenance in animals, but required in small amounts. Since fish cannot synthesize vitamins at all or can only synthesize in insufficient quantity for normal development, growth and maintenance, they must be supplied in the diet. In comparison to terrestrial animals, the information available on vitamin requirement of aquatic species still lacks depth. Other than the 13 known vitamins, i.e. thiamin, riboflavin, niacin, pantothenic acid, biotin, folic acid, vitamin B_6, B_{12}, C, A, D, E and K, their requirements have been quantified in tilapia, the essentiality of choline and inositol for tilapia have also been demonstrated. Vitamin E and B_6 requirements for tilapia are closely related to dietary lipid and protein concentrations, respectively. 42-44 and 60-66 mg vitamin E/kg diet are required for maximal growth of tilapia fed diets with 5 and 12% lipid, respectively; whereas, 1.7-9.5 and 15.0-16.5 mg vitamin B_6/kg diet are required for tilapia fed diets with 28 and 36% protein, respectively. Niacin requirements in tilapia varies with the carbohydrate source in diet. Optimum dietary levels of niacin for maximum growth have been reported to be 26 mg/kg diet for tilapia fed a glucose diet, whereas 121 mg/kg diet is needed for fish fed a dextrin diet. Tilapia produces vitamin B_{12} in their gastrointestinal tract through bacterial synthesis. Thus, no dietary B_{12} is needed for tilapia. Vitamin C (L-ascorbic acid, AA) requirements of tilapia is 79 mg AA/kg diet. When vitamin C stabilizers, L-ascorbyl-2-sulfate (C2S), L-ascorbyl-2-monophosphate-Mg (C2MP-Mg) and L-ascorbyl-2-monophosphate-Na (C2MP-Na), are used as the vitamin C source, the requirement level are 20-23, 17-20 and 16 mg AA/kg diet, respectively. Dietary vitamin A requirements of tilapia is 5,850 to 6,970 IU/kg diet. Tilapia is able to utilize β-carotene to fulfill the dietary vitamin A requirements. The conversion ratio by β-carotene to vitamin A is approximately 19:1.

Shi-Yen Shiau, Yu-Hung Lin. 2006. Vitamin Requirements of Tilapia – A Review. En: Editores: L. Elizabeth Cruz Suárez, Denis Ricque Marie, Mireya Tapia Salazar, Martha G. Nieto López, David A. Villarreal Cavazos, Ana C. Puello Cruz y Armando García Ortega. Avances en Nutrición Acuícola VIII .VIII Simposium Internacional de Nutrición Acuícola. 15 - 17 Noviembre. Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México. ISBN 970-694-333-5.

Introduction

Tilapias are mainly lacustrine fish which are well-adapted to enclosed water. They are fast growing, resistant to disease and handling, easy to reproduce in capacity, and are able to tolerate a wide range of environmental conditions. They are widely cultured in tropical and subtropical regions of the world and constitute the third largest group of farmed finfish, with an annual growth rate of about 11.5% (El-Sayed, 1999). Global production of farmed tilapia has increased more than four-fold since 1984, from 550,327 m.t. to 2,543,017 m.t., representing 4.28% of total farmed finfish in 2004 (FAO, 2005).

Most cultured tilapias are grouped into two genera (Trewavas, 1982): *Tilapia*, which are macrophagous and substrate-spawners, and *Oreochromis*, which are microphagous and mouth-brooders. The main cultured species are *Oreochromis niloticus*, *O. aureus*, hybrid *O. niloticus* × *O. aureus*, *O. mossambicus* and *Tilapia zilli*. For the mouthbrooders of the *Oreochromis* genus, there exists a marked delay in the growth of females, and monosex culture of males is preferred. Thus, it is important to indicate the sex of the cultured fish, and whether sex control was obtained through manual sorting, sex-reversal by hormone treatment, or hybridization.

Originally, for most of the tilapia industry, ponds were fertilized to promote growth of prey items, and formulated feeds were supplemental. Vitamin supplements are often not included in practical diets for tilapias stocked at moderate densities in fertilized ponds. As the industry expands and technology development continues, traditional extensive culture of tilapia is being replaced by semi-intensive and intensive production system, the notion that vitamin needs could always be satisfied through the consumption of natural prey items from the pond environment became questionable. As these alternative sources of nutrients became relatively limited, culturists added a range of vitamins to formulated feeds to ensure requirements for these nutrients were met.
Research on vitamin requirements of tilapia begins in 1982. Since 1990, progress has been substantial. Today nearly all the vitamin requirements have been quantified. Table 1 presents the vitamin requirements of various tilapia.

Water-Soluble Vitamins

Thiamin

Thiamin was the first vitamin to be recognized. In animal tissue, thiamin occurs predominantly in a di-phosphate form known as thiamin pyrophosphate (TPP). TPP is an essential cofactor for a number of important enzymatic steps in energy production, including both decarboxylations and transketolase reactions.

Thiamin deficiency signs observed in red hybrid tilapia (*O. mossambicus* × *O. niloticus*) fingerlings cultured in sea water (32 p.p.t. salinity) were reduced growth and feed efficiency and a low haematocrit. A dietary thiamin level of 2.5 mg/kg of diet was sufficient for maximum growth and prevention of deficiency signs (Lim and LeaMaster, 1991).

Riboflavin

Widely distributed in both plant and animal feedstuffs, riboflavin is generally found complexed with proteins as flavin nucleotides. Within cells, flavin mononucleotide and flavin adenine dinucleotide are typically linked with other organic components into flavoproteins that interact in the metabolism of protein, fat, and carbohydrates. The riboflavin occurring in these enzyme complexes acts as an intermediary in the transfer of electrons in biological oxidation-reduction reactions.

Typically deficiency signs reported for tilapias fed a riboflavin-free diet were anorexia, poor growth, high mortality, fin erosion, loss of normal body colour, short body dwarfism and lens cataracts. The dietary riboflavin requirements were 6 mg/kg of diet for juvenile *O. aureus* grown in fresh water (Soliman and Wilson, 1992a) and 5 mg/kg of diet for *O. mossambicus* × *O. niloticus* grown in 32 p.p.t. sea water (Lim *et al.,* 1993).

Vitamin B_6

Vitamin B_6 in the form of pyridoxal phosphate participates as a prosthetic group of enzymes in a large number of metabolic reactions, particularly those associated with the metabolism of proteins or amino acids.

Fish fed a diet without vitamin B_6 supplementation developed abnormal neurological signs, anorexia, ataxia, convulsions, caudal fin erosion, mouth lesions, hyperirritability, poor growth and high mortality. Weight gain and hepatic alanine aminotransferase activity analyzed by broken-line regression indicated that the optimum dietary vitamin B_6 requirement in juvenile *O. niloticus* × *O. aureus* reared in fresh water were 1.7-9.5 mg/kg of diet and 15.0-16.5 mg/kg of diet containing 28% and 36% protein, respectively (Shiau and Hsieh, 1997). A dietary vitamin B_6 level of 3 mg/kg of diet was reported to be adequate for *O. mossambicus* × *O. niloticus* fed 38% protein diets and reared in sea water (Lim *et al.,* 1995).

Vitamin B_{12}

Vitamin B_{12} is the generic name for a group of cobalt-containing compounds called cobalamins, that have the biological activity of cyanocobalamin. Vitamin B_{12} dependent enzymes play key roles in the synthesis of nucleic acids and proteins and in the transfer of methyl units in carbohydrate and fat metabolism.

Tilapia produced vitamin B_{12} in their gastrointestinal tract through bacterial synthesis and did not have a dietary requirement for this vitamin (Lovell and Limsuwan, 1982; Sugita *et al.,* 1990; Shiau and Lung, 1993).

Niacin

Niacin is a vital part of the coenzymes needed to release energy from carbohydrate. The niacin

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(3-pyridine carboxylic acid and various derivatives) requirement for animal can be met in a number of ways. Some niacin can be synthesized from the amino acid tryptophan. This biosynthesis occurs not only in gut microbes but also in most species of multicellular animals. Synthesis can supply a substantial portion of the total requirement in some animals, however, animal species vary in this ability. Fish apparently do not effectively synthesize niacin as suggested by the relatively rapid development of deficiency symptoms when they are reared on a niacin-free diet (NRC, 1993). Niacin synthesis from tryptophan has yet to be quantified for tilapia.

Niacin is a dietary essential for *O. niloticus* × *O. aureus* hybrid but the level required varies depending on the source of dietary carbohydrate. Optimum dietary level for maximum growth have been reported to be 26 mg niacin/kg of diet for fish fed a glucose diet and 121 mg niacin/kg of diet for fish fed a dextrin diet. Fish deprived of dietary niacin developed haemorrhages, a deformed snout, gill odema and skin, fin and mouth lesions (Shiau and Suen, 1992).

Biotin

Biotin is a coenzyme for several CO_2 fixing enzymes such as pyruvate carboxylase and acetyl CoA carbozylase. Because these enzymes have a role in gluconeogenesis, fatty acid synthesis and degeneration, and function of the tricarboxylic acid cycle, then biotin is important for the metabolism of amino acids, carbohydrate and lipids.
The dietary biotin requirement for maximum growth of *O. niloticus* × *O. aureus* have been estimated to be 0.06 mg/kg of diet (Shiau and Chin, 1999).

Folic acid

Folic acid, when converted to active tetrahydrofolate coenzymes, functions as a one-carbon donor or acceptor in a variety of reactions involved in amino acid and nucleotide metabolism. It is necessary for normal cell division and multiplication, and a deficiency of folic acid is characterized in most animals and in humans by impaired hematopoiesis.
An analysis of the weight gain percentage by broken-line regression indicates that the adequate dietary folic acid requirement of *O. niloticus* × *O. aureus* is 0.82 mg/kg of diet (Shiau and Huang, 2001).

Pantothenic acid

Pantothenic acid functions as a part of the coenzyme A molecule in the metabolic release of energy from all three energy-providing nutrients, carbohydrate, fat, and protein, by way of the tricarboxylic acid cycle.
Pantothenic acid deficiency causes poor growth, haemorrhage, sluggishness, high mortality, anemia and serve hyperplasia of the epithelial cells of gill lamellae in *O. aureus*. A dietary level of 10 mg of calcium-pantothenate/kg of diet is sufficient to prevent these deficiency signs (Soliman and Wilson, 1992b). Roem et al. (1990a) reported that *O. aureus* could satisfy their requirement for pantothenic acid by feeding on bacteria in a recirculating system.

Choline

Choline, a vitamin-like nutrient, is an important component of the phospholipid lecithin and certain other complex lipids. It serves as a source of labile methyl groups for the synthesis of various methylated metabolites and as a precursor of acetylacholine. However, the studies with fish identified choline as essential for maximum weight gain (Craig and Gatlin, 1996; Griffin *et al.,* 1994; Hung, 1989).
While it was suggested that a high level of dietary methionine may have partially satisfied choline requirement in *O. aureus* (Roem *et al.,* 1990a, b), Shiau and Lo (2000) established that the optimum dietary choline requirement for *O. niloticus* × *O. aureus* is 1,000 mg/kg of diet.

***Myo*-inositol**

Myo-inositol is the most prevalent naturally occurring biologically active isomer of inositol and exists as a structural component of phosphatidylinositol in cell membranes. It is also a part of the phosphoinositide system, a signal transduction pathway stimulated by certain hormones, neurotransmitters, or growth factors. Inositol is classified as a vitamin-like nutrient and often a supplement to aquatic feeds.

Shiau and Su (2005) indicated that the intestinal microbial synthesis is not a significant source of inositol for tilapia and that the requirement for dietary *myo*-inositol in growing *O. niloticus* × *O. aureus* is 400 mg/kg of diet.

Vitamin C

Vitamin C (ascorbic acid, AA) acts as a biological reducing agent for hydrogen transport. It is involved in many enzyme systems for hydroxylation, i.e., hydroxylation of tryptophan, tyrosine, and praline. Vitamin C is necessary for the formation of collagen and normal cartilage. Vitamin C is an indispensable nutrient for fish, as they cannot synthesize this nutrient due to the lack of enzyme L-gulonolactone oxidase (EC 1.1.3.8), therefore they depend on exogenous supply through dietary source.

Tilapias showed classical vitamin C deficiency signs when fed a vitamin-deficient diet in the absence of natural foods. The requirement for normal growth of *O. aureus* has been reported to be 50 mg AA/kg of diet (Stickney *et al.,* 1984). In juvenile *O. niloticus* × *O. aureus*, a dietary level of 79 mg AA/kg of diet is needed for maximum growth (Shiau and Jan, 1992). A recommended dietary vitamin C inclusion level for *O. niloticus* is 420 mg AA/kg of diet (Soliman *et al.,* 1994). In all these studies, L-ascorbic acid was used as the source of vitamin C. L-Ascorbic acid (AA) is unstable and most of its activity in practical diets is lost during processing and storage. Shiau and Hsu (1993) found that about 75% of the initial amount of supplemented AA in aquatic diets can be lost during processing and storage at ambient temperature for 1 h. Because of the unstable nature of AA, the use of more stable forms of ascorbic acid is necessary. Several derivatives of AA have been shown to have antiscorbutic activity for tilapia. L-Ascorbyl-2-sulfate (C2S) has been shown to have equal antiscorbutic activity as L-ascorbyl-2- monophosphate (C2MP-Mg) for *O. niloticus* × *O. aureus*. The optimum dietary level is 41-48 mg C2S/kg of diet and 37-42 mg C2MP-Mg/kg of diet (Shiau and Hsu, 1995). The optimum dietary L-ascorbyl-2- monophosphate-Na (C2MP-Na) for *O. niloticus* × *O. aureus* is 63.4 mg/kg of diet. It was suggested that C2MP-Mg is about 85% as effective as C2MP-Na in meeting the vitamin C requirement for tilapia (Shiau and Hsu, 1999). The requirement for optimum growth of *O. spilurus* in seawater has been determined to be between 100 to 200 mg C2S/kg of diet (Al-Amoudi *et al.,* 1992).

Lipid-Soluble Vitamins
Vitamin A

Vitamin A is important in a number of physiological processes such as vision, reproduction, and in the maintenance of differentiated epithelia in vertebrates.
Requirements for vitamin A to *O. niloticus* × *O. aureus* is 5,850-6,970 IU/kg of diet based on weight gain and liver vitamin A retention (Hu *et al.,* 2006). From the chemical structure, β-carotene can be hydrolyzed to two molecules of vitamin A. The vitamin A activity of β-carotene has been demonstrated in tilapia (Katsuyama and Matsuno, 1988). The requirement of dietary β-carotene for tilapia is 28.6 to 44.3 mg/kg of diet at a dietary vitamin A content of 84/IU kg of diet. The conversion ratio of β-carotene to vitamin A is approximately 19:1 (Hu *et al.,* 2006).

Vitamin D

Vitamin D functions as a precursor 0f 1,25-dihydrocholecalciferol, which stimulates the absorption of calcium from the intestine. Vitamin D is essential for maintaining homeostasis of calcium and inorganic phosphate. It is involved in alkaline phosphatase activity, promotes intestinal absorption of calcium, and influences the action of parathyroid hormone on bone.
Optimum vitamin D_3 (cholecalciferol) requirement for maximum growth of *O. niloticus* × *O. aureus* is 374.8 IU/kg of diet (Shiau and Hwang, 1993). O'Connell and Gatlin (1994) reported that dietary vitamin D_3 is not dietary essential for *O. aureus*.

Vitamin E

Vitamin E functions as a lipid-soluble antioxidant, protecting biological membranes, lipoproteins and lipid stores against oxidation. The antioxidative functions of vitamin E include scavenging of free radicals to terminate lipid peroxidation, which can initiate damage to unstable intracellular components including membranes, nucleic acids and enzymes, and thereby result in pathological conditions.

Vitamin E-deficient *O. aureus* exhibited anorexia, reduced weight gain and diet efficiency, skin hemorrhages, impaired erythropoiesis, muscle degeneration, ceroid in liver and spleen and abnormal skin coloration. The dietary vitamin E requirement increased with increasing levels of dietary lipid. The requirement of *O. aureus* was estimated at 10 mg and 25 mg dl-α-tocopheryl acetate/kg of diet in diets containing 3% and 6% dietary lipid, respectively, or 3 to 4 mg α-tocopheryl acetate for each per cent of dietary lipid (Roem *et al.,* 1990c). The dietary vitamin

E requirement of *O. niloticus* was reported to be 50 to 100 mg/kg of diet for a diet containing 5% lipid, increasing to 500 mg/kg of diet for a diet containing 10-15% lipid (Satoh *et al.,* 1987). Recently, tilapia has been shown to require both n-3 highly unsaturated fatty acid and n-6 fatty acid for maximal growth (Chou and Shiau, 1999; Chou *et al.,* 2001). Consequently, the vitamin E requirement of tilapia has been re-evaluated to be 42-44 mg/kg of diet and 60-66 mg/kg of diet in 5% and 12% lipid diets, respectively (Shiau and Shiau, 2001).

Vitamin K

Vitamin K is typically associated with its role in coagulation of blood, it also has an important role in calcium transport. In vertebrates, the action of osteocalcin, the major bone matrix protein, is vitamin K-dependent. Vitamin K is required for the posttranslational carboxylation of specific glutamate residues to gamma-carboxyglutamate residues. These residues interact with calcium allowing osteocalcin to regular the incorporation of calcium phosphates into bone tissue.
Tilapia showed poor growth and low plasma prothrombin concentration when fed a vtamin K-free diet for 8 weeks. The dietary vitamin K requirement of *O. niloticus* × *O. aureus* is estimated to be 5.2 mg/kg of diet (Lee, 2003).

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Table 1. Vitamin requirements in tilapia (*Oreochromis* spp.)

Vitamins	Requirement (mg kg^{-1} diet)				
	O. niloticus × *O. aureus*	*O. aureus*	*O. niloticus*	*O. mossambicus* × *O. niloticus*	*O. Spilurus*
Thiamin	-	-	-	2.5[B]	-
Riboflavin	-	6[o]	-	5[C]	-
Vitamin B_6	1.7-9.5 (28% C.P.)[a] 15-16.5 (36% C.P.)[a]	-	-	3[D] (38% CP)	-
Vitamin B_{12}	dispensable[b]	-	dispensable[w,x]	-	-
Niacin	121[c]	-	-	-	-
Biotin	0.06[d]	-	-	-	-
Folic acid	0.82[e]	-	0.5-1[y]	-	-
Pantothenic acid	-	6-10[p,q]	-	-	-
Choline	1000[f]	dispensable[r,s]	-	-	-
Inositol	400[g]	-	-	-	-
Ascorbic acid	79 (AA)[h] 41-48 (C2S)[i] 37-42 (C2MP-Mg)[i] 63.4 (C2MP-Na)[j]	50 (AA)[t]	420 (AA)[z]	-	100-200 (C2S)[E]
Vitamin A	5850-6970 IU/kg [k]	-	-	-	-
Vitamin D	0.00937 (374.8 IU/kg)[l]	dispensable[u]	-	-	-
Vitamin E	42-44 (5% lipid)[m] 60-66 (12% lipid)[m]	10 (3% lipid)[v] 25 (6% lipid)[v]	50-100 (5% lipid)[A]	-	-
Vitamin K	5.2[n]	-	-	-	-

[a] Shiau and Hsieh (1997)
[b] Shiau and Lung (1993)
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