



Review

L-Carnitine and its attributed functions in fish culture and nutrition—a review

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Abstract

This review summarizes the different studies on L-carnitine supplementation and its various attributes in fish culture and nutrition. Based on its role in vertebrates, the use of L-carnitine supplementation in fish diets in aquaculture has been advocated for multi functional purposes: as a growth promoter, specifically aiding in the utilization of high fat levels in the diet and thus providing a protein sparing effect; providing protection against toxic levels of ammonia and xenobiotics; alleviating stress related to water temperature extremes and facilitating better acclimation to water temperature changes. Levels of dietary L-carnitine supplementation examined ranged from a few hundred to over 4000 mg/kg of diet.

The studies on fish exhibit conflicting results: in a number of cases L-carnitine supplementation led to better growth and changes in lipid utilization while in others no effect could be detected. Contradicting results have been reported even with the same fish species. Explanations are provided in an attempt to clarify the discrepancies. Cost effectiveness is an important issue to be considered, and even in cases where L-carnitine has shown positive effects, the levels of this rather expensive substance required might not be economically justifiable.

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1. Introduction

During the past two decades, the effects of L-carnitine supplementation on fish culture and nutrition have been studied in different cultured fish species. While some studies show positive results, others have contradicted them and the reasons for these conflicting reports need to be elucidated. This review will summarize the different studies and various attributes of L-carnitine while attempting to provide an explanation for the discrepancies. A general description of L-carnitine, its sources and synthesis will be given, followed by a discussion on its properties and role in vertebrates including humans. This background will help us understand the reasons why L-carnitine was originally thought to be a wonder substance and why it has been advocated for fish. The question of its effectiveness needs to be carefully considered.

1.1. General description of L-carnitine

L-Carnitine (L- β -hydroxy- γ -*N,N,N*-trimethylaminobutyric acid) is a non-essential organic nutrient, sometimes referred to as a quasi amino acid, required for entry of long-chain fatty acids (as acylcarnitine

esters) into the mitochondria. It is a derivative of the lysine, has a molecular weight of 161.2 and is a very hygroscopic compound, easily soluble in water. Its name is derived from the fact that it was first isolated from meat (carnus), 100 years ago. Since L-carnitine appeared to act as a vitamin in the mealworm (*Tenebrio molitor*), it was named vitamin B_T. This term “vitamin” turned out to be misleading when scientists later discovered that other higher organisms including humans are capable of synthesizing L-carnitine. Under certain conditions, the demand for L-carnitine may exceed an individual’s capacity to synthesize it, making it a conditionally essential nutrient. As in many other substances, the D-isomer of carnitine is either less active or biologically inactive (Gross and Henderson, 1984). Santulli and D’Amelio (1986a) studied the effects of supplementing the diet of the European sea bass with a daily ration of 250 mg of either L-carnitine or D-carnitine per kilogram of wet body weight. Their results show that the use of the D-carnitine supplement resulted in significantly lower growth rate of the fish compared with the control group which had no carnitine supplementation. On the other hand the fish fed the L-carnitine supplementation (under the same experimental conditions) exhibited significantly better growth results compared

with the control group. This might be due to the fact that orally administered carnitine must be transported to the circulation via the intestine and at that stage the L-carnitine is taken up much faster (more than double the rate of D-carnitine) as demonstrated in rats (Gross and Henderson, 1984).

1.2. Synthesis of L-carnitine

L-Carnitine is synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and other secondary compounds produced in the body. The first convincing evidence for carnitine biosynthesis in animals was obtained from chick embryos, which contained significant amounts of carnitine, whereas none was found in eggs. When grown on a carnitine-free synthetic medium, the microorganism *Neurospora crassa* also contained carnitine. As for carnitine biosynthesis, it was shown that the methyl groups of carnitine come from methionine (but not from choline) and that γ -butyrobetaine (but not γ -aminobutyric acid or γ -dimethylaminobutyrate) is converted to carnitine. It was also shown that lysine is converted to carnitine with 6-*N*-trimethyllysine as an intermediate (Bremer, 1983). The endogenous formation of carnitine in vertebrates occurs primarily in the liver, as well as in the kidneys and the brain, as a result of the occurrence there of the required enzyme 4-butyrobetaine hydroxylase. This synthesis of L-carnitine is catalyzed by the concerted action of five different enzymes. The process requires two essential amino acids (lysine and methionine), iron (Fe^{2+}), vitamin C, vitamin B₆ and niacin in the form of nicotinamide adenine dinucleotide (NAD). One of the earliest symptoms of vitamin C deficiency is fatigue, thought to be related to decreased synthesis of L-carnitine (Lohninger et al., 1987).

1.3. Effects on fat metabolism

After synthesis, the L-carnitine must be transported to other tissues. It is most concentrated in tissues that use fatty acids as their primary dietary fuel, such as skeletal and cardiac muscle. In this regard, L-carnitine plays an important role in energy production by chaperoning activated fatty acids (acyl-CoA) into the matrix for and accompanying intermediate compounds out of the mitochondrial matrix to prevent

their accumulation. Carnitine-acylcarnitine translocase is responsible for the transport of carnitine and its esters across the inner mitochondrial membrane (Fritz and Yue, 1963). Carnitine is therefore a normal constituent of animal tissues and plasma, which is required for the transport of long-chain fatty acids to the site of oxidation. Carnitine also facilitates removal of short-chain organic acids from mitochondria, thereby freeing intramitochondrial coenzyme A to participate in the β -oxidation and tricarboxylic acid cycle pathways. It is a substrate for carnitine palmitoyltransferases I and II and carnitine acetyltransferase, enzymes that participate in and regulate fatty acid utilization (Borum, 1987).

1.4. Sources of carnitine

Red meats are the best source of carnitine (containing 500 to 1200 mg/kg), followed by fish, chicken and milk-derived substances (containing 16 to 64 mg/kg). On the other hand vegetables, fruits, grains and other plant-derived food sources contain very little carnitine (usually < 0.5 mg/kg). Reports of nutritional carnitine deficiency are very rare. During the early stages of life, infants are particularly susceptible to carnitine depletion, because the demands of tissue accretion associated with rapid growth exceed the ability of the body to synthesize carnitine. However, the substantially lower plasma carnitine concentrations (and, presumably, lower tissue carnitine concentrations) do not impair growth or other indicators of normal development (Bremer, 1983; Lohninger et al., 1987).

In carnitine deficiency, fatty acid oxidation is reduced and fatty acids are diverted into triacylglycerol synthesis, particularly in the liver. Mitochondrial failure develops in carnitine deficiency when there is insufficient tissue carnitine available to buffer toxic acyl-coenzyme (CoA) metabolites. Toxic amounts of acyl-CoA impair the citrate cycle, gluconeogenesis and fatty acid oxidation. These processes are reversible with the addition of sufficient quantities of carnitine to the diet (McDowell, 1989).

1.5. Role of L-carnitine in vertebrates including humans

The role of carnitine in vertebrates has been studied extensively and most studies relate to its role in fatty

acid oxidation. However, carnitine may have functions in cellular metabolism, independent of its role in fatty acid oxidation, such as plasma membrane fatty acid remodeling and gene regulation (modulation of the transcriptional response to triiodothyronine of genes for malic enzyme and fatty acid synthase). Esters of carnitine (acetyl- and propionylcarnitine) may have pharmacological value, by virtue of their antioxidant properties and/or ability to deliver readily oxidizable carbon units to mitochondria, in chronic disorders such as ischemia-induced myocardial dysfunction in angina pectoris (Sinatra and Sinatra, 1999). Carnitine, due to its role in fatty acid oxidation, may diminish modulation of transcription of urea cycle enzymes by long-chain fatty acids, suggesting a mechanism for its ameliorating effect on experimentally-induced hyperammonemia in higher vertebrates (O'Connor et al., 1984). Carnitine and acetyl-L-carnitine both enhance immune function (Rebouche, 1998). For example the effect of short and long term feeding with L-carnitine, L-acetyl carnitine and L-propionyl carnitine on the production of eicosanoids from in vitro stimulated carrageenan-induced rat peritoneal macrophages was investigated by Garrelds et al. (1994), who found that plasma carnitine levels were higher in young rats given carnitine, both chronically and acutely. Carnitine derivatives on the other hand had no effect. In addition, acetyl-L-carnitine protects nerve cells from stress and deterioration (Crayhon, 1998).

2. The role of L-carnitine in fish

Based on its role in vertebrates, the use of L-carnitine supplementation in fish diets in aquaculture has been advocated for multi functional purposes:

- As a growth promoter, specifically aiding in the utilization of high fat levels in the diet and thus providing a protein sparing effect;
- Providing protection against toxic levels of ammonia and xenobiotics;
- Alleviating stress related to water temperature extremes and facilitating better acclimation to water temperature changes;
- Changes in muscle structure/texture related to higher levels of swimming activity;
- Enhancing reproduction.

The aforementioned points will be discussed in detail, in view of the fact that studies carried out on L-carnitine supplementation in fish have shown conflicting results, even with the same species. The number of reports showing no effect of L-carnitine supplementation on the growth of the tested fish is remarkable considering the fact that usually researchers abstain from publishing negative results, which are perceived as less lucrative. Our personal experience with L-carnitine studies on ornamental fish has also shown conflicting results and my discussions with colleagues have brought to light many similar experiences. This leads me to believe that the number of studies showing negative results is actually far higher than has been reported in the literature.

2.1. Effects on fish growth

Growth (measured as weight gain) is of great importance to fish growers and therefore deserves special attention with respect to carnitine supplementation. The growth promoting effects of carnitine supplementation in fish feeds have been attributed to the increase in utilization of energy as a result of the increase in fatty acid oxidation by the mitochondria. This process has been demonstrated in isolated mitochondria of trout (Bilinski and Jonas, 1970).

A growth promoting effect of L-carnitine supplementation was found by the following researchers: Santulli and D'Amelio (1986a) in European sea bass; Twibell and Brown (2000) in hybrid striped bass; Torreele et al. (1993), in African catfish; Chatzifotis et al. (1995) in red sea bream; Keshavanath and Renuka (1998) in the Indian major carp rohu; Jayaprakas et al. (1996) in Mossambique tilapia; and Becker et al. (1999) in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). An indication of growth promotion (though not significant) was found by Becker and Focken (1995) and Focken et al. (1997) in common carp.

On the other hand, no effect of L-carnitine supplementation on growth was observed by Dias et al. (2001) in European sea bass; by Ozorio (2001) and Ozorio et al. (2001a,b) in African catfish; by Rodehutsord (1995) and Chatzifotis et al. (1997) in rainbow trout; by Burtle and Liu (1994) in channel catfish; by Gaylord and Gatlin (2000a,b) in hybrid striped bass; by Ji et al. (1996) in Atlantic salmon; by

Harpaz et al. (1999) in the ornamental cichlid (*Pelvicachromis pulcher*); Dzikowski et al. (2001) in guppy fish (*Poecilia reticulata*); and by Schlechtriem et al. (2004) with hybrid tilapia (*O. niloticus* × *O. aureus*). Interpretation of the conflicting results is more complicated than originally meets the eye.

Fish require a very high level of protein in their diet as compared with other farmed animals. The cost of the protein ingredient is high and some of it is used for energy and not utilized for growth (Wilson, 2002). Over the last two decades there has been increasing pressure to reduce the level of fish meal in diets prepared for fish. In many carnivorous fish, high-energy feeds are being used and the level of fat in the diet has increased tremendously, reaching 35% and above. This has been done in an effort to reduce fish meal content and to achieve protein sparing. These high fat levels have triggered interest in the fish's capability of fully utilizing high-energy feeds. Since carnitine is so closely associated with fat metabolism, many researchers have tried to establish correlations between higher levels of carnitine in the fish diets and fat metabolism (Santulli et al., 1988; Kiessling and Kiessling, 1993; Burtle and Liu, 1994; Chatzifotis et al., 1995; Froyland et al., 1998). Oxidation of fat provides the highest and most cost effective energy yield per unit weight of the dietary ingredients. Carnitine promotes the oxidation of fat and therefore it was assumed that the addition of carnitine to the diet of fish would result in the enhancement of the protein sparing action of fat and thus lead to better growth on diets containing less protein. In most instances the results varied, and although elevated carnitine levels facilitated changes in lipid metabolism (as could be expected) this did not necessarily result in better growth of the fish.

To avoid accumulation of lipids, supplementation of dietary carnitine may be used to stimulate fatty acid oxidation and to regulate lipolysis as described by Ozorio (2001) and Ozorio et al. (2003). Accelerated growth and reduced body fat have been reported in a number of fish species reared on diets containing supplementary L-carnitine: European sea bass (Santulli and D'Amelio, 1986a; Santulli et al., 1988, 1990) and African catfish (Torreele et al., 1993; Ozorio et al., 2001b). However, Burtle and Liu (1994) who studied the effects of L-carnitine supplementation (at a level of 1000 mg/kg) to the diet of fingerling chan-

nel catfish at different levels of dietary lysine, showed no effect of the carnitine on growth, but carnitine did significantly reduce the muscle and liver lipid levels.

Ji et al. (1996) who studied the effects of L-carnitine supplementation on the metabolism of fat in Atlantic salmon demonstrated that fish fed L-carnitine exhibit altered intermediary metabolism and reduced tissue lipid. Elevated levels of palmitate oxidation in the liver and significantly higher incorporation of labeled lactate into glucose by liver cubes (120%) and isolated hepatocytes (210%) were also found. Higher levels of methionine incorporation were observed as well, but no changes were found in the growth of the fish. The authors conclude that their results showed enhanced protein synthesis in the mechanism of carnitine induced changes in gluconeogenesis and nitrogen metabolism.

Cost effectiveness is another important issue to be considered. Even in the cases where L-carnitine has shown positive effects, the required levels of this rather expensive substance might not justify the additional growth obtained. Becker et al. (1999) addressed this point stating that the better growth obtained by the addition of 150 mg/kg carnitine to the diet of hybrid tilapia was a result of better feed conversion ratio and 13% less feed was required to attain the same weight. Thus, according to their calculations, despite the high price of carnitine the farmer would still benefit from supplementing the diet with L-carnitine. Unfortunately, most of the studies exhibiting positive effects of L-carnitine supplementation in fish diets show that the levels of supplementation required are much higher than 150 mg/kg and at these high levels the economic viability of this practice should be carefully evaluated. The ecological aspects of better feed conversion should also be taken into account in this evaluation.

2.2. Protection against toxic levels of ammonia and xenobiotics

Ammonia toxicity is known to be one of the common stressors in fish culture. Carnitine has been shown to provide protection of fish against acute ammonia toxicity. An example of controversial results in the role of L-carnitine as a protecting agent against the detrimental effects of ammonia toxicity is described by Tremblay and Bradley (1992). Their experimental work in fish was carried out following a

controversy between the work of O'Connor et al. (1984) and that of Kloiber et al. (1988). O'Connor et al. (1984) suggested that L-carnitine fully protects mice against acute ammonia toxicity when the mice were injected with a lethal dose of ammonium acetate. The mode of protection was suggested to be a result of rapid restored energy status and stimulation of the urea cycle in the mice. On the other hand, Kloiber et al. (1988) showed that similar protection is provided by other quaternary amines, meaning that the mechanism of action might be different than the one suggested by O'Connor et al. (1984). Deshmukh and Rusk (1988) who failed to repeat O'Connor et al.'s (1984) work claim that since in the experiment the ammonium challenge was carried out by injection, the injected carnitine solvent (and not the carnitine itself) was enough to prevent the toxic effect simply by diluting the level of the ammonium challenge in the blood of the experimental animals. Similar such discrepancies in results are found in fish research although in most instances the lack of effect is found in different species.

Tremblay and Bradley (1992) examined juvenile chinook salmon and showed an impressive protecting effect of L-carnitine injected into fish at a dose of 10–16 mmol/kg body weight against a subsequent 10.75 mmol/kg injection of ammonium acetate. Their results showed that 98% of the untreated fish showed signs of ammonia toxicity while only 33% exhibited such signs in the fish injected with L-carnitine. The carnitine injected fish had only 4% mortality while the control (manitol injected fish) exhibited 69% mortality. Their results also showed that of the quaternary amines tested for the protective effect, based on the results of Kloiber et al. (1988) in mice, trimethylamine oxide (TMAO) had similar protective properties while betaine and choline were toxic to juvenile chinook salmon. Li et al. (1992) tested human subjects and demonstrated that dietary carnitine is primarily absorbed by active transport whereas a pharmacological dose is largely assimilated by passive means. When the rate of carnitine absorption was measured in situ, it appeared adequate for the physiological amount (derived from the diet) while it was marginal for pharmacological doses. The experimental results with fish showed that high levels of L-carnitine in the fish circulation system can have a protective role when the fish are exposed to high concentrations of

this stressor. Yet, simulation of carnitine role through its injection into the fish at very high levels, equivalent to 1600–2600 mg/kg, is by no means the same as feeding a diet supplemented with carnitine at those high levels. This aspect should be taken into account when conducting experiments in which the researchers inject the various test substances, rather than having them delivered via the diet, as was done by Tremblay and Bradley (1992).

It appears that in the event that the fish are exposed to extreme stress in the course of an experiment or during the production cycle, the presence of higher levels of carnitine can lead to better “handling” of the stress resulting in better growth. In order to better evaluate the results of the experiments which did indeed show an advantage of the addition of carnitine to the diet, it is necessary to verify whether the fish were not exposed to stress caused by an episode of severe oxygen depletion or a transient state of elevated ammonia/nitrite levels in the course of the study. Usually it is not possible to obtain that information.

The carnitine system can be altered by several xenobiotics (drugs and chemicals). These alterations are responsible for most toxic effects, which can be reverted or minimized by L-carnitine administration. Formation of nonmetabolizable acyl coenzyme A (CoA) is a typical step in the biotransformation of pivaloyl antibiotics. L-Carnitine acts as an acceptor of specific, nonmetabolizable acyl CoA. The consequence of this process is a secondary carnitine deficiency. L-Carnitine interacts with cardiolipin, modifying membrane permeability and protecting the functions of the mitochondria. This mechanism can be proposed to explain the protective effects of L-carnitine against cardiotoxicity, ammonium acetate-induced mitochondrial ultrastructural and functional alterations (Arrigoni-Martelli and Caso, 2001). Similarly this could also provide an explanation for the role carnitine plays in the protection of gills and skin of guppy fish against anionic xenobiotics. Schreiber et al. (1997) used contact fluorescent microscopy to determine the level of fluorescein penetration from the water via the gills or skin and showed that L-carnitine treatment reduced the level of penetration of this marker in guppy fish which were fed a diet supplemented with 1100 mg L-carnitine/kg diet. Similar protection, provided by a lower level (150 mg/kg) of carnitine supplementation for hybrid tilapia reared

under intensive pond culture conditions, was found by [Schlechtriem et al. \(2004\)](#). The results obtained in this study with hybrid tilapia ([Schlechtriem et al., 2004](#)) are interesting. Halfway through the experiment an accident occurred which resulted in the dumping of a tremendously excessive amount of food to the pond in which the experimental fish were held. This caused a sharp depletion of oxygen and a surge in the level of ammonia in the rearing water. Previous work has shown that L-carnitine has a protective role in cases of exposure to high levels of ammonia ([Santulli and D'Amelio, 1986b](#); [Tremblay and Bradley, 1992](#)). However, there was no indication that the L-carnitine treatments (150 and 450 mg/kg added to the feed) administered by [Schlechtriem et al. \(2004\)](#) had any effect on the growth or survival of the fish compared with the control group that had no carnitine added to its feed. Their results only showed that the L-carnitine (150 mg/kg) treated fish exhibited better resistance to xenobiotics. It is possible that the short duration of the experiment (only 31 days past the stressful stage) was not long enough for the beneficial effects of carnitine to be expressed.

2.3. *Alleviating stress related to water temperature extremes and facilitating better acclimation to water temperature changes*

Due to its marked effects on biochemical activities, temperature is of crucial importance to ectotherms. Temperature shifts may alter the equilibrium between synthesis and degradation of biological structures, and change metabolic requirements. Given the extent of these thermal effects, it is not surprising that fish exhibit a variety of biochemical strategies to cope with thermal change. How animals and fish regulate lipid metabolism is an important part of the enantiostatic mechanism (conserved function) used by animals in response to stress (such as temperature stress). The interaction between environmental stress, exercise and lipid oxidation still requires elucidation. There are many functional and structural steps as fatty acids are mobilized, transported and oxidized in working muscle and these may serve as regulatory points for responding to acute or chronic stimuli. Determining which of these factors help regulate the fatty acid pathway and what impact they have on whole-animal lipid oxidation and performance is an

important area of future research. More studies need to attempt to integrate gene expression with physiological function, especially in response to environmental stress ([McClelland, 2004](#)).

During the course of an annual cycle, fish from temperate zones cope with a wide thermal range. Many species modify their biochemical and physiological properties to offset these thermal shifts. A number of reports have addressed this issue in fish. In cold temperate zones the fish often shift the level of the aerobic capacity of their skeletal muscles. During cold acclimation, fish tissues can increase mitochondrial volume density ([Egginton, 1996](#); [Cordiner and Egginton, 1997](#)) and the activities of mitochondrial enzymes ([Rodnick and Sidell, 1994](#); [St-Pierre et al., 1998](#)) or protein-specific oxidative capacities of their mitochondria ([Guderley and Johnston, 1996](#); [Guderley et al., 1997](#)). In rainbow trout, cold acclimation and acclimatization increase the capacity of skeletal muscle mitochondria to oxidize pyruvate and acyl carnitines and increase polyunsaturation of mitochondrial phospholipids ([Guderley et al., 1997](#)). Cold acclimation and acclimatization also increases the activity of some mitochondrial enzymes such as citrate synthase and carnitine palmitoyl transferase ([St-Pierre et al., 1998](#)). However, cold acclimation of rainbow trout does not increase the proportion of oxidative fiber volume occupied by mitochondria ([St-Pierre et al., 1998](#)). During fish culture a more common situation is a sudden drop or elevation in temperature rather than a slow change.

A marked reduction in stress related cold shock deaths has been shown in the case of the warm water ornamental cichlid fish *P. pulcher* fed varying levels of L-carnitine supplementation (0, 500, 1000 and 2000 mg/kg diet). Fish fed diets supplemented with L-carnitine (at all levels of supplementation) exhibited a higher survival after exposure to a severe cold shock, compared with the control fish fed a diet that was not supplemented with L-carnitine ([Harpaz et al., 1999](#)). The results from other animals such as chicken follow the same pattern. Laying hens exposed to extreme high temperature exhibited higher albumin quality of their eggs during the stress involved in this exposure when L-carnitine at a level of 50 mg/l was added to their drinking water. However, it should be pointed out that weight gain, egg weight and mass as well as other traits were not affected by

the addition of L-carnitine to the drinking water (Celik et al., 2004). In humans the effects of L-carnitine were noticeable in cases of stress related to heavy exercise, in which both higher temperatures are involved as well as faster usage of lipid resources (McClelland, 2004).

In a study conducted by Guderley and Johnston (1996) short-horned sculpin *Myoxocephalus scorpius* were acclimated to 5 and 15 °C to evaluate the impact of thermal acclimation upon maximal rates of substrate oxidation by mitochondria. Cold acclimation virtually doubled maximal rates of pyruvate oxidation at all experimental temperatures (2.5, 7.5, 12.5 and 20 °C). Rates of palmitoyl carnitine oxidation were also enhanced by cold acclimation.

Rodnick and Sidell (1994) studied the effect of thermal acclimation on the activity of carnitine palmitoyltransferase I (CPT I). The level of this rate-limiting enzyme for beta-oxidation of long-chain fatty acids, was determined in oxidative red muscle of striped bass acclimated at 5 or 25 °C. Cold acclimation increased citrate synthase activity 1.6-fold and total CPT activity 2-fold in homogenates of red muscle; free carnitine increased by 62%, and specific activity of CPT I in mitochondria increased 2-fold. Thermal sensitivity of CPT I and its preference for different long-chain fatty acyl-CoA substrates (16:1-CoA=16:0-CoA>18:1-CoA) were not altered by thermal acclimation. Rodnick and Sidell (1997) also examined the effects of temperature acclimation on ultrastructural characteristics of cardiac myocytes and maximal activities of metabolic enzymes in cardiac tissue of striped bass. Ventricular mass and ventricular mass divided by body weight were significantly increased (29% and 40%, respectively) in fish acclimated to cold (5 °C) vs. warm temperatures (25 °C). Ventricular enlargement did not alter volume densities of mitochondria, myofibrils, protein concentration, or citrate synthase activity. Thus the total volume of mitochondria and myofibrils increased proportionately with cardiac mass in cold animals. Activity of carnitine palmitoyltransferase increased in cold animals (42%), suggesting positive compensation and increased aerobic capacity for utilization of glucose and fatty acids for energy production. Enlargement of the ventricle and an increased capacity for ATP production in striped bass may help compensate for kinetic constraints at cold temperatures and maintain

circulatory support to oxidative axial musculature for swimming activity.

In a similar manner Hicks et al. (1996) demonstrated that acclimation to low temperature in rainbow trout is associated with a two-fold increase in long-chain acyl-CoA synthetase in the heart. Interestingly, in an effort to explore the effects of carnitine supplementation on chicken reared under normal and low temperature (a rapid decrease from 28 °C to 20 °C) Buyse et al. (2001) conducted a study in which they examined the effects of the addition of 100 mg L-carnitine/kg to the basal starter and finisher (containing 17.8 and 22.9 mg L-carnitine/kg, respectively) diets on performance, organ weights of male and female broiler chickens. Dietary L-carnitine supplementation had no significant effects on any of these production parameters, except for a reduction in the abdominal fat content of females in one group of treated chickens. However, dietary L-carnitine supplementation greatly increased absolute and proportional heart weights.

Scombroid fishes possess the ability to slightly elevate their body temperature utilizing special “heater organs”. These fish have therefore been the focus of research aimed at better understanding the metabolic pathways utilized in this process. Tullis et al. (1991) conducted a study designed to elucidate the activities of key metabolic enzymes and to determine which metabolic substrates could fuel heat production in these heater organs. They tested the in vitro activities of the metabolic enzymes in the brain and eye of five species of scombroid fishes. Their findings showed that most of the fish examined showed extremely high oxidative capacity. The activity level of citrate synthase, a commonly measured index of oxidative metabolism, was found to be one of the highest values ever reported for vertebrate tissue. Carnitine palmitoyltransferase and 3-hydroxyacyl-CoA dehydrogenase (measured as indicative enzymes for fatty acid metabolism) activity were also high, with carnitine palmitoyltransferase being among the highest reported for vertebrates. These results indicate that heat production could be fueled aerobically by either lipid or carbohydrate metabolism. Heater organs of fishes from the colder Mediterranean waters were found to have a higher aerobic capacity and as a result, a greater heat-generating potential, than fishes from the warmer waters of the Pacific. Yet, this dif-

ference may be due to the fact that tested fishes caught in the Mediterranean were considerably smaller than those caught in the Pacific and the difference might be related to fish size and not necessarily attributed to different thermal environments. Energy utilization in fish living in extremely cold water and the fatty acids involved in the process could shed more light on the possible involvement of carnitine. Crockett and Sidell (1993) examined hepatic mitochondrial and peroxisomal beta-oxidation in an Antarctic marine teleost, *Notothenia gibberifrons*. Mitochondria showed marked preference for the oxidation of a monounsaturated substrate. Carnitine palmitoyltransferase activities with palmitoleoyl-CoA (C16:1) were found to be 2.4-fold higher than activities with palmitoyl-CoA (C16:0). Their findings suggest that the polyunsaturates, eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6), found in high concentrations in Antarctic fishes, are utilized as fuels to support aerobic energy metabolism.

Carnitine, through its involvement in lipid metabolism, has an important role in the process of temperature acclimation and can provide some protection to fish kept under aquaculture conditions with rapid environmental changes resulting in high temperature fluctuations.

2.4. Changes in muscle structure/texture related to higher levels of swimming activity

Exercising the muscles (as is the case in fast swimming fish) in the presence of higher levels of carnitine (derived from dietary supplementation) could enhance the lipid utilization and alter muscle structure and texture (the main edible part in cultured fish). A study investigating the effect of L-carnitine supplementation on carnitine content in muscle fiber and fatty acid metabolism and on performance of exercise trained rats was conducted by Bacurau et al. (2003). Carnitine supplementation increased time until exhaustion (14.0%), but the effect was even greater (30.3% increase) in the supplemented and trained rats. Carnitine supplementation increased oleate decarboxylation (17% for sedentary rats and 119% for trained rats) These results show that carnitine supplementation increases fatty acid oxidation in skeletal muscle by a mechanism that includes increasing total carnitine content in soleus muscle mi-

tochondria and the total content of acyl-carnitine. Moyes et al. (1992) studied mitochondrial metabolism of cardiac and skeletal muscles derived from a fast tuna (*Katsuwonus pelamis*) and a slow common carp fish. They found that the tissue of tuna (which has a higher aerobic capacity) had between 30% and 80% more mitochondrial protein per gram of tissue than the carp. The mitochondrial volume was the same but in the tuna the mitochondria were extremely densely packed. The highest rate of oxygen consumption by ventricle mitochondria was 2 times higher in the tuna. Activities of carnitine palmitoyl transferase per milligram of protein were 2–2.5 times higher in tuna red muscle and ventricle mitochondria. Their results show that muscle aerobic capacity can be increased at several levels: tissue mass, mitochondrial volume density as well as mitochondrial specific activity and these have an impact on the muscle structure.

2.5. Enhancing reproduction

Jayaprakas et al. (1996) studied the effects of 4 levels (300, 500, 700 and 900 mg/kg) of dietary L-carnitine supplementation on growth and reproductive performance of male Mossambique tilapia. The study was carried out with juvenile (2.2 g fish) that were reared in open concrete tanks for a period of 252 days on the experimental diet administered at a level of 5% of the fish biomass. Their results showed an effect on both growth and reproductive performance of the fish, this effect being correlated with the level of carnitine supplementation. The best effect was obtained with the diet supplemented with 900 mg L-carnitine/kg diet (net weight gain of 91.83 ± 3.91 g compared with 75.83 ± 2.31 g in the control group with no L-carnitine supplementation). Food consumption after 1 month of feeding was also positively affected by the level of added carnitine in the diet (11.01 g in the control group compared with 16.69 in the group fed 900 mg carnitine/kg diet). Similarly they showed that the L-carnitine supplementation had a positive effect on the reproductive performance of the fish, significantly increasing both testis weight (0.45 g in the 900 mg/kg vs. 0.1 g in the control) and sperm cell concentration per ml (39.66×10^9 in the 900 mg/kg vs. 24.4×10^9 in the control). However the better reproductive performance might be attributed to the better growth of fish

fed a high quality diet rich in carnitine and not necessarily to a direct influence of L-carnitine on reproduction, since a direct correlation between feed quality and sperm viability in tilapia has been shown by [Chao et al. \(1987\)](#). Interestingly, the results of a study carried out on humans by [Matalliotakis et al. \(2000\)](#) found differences between L-carnitine concentrations in fertile and infertile human males, pointing towards a potential relationship between L-carnitine and semen quality. They reported L-carnitine levels which differed significantly ($P < 0.0001$) between controls and the infertile patient groups. The group with normal spermiogram had a mean value of 478.4 for L-carnitine, while the abnormal ones had only 100.58. They also showed a statistically significant, positive correlation between L-carnitine and the number of spermatozoa, the percentage of motile spermatozoa and the percentage of normal forms.

On the other hand [Dzikowski et al. \(2001\)](#) who studied the effect of temperature and diets supplemented with 1100 mg L-carnitine/kg food on the reproductive performance of female live-bearer guppy fish found that temperature had a significant effect on brood size and brood interval, but that L-carnitine did not. These results do not corroborate the findings of [Schreiber et al. \(1997\)](#) who reported that in guppy fish kept under a temperature range of 26–32 °C, the addition of L-carnitine at a level of 1100 mg/kg to the diet resulted in significantly better average brood size per female. It should be noted that in [Schreiber et al.'s \(1997\)](#) study, this positive effect of L-carnitine was found only during summer and no effect was found during winter. Similarly, in laying hens kept under heat stress environment (35–37 °C), the addition of 50 mg L-carnitine/l to the drinking water over a period of 8 weeks affected egg quality, resulting in a significant increase in the relative albumen weight ([Celik et al., 2004](#)).

3. Other aspects of L-carnitine function not yet researched in depth in fish

There are a number of aspects of L-carnitine functions which have not yet been researched in depth in fish. Experience from studies conducted on vertebrates including humans could be useful in understanding the role of L-carnitine in fish.

3.1. Concentrations of L-carnitine in body tissues

The concentration of carnitine in different species and in different tissues vary over a wide range. The highest concentrations reported have been found in horseshoe crab muscle and in rat epididymal fluid, in which carnitine can reach a concentration of 60 mmol/l. In mammalian tissues the concentration usually varies between 0.1 mmol/l and a few millimoles per liter (with the highest concentrations in heart and skeletal muscle), and there are relatively great inter-species variations. The carnitine concentration is 1 mmol/l in rat skeletal muscle; 3 mmol/l in human muscle; and may be up to 15 mmol/l in ruminant muscle. In plants on the other hand, the concentration is only a few micromoles per liter ([Bremer, 1983](#)). [Miyasaki et al. \(1994\)](#) determined the level of free and esterified carnitine in tissues of rainbow trout and found the levels of acid soluble carnitine (containing free carnitine acetylcarnitine and short-chain acylcarnitines) to be 188 µg/g wet tissue in an ordinary muscle compared with 133 µg/g wet tissue in the liver or 5.6 µg/g wet tissue in the plasma of the fish. This level corresponds to a concentration of about 1 mmol/l in the muscle of the fish ([Bremer, 1983](#)).

In humans, carnitine is concentrated in most tissues of the body, principally in skeletal muscle which has about 40 times the concentration of carnitine in the blood. In rainbow trout the concentration in the muscle is around 35 times the concentration found in the plasma ([Miyasaki et al., 1994](#)). The final enzymatic reaction in the pathway occurs primarily in the liver and kidneys, but not in skeletal and heart muscle. Normal adults synthesize and ingest carnitine in amounts totaling 100 mg/day. The normal rate of carnitine synthesis in humans is 0.16 to 0.48 mg/kg body weight/day. Thus, a person weighing 70 kg, s from 11 to 34 mg/day. This rate of synthesis combined with the re-absorption of about 95% of the L-carnitine filtered by the kidneys is enough to prevent deficiency in generally healthy people, including strict vegetarians ([Bremer, 1983](#)). In most studies conducted with fish, the carnitine inclusion level in the fish diets was in the range of a few hundred to above 4000 (!) mg/kg diet (e.g. [Chatzifotis et al., 1995, 1996](#); [Jayaprakas et al., 1996](#); [Harpaz et al., 1999](#); [Gaylord and Gatlin, 2000a,b](#); [Dias et al., 2001](#)). Compared with the requirements for human nutrition, this is clearly an

excessive level of supplementation. However, in humans approximately 75% of the dietary carnitine is absorbed, and unabsorbed carnitine is almost entirely degraded by bacteria in the large intestine. No dietary components are known to impair absorption. There is no known toxicity associated with excessive ingestion from normal dietary components (Bremer, 1983). It is likely that the same exists in fish and the excess carnitine is degraded so that the negative impact is mainly on the cost of the feed.

3.2. *Effects of carnitine at different developmental stages*

The required quantities of carnitine in fish during their developmental stages have not been as thoroughly studied, but it is assumed that they follow a similar pattern to those reported in other vertebrates. Studies carried out with mammals at different developmental stages showed some effects of L-carnitine supplementation on growth in the early life stages (Rebouche, 1992). Rincker et al. (2003) showed that the addition of 50 to 100 mg L-carnitine/kg diet improved growth performance (average daily gain) of weanling pigs during phase 2 of growth, but had only minor effects on growth during phases 1 and 3 of their growth. Supplemental L-carnitine tended to be more effective when soybean oil was provided in the diet. In another study designed to examine the kinetics of carnitine palmitoyltransferase-I (CPT-I) and the influence of dietary variables in young pigs (18 kg), Heo et al. (2000) fed diets supplemented with either 0 or 500 mg L-carnitine/kg. Their findings showed that CPT-I activities in the skeletal muscle mitochondria were not affected by diet. In contrast, the K_m for carnitine in liver was significantly increased by dietary L-carnitine supplementation and by high protein feeding. It was found that dietary L-carnitine increased muscle and liver free carnitine concentrations by 72% and 158% over control concentrations. Lin and Odle (2003) measured changes in carnitine and carnitine palmitoyltransferase concentrations activity in tissue homogenates of dogs in six developmental age categories: newborn; 24 h old; 3, 6 and 9 weeks old; and adult. Hepatic carnitine palmitoyltransferase activity was low at birth, increased by 100% during the suckling period and then declined after weaning to adult levels. In contrast, carnitine palmitoyltransferase activity in

the muscle continued to increase with age, reaching adult levels after 9 weeks. They therefore concluded that increased dietary carnitine may improve fatty acid oxidative capacity in developing dogs.

Further studies along this line will enable a clearer understanding of the metabolic activity during the developmental stages of fish since studies conducted on L-carnitine diet supplementation with small fish, tend to exhibit better results.

3.3. *Effect on old age*

A major cause of aging is the deterioration of the energy-producing components of the cell which results in reduced cellular metabolic activity, the accumulation of cellular debris and eventual death of the cell. L-Carnitine has been shown to have effective anti-aging therapeutic properties for maintaining youthful cellular energy metabolism, exhibiting several mechanisms for protecting cells from the effects of aging. The acetyl ester of the L-carnitine (acetyl-L-carnitine) is absorbed into the bloodstream more efficiently than L-carnitine, it passes more easily through cell membranes, and is utilized more efficiently in the mitochondria of the cell (Bremer, 1983). Hagen et al. (1998) monitored mitochondrial function and ambulatory activity after feeding rats acetyl-L-carnitine. Young (3–5 months) and old (22–28 months) rats were given a 1.5% (wt/vol) solution of acetyl-L-carnitine in their drinking water for 1 month. Acetyl-L-carnitine supplementation significantly reversed the age-associated decline of mitochondrial membrane potential. It was also found to increase cellular oxygen consumption, which declines with age, to the level of young rats. Thus, acetyl-L-carnitine supplementation in old rats markedly reversed the age-associated decline in many indices of mitochondrial function.

Similar effects were found by Rabie et al. (1997). They tested the effects of L-carnitine supplementation (50–500 mg/kg diet) of a practical layer diet, on the performance of laying hens and some indices of egg quality using 65-week-old hens kept in cages. Albumen quality was improved; while yolk index and yolk color score were not affected by dietary L-carnitine. Dietary L-carnitine also did not influence laying performance (egg production rate, mean egg weight, daily feed intake and daily egg mass and feed conversion) or external egg quality. L-Carnitine had a

beneficial effect on albumen quality and could modify the components of the edible part of the egg, during the late laying period.

It is not known if these beneficial effects of L-carnitine on old aged fish exist, but if a similar trend exists in fish, the addition of carnitine or acetyl-L-carnitine to the diet might help in maintaining fish brood-stocks viable and more productive.

4. Possible explanations for conflicting results of L-carnitine supplementation

4.1. Correlation between carnitine and lysine/methionine

The effects of dietary lysine levels on growth and carnitine concentrations in rainbow trout were examined by [Walton et al. \(1984\)](#). Trout at an average mean weight of 5 g were fed diets containing 10, 12, 14, 17, 21, 24 and 26 g lysine/kg diet for 12 weeks. By analysis of the growth values the dietary requirement of lysine was determined to be 19 g/kg diet. Their results also showed that the liver concentrations of total lipid and carnitine were not significantly different in fish from the different dietary treatments. The effects of L-carnitine addition at a level of 450 mg/kg to diets (of 50% crude protein) containing different levels of lysine (4.7 vs. 5.3 g/16g N) and sulfur amino acids (3.2 vs. 3.5 g Met+Cys/16 g N) on growth and feed efficiency of rainbow trout fingerlings (mean weight 20 g) were studied by [Schuhmacher and Gropp \(1998\)](#). Their results showed that in the fish that received diets marginally deficient in lysine and methionine, the addition of L-carnitine improved the specific growth rate by 4% and feed efficiency was significantly improved by 8%. Yet, the effect of L-carnitine supplementation on body weight, weight gain and feed consumption was not significant. These results are very similar to the findings of a study carried out with young pigs and quail chicks described by [Schuhmacher and Gropp \(1998\)](#). The findings suggest that a lysine sparing effect of carnitine is limited to cases in which the level of this limiting amino acid is only marginally deficient while in cases that lysine is much below the requirements of the fish, the addition of carnitine does not result in a positive outcome. This can also be seen in the results of a study carried out

with fingerlings of red sea bream in which the addition of 2000 mg/kg was tested at 2 levels of dietary lysine (10 and 14 g/kg). Carnitine increased growth in fish fed a diet containing lysine at a level of 14 g/kg but had no effect on fish fed a lysine deficient (10 g/kg) diet ([Chatzifotis et al., 1996](#)). [Burtle and Liu \(1994\)](#) studied the effects of L-carnitine supplementation (at a level of 1000 mg/kg) to the diet (containing 30% protein) of fingerling channel catfish at 3 levels of dietary lysine (1.1%, 1.4% and 1.7%). Their results also show no effect of the carnitine on growth, but carnitine did significantly reduce muscle and liver lipid levels. It is possible that the fluctuations in the results obtained with L-carnitine supplementation are merely a reflection of the content of the other ingredients in the diet and more specifically the level of the essential amino acids lysine and methionine from which the fish can synthesis its required L-carnitine needs.

Correlation between carnitine levels and methionine and lysine intake was studied in human subjects ([Krajcovicova-Kudlackova et al., 2000](#)). Plasma carnitine levels were measured in two alternative nutrition groups: strict vegetarians and lactoovo vegetarians (vegetarians consuming limited amounts of animal products such as milk products and eggs). The results were compared to an average sample of people who consumed a mixed nutrition diet (omnivores). Carnitine levels were correlated with the intake of essential amino acids, methionine and lysine (as substrates of its endogenous synthesis), since the highest carnitine content is in meat, lower in milk products, while fruit, cereals and vegetables contain low levels or no carnitine. Significant positive correlation of carnitine levels with methionine and lysine intake in alternative nutrition groups indicates that a significant portion of carnitine needs are covered by endogenous synthesis. Approximately two thirds of the carnitine needs in omnivores come from exogenous sources. These results demonstrate the importance of the essential amino acids methionine and lysine, with respect to the intake and biosynthesis of carnitine especially in diets low in carnitine.

4.2. Duration of the experiments

One of the possible explanations for the conflicting results could be the fact that many of the experiments in which the positive data were obtained were con-

ducted over long periods of time (over 120 days) and they were kept under natural photoperiod and temperature conditions resulting in large temperature fluctuations. Thus, in the study conducted with hybrid tilapia the temperature range was 22–28 °C (Becker et al., 1999). In a study with the European sea bass the temperature variation was 12–21 °C (Santulli and D'Amelio, 1986a), while in a study carried out with rohu fingerlings, the temperature fluctuation during the course of the experiment was from 23.5 to 34.0 °C (Keshavanath and Renuka, 1998). Jayaprakas et al. (1996) conducted an experiment with Mossambique tilapia held in outdoor conditions for a period of 252 days and found a positive effect on the fish growth as well as other factors when the diet was supplemented with 900 mg L-carnitine/kg. They do not provide detailed information about the temperature at which the fish were kept but since the experimental system and the area at which the experiment was carried out were similar to those in which Keshavanath and Renuka (1998) conducted their work, it is probably safe to assume that the temperature was in the range of 23–34 °C as well. These temperature fluctuations coupled with the long exposure of the fish to other stressors during the course of the experimental period might have given the L-carnitine treated fish an advantage which would not be the case had the fish been kept under more controlled conditions.

4.3. Level of supplementation and fish size

Interestingly, the level of supplementary L-carnitine which exhibited a positive growth enhancing effect in some of the fish was found to be limited to a certain concentration, out of the range tested, while the other concentrations tested did not show such positive results. In the case of the hybrid tilapia a low dose of 150 mg L-carnitine/kg supplementation yielded positive growth enhancement while a higher dose of 300 mg/kg oddly enough, did not result in better growth (Becker et al., 1999). In Mossambique tilapia (Jayaprakas et al., 1996) out of the following tested range: 150, 300, 500, 700 and 900 mg/kg supplementation, only the 900 mg/kg level exhibited significant growth enhancement. Chatzifotis et al.'s (1995) study with red sea bream fingerlings of 9.6 g showed that out of the range tested (75, 545, 1087, 2088 and 4162 mg carnitine/kg diet) only the 1087

and 2088 mg/kg levels of supplementation resulted in significantly better growth. In a study conducted with rohu fingerlings, at an initial average weight of 3.38 g, were fed a diet containing 30% protein and only 6% fat supplemented with 0, 250, 500, 750 and 1000 mg L-carnitine/kg diet for a total of 126 days (Keshavanath and Renuka, 1998). Surprisingly only the 500 mg/kg level resulted in a positive growth enhancement and a significantly higher final weight was obtained only in this group (that attained a final weight of 89 g) compared to a final weight of 51–56 g in all other diet supplementation groups. The positive effects of the diet supplemented with 500 mg L-carnitine/kg were visible after 56 days of growth. The study also compared flesh characteristics between the fish fed the control diet and those fed a diet supplemented with different levels of L-carnitine and found no significant differences between them.

The effects of carnitine in diets fed to hybrid striped bass were evaluated by Twibell and Brown (2000). Their basal diet contained 34.6% crude protein and 6.0% lipid. The four dietary treatments contained L-carnitine concentrations of 2.1, 41.0, 212.0 or 369.7 mg/kg diet. Dietary treatments were fed to apparent satiation twice daily to fish initially weighing 13.5 g. At the end of the 8-week feeding trial, feed intake and weight gain of fish fed 369.7 mg carnitine/kg diet were significantly higher than fish fed the basal diet containing 2.1 mg carnitine/kg diet. However, there were no significant differences in feed intake or weight gain among fish fed 2.1, 41.0 or 212.0 mg carnitine/kg diet. Feed efficiency, total liver lipid concentration, intraperitoneal fat ratio and proximate composition of muscle and carcass were not significantly affected by dietary carnitine concentration. Serum concentrations of total and free carnitine and carnitine esters were not significantly different among fish fed any of the diets, although values tended to increase with increasing dietary carnitine. The results of this study indicate that growth rate, but not body composition, of hybrid striped bass can be slightly improved with moderate concentrations of dietary carnitine. When a much higher dose of carnitine supplementation was used in the same species, although under a total different experimental set up, the results did not show growth improvement.

Gaylord and Gatlin (2000b) added a level of 3000 mg carnitine/kg diet of juvenile (2.5 g) hybrid striped

bass kept in 5‰ brackish water. The fish were fed at 4 dietary lipid levels (5%, 10%, 15% and 20%) in order to explore the effect on growth and body lipid level at higher lipid levels, after a previous study with low (5–10%) lipid level and a supplementation of 500 and 1000 mg/kg did not reveal any positive effect of carnitine supplementation on growth (Gaylord and Gatlin, 2000a). The results of that study only showed a positive effect of the higher lipid level (growth on a diet containing 10% lipid was significantly higher than the 5% lipid level). The results of the second study again showed no effect of the carnitine supplementation on growth. The level of lipid in the diet was again the main determinant of growth (the higher the better) and not the presence of L-carnitine supplementation in diet even at this very high level of 3000 mg/kg.

The only study that refers to changing/adjusting the carnitine levels in fish diets during the course of the growth period is that of Santulli and D'Amelio (1986a) in which the carnitine level given in the pellets was adjusted on the basis of ration consumed, so as to administer a daily dose of 250 mg carnitine/kg of wet fish weight. It is possible that changes in feed intake levels are responsible for some of the variations obtained in the different studies.

Many of the studies on L-carnitine supplementation that exhibited positive growth enhancement were carried out with fingerlings or small juvenile fish. Initial size of the tested fish plays an important role in the outcome of the growth since growth decrease (as percent body mass) when fish increase in size. A deficiency in carnitine might be easier expressed in fish of small size that also require more food per unit of body weight. This can explain the different results obtained by Torreele et al. (1993) showing positive growth effects in juvenile African catfish of 5 g initial weight, while such an effect was not detected by Ozorio (2001) examining the same fish species with an initial weight of 23 g.

One of the first reports on the positive effects of L-carnitine supplementation on the growth of fish was that of Santulli and D'Amelio (1986a) who studied the effects on the growth of the European sea bass. Many fish species show a high growth variation even when hatchery-reared. A close look at the growth data presented by Santulli and D'Amelio (1986a) shows that the experimental groups varied in their initial

weight: the L-carnitine treated group had an initial average weight of 35.5 ± 6.7 g while the D-carnitine treated group started at 29.4 ± 6.7 g and the control group at 32.0 ± 9.0 g. Although the results show positive slope values of weight increment (0.1, 0.06 and 0.08 for the L-carnitine, D-carnitine and control, respectively) this might be a reflection of the better "head start" of the L-carnitine treated fish.

4.4. Activities related to carnitine: acetyl-L-carnitine and carnitine palmitoyl transferase

Acetyl-L-carnitine is reported to enhance motor activity in animal models and to modulate membrane phospholipid metabolism and high-energy phosphate metabolism back to normal. Fish present unique animal models for the in vivo study of high-energy phosphate and membrane phospholipid metabolism by noninvasive in vivo labeled ^{31}P nuclear magnetic resonance spectra (NMR). A study conducted with free-swimming zebra fish using ^{31}P NMR spectroscopy showed that acetyl-L-carnitine caused a decrease in levels of phosphodiester and inorganic orthophosphate in the fish. The findings demonstrate that acetyl-L-carnitine modulates membrane phospholipid and high-energy phosphate metabolism in free-swimming zebra fish (Levine et al., 2003).

The enzyme carnitine palmitoyltransferase (CPT) mediates the transport of fatty acids across the outer mitochondrial membrane. This enzyme is able to use a range of fatty acids as substrates, with some variation in catalytic rate among tissues. In mammals, there are two different proteins CPT I in the skeletal muscle and in the liver, encoded by two genes. Carnitine is an essential component in the process of mitochondrial fatty acid oxidation and, with the cooperation of two carnitine palmitoyltransferases (CPT-I and CPT-II) and a carnitine acylcarnitine transporter across the inner mitochondrial membrane, it acts as a carrier for acyl groups into the mitochondrial matrix where beta-oxidation occurs. However, limited information is available on required carnitine levels and for differentiating between CPT-I and CPT-II activities in fish.

In a study carried out by Froyland et al. (1998) the potential for fatty acid catabolism, and the activities of the key enzymes acyl-CoA oxidase (ACO) and CPT-I and CPT-II involved in fatty acid oxidation, were

determined in different tissues of farmed Atlantic salmon. They demonstrated that malonyl-CoA was a potent inhibitor of CPT-I activity not only in red muscle but also in liver, white muscle and heart. By expressing the enzyme activities per wet tissue, the CPT-I activity of white muscle equaled that of the red muscle, both being much higher than in the liver. CPT-II dominated in red muscle whereas the liver and white muscle activities were comparable. ACO activity was high in the liver regardless of how the data were calculated. Based on their results and the CPT-II activity and total palmitoyl-L-carnitine oxidation in white muscle, Froyland et al. (1998) concluded that the white muscle might have a profound role in the overall fatty acid oxidation capacity in fish. Gutiérrez et al. (2003) examined CPT I cDNA and CPT activity in different tissues of rainbow trout. Their results demonstrate the existence of at least one gene encoding for CPT I present in both the liver and the muscle of rainbow trout.

Egginton (1996) studied the enzymatic activity of (CPT) which is capable of using a range of fatty acids as substrates, with some variation, in catalytic rate among tissues. The substrate giving maximal activity of the enzyme under optimal conditions (C16:1), was found to be similar in trout, salmon, goldfish, eel and tilapia. In addition this study found no evidence that thermal acclimation affected substrate preference for β -oxidation. Carnitine stimulation of β -oxidation of palmitate and oleate has been demonstrated in mitochondrial suspensions from several tissues of rainbow trout (Bilinski and Jonas, 1970).

Better understanding of the interaction between CPT-I and CPT-II as well as acetyl-L-carnitine activities and carnitine, is perhaps the key for elucidating the overall results obtained in L-carnitine research in fish.

4.5. Leaching of carnitine—the stability of the pellet

In many cases L-carnitine was added to the fish diets after the pellets were prepared or to pellets that were not extruded or coated. It is possible that due to the fact that L-carnitine is easily soluble in water and has a relatively low molecular weight a large proportion of the supplemented carnitine leached into the water and only a small portion actually reached the fish. This might explain the very high levels of car-

nitine supplementation required in fish compared with terrestrial animals.

4.6. Possible role as a chemoattractant

The structure of carnitine (3-hydroxy-4-*N*-trimethyl-ammoniumbutanoate, see Fig. 1) is similar to quaternary ammonium substances such as betaine and tri-methyl amine (TMA) known to act as powerful attractants for fish and crustaceans (Harpaz, 1997). An excess of carnitine in human diet might lead to the fishy odor syndrome associated with TMA or TMA oxide. Fish odor syndrome (trimethylaminuria) is a metabolic syndrome caused by abnormal excretion of trimethylamine in the breath, urine, sweat, saliva. Trimethylamine is derived from the intestinal bacterial degradation of foods rich in carnitine and choline (see Fig. 1). It is normally oxidized by the liver to odorless trimethylamine *N*-oxide which is then excreted in the urine (Holmes et al., 1997). Impaired oxidation of trimethylamine is thought to be the cause of the fish odor syndrome and is responsible for the smell of rotting fish. Certain foods rich in carnitine or choline exacerbate the condition while dietary adjustments reduce the excretion of trimethylamine (Rehman, 1999).

The mechanism of biosynthesis of trimethylamine oxide (TMAO) from dietary precursors in Nile tilapia was investigated by Niizeki et al. (2002). They utilized diets supplemented with quaternary ammonium choline, carnitine, glycine, betaine or phosphatidylcholine. Significant increases in TMAO levels in the muscle were only observed with choline. Tilapia possess the ability to produce TMAO from choline, which is related to intestinal microorganisms and tissue mono-oxygenase under freshwater conditions. It has been shown that the presence of a powerful attractant such as TMA or TMAO in the environment leads to intense food search (Harpaz et al., 1987). This in turn leads to higher food consumption in crustaceans resulting in significantly better growth (Harpaz, 1997). It is possible that the extremely high dietary supplementation of L-carnitine in the experimental diets used, led to the excretion of high levels of TMA and TMAO into the environment and these in turn acted as chemostimulants increasing the consumption and utilization of the food by the fish. This effect would be especially pronounced in the

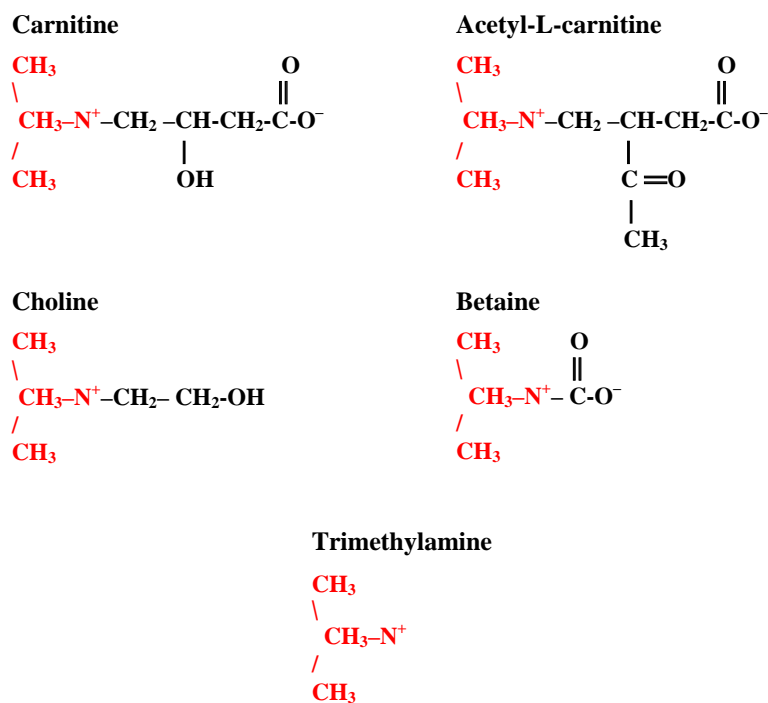


Fig. 1. Structure of L-carnitine, its derivatives and other quaternary ammonium substances.

diets with low levels of fish meal or lacking sufficient levels of natural attractants.

5. Conclusions

There are some cases in which the use of supplemental L-carnitine in fish diets led directly to better growth of fish especially when small fish were tested. However, great variations were found in the carnitine levels shown to be effective in improving the fish performance. These differences are evident not only among the various species studied but also for the same species when studied by different research groups. In addition, the existence of a carnitine effect is often inconsistent, adding to the confusion.

The cases in which L-carnitine supplementation has shown positive results have been either in very young fish and/or long term experiments lasting for over 4 months. Presumably during the long time span of the experiment the fish were exposed to a combination of stressors giving the L-carnitine treated fish a slight edge over the nontreated fish. There

is evidence showing that the addition of L-carnitine to the diet of fish can protect the fish in the event they are exposed to acute cold stress or when exposed to high and stressful levels of ammonia. L-Carnitine might be used in advance as a preventative measure to reduce stress resulting from exposure to harsh conditions or stressful activity such as size grading or selective harvest.

Lack of sufficient levels of the essential amino acids lysine or methionine in the diets of the experimental fish might have also contributed to the positive growth results obtained when L-carnitine supplementation was carried out. Examination of the levels of methionine and lysine in the diet, as a possible source for carnitine, can provide information about the necessity to supplement the diet with carnitine. The current trend is to replace animal protein sources with proteins derived from plant material. This practice should take into account the significantly lower levels of L-carnitine existing in plants and see to it that the carnitine needs are covered by either supplying carnitine or alternatively providing diets rich in the essential amino acids lysine and methio-

nine, thereby enabling the fish to synthesize the required level of L-carnitine.

It should be noted that increased carnitine concentrations may be required for oxidation of lipids when there is a clear need for energy as a result of starvation (Miyasaki et al., 1995; Chatzifotis and Takeuchi, 1997); cold acclimation (Harpaz et al., 1999) and similar acute environmental conditions. Carnitine can then increase lipid catabolism and might also lead to a protein sparing effect.

Evidence of the positive effects of L-carnitine on reproduction exists in a number of vertebrates including fish and it could be used specifically for brood stock fish both to enhance fertility and to revitalize and slow down the ageing of the fish as has been shown in laying hens.

Cost effectiveness is an important issue to be considered, and even in the cases where L-carnitine has shown positive effects, the levels of this rather expensive substance required, might not be economically justifiable.

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