

**THE EFFECT OF DIETARY L-CARNITINE
SUPPLEMENTATION ON PRODUCTION
PERFORMANCE PARAMETERS OF MOZAMBIQUE
TILAPIA, *Oreochromis mossambicus*, AT SUB-OPTIMAL
WATER TEMPERATURE**

By
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The crest of the University of Stellenbosch, featuring a shield with various symbols, a crown on top, and a banner at the bottom with the motto "Veritas liberabit vos".

Assignment presented in partial fulfilment of the requirements for the
degree of Master of Philosophy in Livestock Industry Management:
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Declaration:

I, the undersigned, hereby declare that the work contained in this assignment is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

Date:

Abstract

A 60-day growth experiment was conducted to investigate the effect of dietary L-carnitine supplementation on the production performance parameters of Mozambique tilapia, *Oreochromis mosambicus*. A number of approximately 140 tilapia fry with average weight of $1.4\text{g} \pm 0.71\text{g}$ were stocked in each of 40 fine-meshed hapas (1m x 1m x 1.5m) submerged within a complete recirculation pond system. During the first 30 days of the experiment water temperatures ranged from 19 to 23°C where after it decreased to 16-20°C for the consecutive 30-day period. Dietary treatments consisted of 8 replicates of 5 levels of L-carnitine supplementation labelled as C₀, C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀ represented 0mg, 250mg, 500mg, 750mg and 1000mg L-carnitine supplementation per kg feed respectively. Results were analyzed for significant differences using one-way analysis of variance (ANOVA) and Tukey's pairwise comparison test for growth rate, feed intake (FI) and feed conversion efficiency. After completion of the trial 8 fish from each hapa were sacrificed and analyzed for cephalosomatic index (CSI), dress out percentage (viscera, gills and head excluded), viscerosomatic index (VSI) and hepatosomatic index (HSI).

Poor production performance results were generally observed as water temperatures were sub-optimal, especially during the second 30-days period. Results from the trial indicate no significant differences ($P > 0.05$) between treatments for weight gain, FCR, FI and VSI. A negative trend was observed for FCR with increasing level of L-carnitine supplementation for both the first 30-day period (1.50 ± 0.07 , 1.53 ± 0.08 , 1.58 ± 0.09 and 1.61 ± 0.17 for C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀) as well as for the consecutive lower temperature 30-day period (2.22 ± 0.10 , 2.25 ± 0.11 , 2.27 ± 0.28 and 2.29 ± 0.21 for C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀). Although statistically not significant, fish fed the C₂₅₀ showed better performance in dress out percentage weight either than the control or the higher levels. The increasing trend for head weight with increasing level of L-carnitine supplementation were significant ($P < 0.05$) from C₀ and C₂₅₀ with and above C₅₀₀. The decreasing trend for liver weight with increasing level of L-carnitine supplementation became significant ($P < 0.05$) with and above C₇₅₀. The results of the current study showed a trend in the improvement of L-carnitine on the production performance parameters. However, the natural content of L-carnitine in the basal diet impaired with the inclusion levels, thus further research at lower inclusion levels is recommended.

Samevatting

'n Proef oor 'n tydperk van 60-dae is onderneem om die effek van L-karniten aanvulling op produksie prestasie parameters van Mosambiek tilapia (*O. mosambicus*) te ondersoek. 140 tilapia vingerlinge met 'n gemiddelde massa van $1.4g \pm 0.71g$ is ewekansig uitgeplaas in 40 eksperimentele hapa-hokkies (1mx1mx1.5m) in 'n hersirkulasie sementdam-stelsel. Gedurende die eerste 30 dae van die proef het water temperatuur gewissel tussen 19 to 23°C waarna dit gedaal het na tussen 16-20°C vir die opeenvolgende 30-dag periode. Proef-rantsoen behandelings het bestaan uit 8 herhalings van 5 vlakke van L-karnitien aanvulling, naamlik C₀, C₂₅₀, C₅₀₀, C₇₅₀ en C₁₀₀₀ vir 0mg, 250mg, 500mg, 750mg en 1000mg L-karnitien aanvulling per kg voer afsonderlik. Resultate was ontleed vir betekenisvolle verskille deur gebruik te maak van analise van variansie (ANOVA) ontleding en die Tukey se vergelykende toets vir groeitempo, voerinnname en voeromsettingsverhouding. Aan die einde van die proefperiode is 8 visse van elke hapa ontleed vir liggaamskomponent-samestelling (kop-, ingewande- en heptosomatiese indekse).

Ondergemiddelde produksie resultate is waargeneem wat toegeskryf kan word aan onder-optimale water temperature, veral gedurende die tweede 30-dag periode van die proef. Proef resultate het geen betekenisvolle verskille ($P > 0.05$) in massatoename, voeromsettingsverhouding (VOV) of visserosomatiese indeks tussen behandelings getoon nie. 'n Negatiewe neiging is waargeneem vir VOV met toenemende vlakke van L-karnitien insluiting vir beide die eerste 30 dag periode (1.50 ± 0.07 , 1.53 ± 0.08 , 1.58 ± 0.09 and 1.61 ± 0.17 for C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀) sowel as vir die opvolgende 30-day periode nie (2.22 ± 0.10 , 2.25 ± 0.11 , 2.27 ± 0.28 and 2.29 ± 0.21 for C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀). 'n Toenemende neiging vir kop-massa met toenemende L-karnitien insluiting was betekenisvol ($P < 0.05$) vanaf C₀ en C₂₅₀ met en hoër as C₅₀₀. 'n Dalende neiging vir lewermassa met toenemende L-kinsluiting was betekenisvol ($P < 0.05$) met en hoër as C₇₅₀. Resultate van die proef dui oor die algemeen op 'n neiging tot verbeterde produksie prestasie parameters van tilapia vingerlinge met toenemende insluiting van L-karnitien. Verdere navorsing word aanbeveel om die invloed van natuurlike L-karnitien in die proteïen-bronne van die basaalrantsoen te op die gebrek aan betekenisvolheid van hierdie neiging te verklaar.

Dedicated to my family, in particular to my father and my elder sister

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1 INTRODUCTION

1.1 Scope of the study and economic importance of optimal aquafeed

Nowadays, tilapia is becoming a choice of species in the freshwater aquaculture industry for its hardiness to wider environmental and physiological variations. It is raised in a variety of production systems from extensive and semi-intensive to highly intensive recirculation systems. However, in the temperate regions extended cold winter temperatures affect the growth performance of fish consequently resulting in a reduced growth rate and poor feed conversion ratio (FCR). This makes the venture uneconomical as the production cost is increased due to the increase in feed cost which comprises for more than 50% of the total production cost (Cunningham *et al.*, 1985). Researches on aquaculture nutrition are therefore constantly investigating ways to optimize economic feed utilization through enhanced growth performance of fish and better feed conversion efficiency.

One way of enhancing growth performance and improving the feed conversion efficiency is supplementation of the basal feed of fish with dietary L-carnitine. Santulli and D'Amelio (1986) indicated the positive role of L-carnitine in the growth and lipid metabolism of hatchery reared sea bass, *Dicentrarchus labrax* L., and suggested the possible utilization of the product for the improvement of culture techniques in the aquaculture industry.

Because animal protein is the most expensive component of fish diets, a number of studies have been carried out to investigate ways of replacing fish meal with plant proteins and to spare dietary protein by increasing dietary non-protein energy (NPE) levels. However, as a result of increasing the NPE supplementation, farmed fish accumulate more lipids than wild ones which is associated with a fall in food conversion efficiency (Bradley and Smart, 1981). Moreover, its effect is believed to improve product quality by limiting undesirable lipid accumulation in the tissue of farmed fish (Santulli and D'Amelio, 1986). Therefore, the supplementation of L-carnitine to the diets of fish feed will be indispensable in order to enhance the oxidation of the accumulated lipid. In agreement to this, Ozario *et al.* (2001) ascertained that the supplementation of L-carnitine to fish diets increases the protein to fat ratios of the carcass of fish under intensive aquaculture systems. Its use in

aquafeed is therefore believed to enable the utilization of high-energy diets with less accumulation of intramuscular lipid and fat depots thereby improving flesh quality, minimizing production costs and reducing nitrogen losses from the fish farm in effluents.

1.2 Objective

The aim of the current study is to evaluate the effect of dietary L-carnitine supplementation on some production performance parameters of Mozambique tilapia, *Oreochromis mossambicus*, at suboptimal temperatures. The growth rate, weight gain, dress out percentage, hepatosomatic index (HIS) and viscerosomatic index (VSI) are incorporated as a measure of production performance in relation to five levels of inclusion of L-carnitine to the basal diet of tilapia.

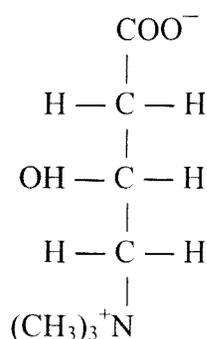
1.3 Chemical explanation of L-carnitine

As described by Williams (2002), carnitine is a water soluble, vitamin-like naturally occurring compound synthesized from the two essential aminoacids lysine and methionine and is also known as vitamin BT to indicate its place in the B group vitamins. Moreover, he indicated two isomeric forms of carnitine, L-carnitine and D-carnitine the L- form being physiologically active form in the body of animals. Its chemical name is represented as β -OH-(γ -N-trimethyl-amino)-butyrate (Ozario *et al.*, 2001).

In the body of animals L-carnitine is synthesized *de novo* in the brain liver and kidney from the two essential precursors, methionine and lysine (Dunn, 1981). It was first discovered in 1905 in Russia by the two scientists Gulewitch and Krimberg when they isolate it from red meat extract (Williams, 2002).

The chemical structure of L-carnitine is as follows (Montgomery *et al.* 1980)

L-carnitine



D-carnitine

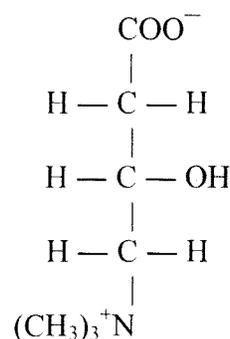


Figure 1.1 Chemical structure of carnitine showing the two isomeric forms, L-carnitine and D-carnitine

1.4 History and background on the use of L-carnitine

The following background information on the use of L-carnitine was obtained from Lonza Group¹ (<http://www.carnipure.com/carnipure/en/what/historical.html>) released on the internet.

A century has elapsed since the discovery of L-carnitine in 1905 when Gulewitch and Krimberg isolated it from muscle extract and it was so named from the Latin word *caro, carnis* meaning flesh or meat. After the disclosure of its chemical structure in 1927, Strack published his first article on L-carnitine in 1935 and this initiated numerous studies on its physiological importance to animals. In 1952, Fraenkel after conducting a research on the importance of L-carnitine as a growth factor in mealworms, *Tenebrio molitor*, isolated L-carnitine from liver extract and gave it the name “vitamin BT”. In 1958, Fritz for the first time discovered the fundamental role of L-carnitine in fat oxidation which is an important process of energy production in the mitochondria of the cells.

In the 1970's, intensive researches on the function of L-carnitine have been conducted thereafter it became commercially available in the 1980's. In 1983, Lonza the world's largest supplier of L-carnitine (CARNIPURE[®]) patented a unique method for commercial production of the product. In 1993, L-carnitine was evaluated by an

¹ Lonza Group is a company based in Switzerland which is involved in research and production of chemicals for pharmaceuticals and biotechnological purposes.

independent group of scientists for its GRAS (Generally Recognized As Safe) status for a dietary supplement. In 2000, the U.S. Food and Drug Administration (FDA) and the national authorities for production of pharmaceuticals inspected and approved Lonza's plants.

1.5 Sources of L-carnitine

The requirement for certain components involved in lipid metabolism increases with the increase in the lipid content of the diet (Chatzifotis and Takeuchi, 1997). To fulfil the enhanced requirement for these components fish and other animals including humans either have to synthesize them from within their body (from indigenous sources) or obtain them from their diet. L-carnitine is one of the most important components involved in the process of lipid catabolism (Ozario *et al.*, 2001).

Although L-carnitine is not an essential dietary nutrient it is synthesized from other nutrients and is found in substantial quantities in animal feed. Meat products, especially beef and pork, are the primary sources with minimal amounts being obtained from fruits, vegetables and grains (Williams, 2002). Milk is also a good source of carnitine. According to Arenas *et al.* (1998), about 75% of the carnitine in human comes from the diet where red meat in adults and breast milk in infants are the main sources.

An increase in the triglyceride concentrations in the liver initiates increased synthesis of carnitine in the body of animals (Chien *et al.*, 2000). However, in normal physiological and nutritional conditions where the natural sources are found to be insufficient due to the inability to produce the required amount of L-carnitine in the body, dietary sources of carnitine are inevitable for the facilitated transport of fatty acids into the mitochondria of the cells (Krajcovicova *et al.*, 2000).

In the feed of livestock, the L-carnitine content of feedstuffs varies based on whether the ingredients are of plant origin or animal based products. According to Baumgartner (2003), the L-carnitine content for selected feedstuffs is presented in Table 1.

Table 1.1 L-carnitine concentrations of selected feedstuffs (Eder *et al.*, 2002; Baumgartner, 2003)

Origin of Feedstuffs		L-carnitine content (mg/kg)
Plant based Products	Corn, rice, sorghum, wheat	5
	Barley, oats	5-10
	Rice bran	5-15
	Wheat bran	10-15
	Soybean meal	10-20
	Cotton seed	5
	Rape seed	5-10
	Sunflower seed, groundnut	10
	Linseed	10-15
	Vegetable fats	0
Animal based Products	Fish meal	60-120
	Fish and bone meal	50-80
	Meat and bone meal (40%)	50-80
	Feather meal	10
	Blood meal	5-10
	Plasma protein	15
	Animal fat	0
	Milk	20
	Skim milk	10-30
	Skim milk powder	100-300
Whey powder	300-500	

2 THE METABOLIC FUNCTION OF L-CARNITINE AND ITS USE IN ANIMAL NUTRITION

2.1 Metabolic function of L-carnitine

The primary metabolic function of L-carnitine is to facilitate the transport of long-chain fatty acids into the energy sites of the cells (mitochondria) so as to enhance the energy production by the cells. Various enzymatic actions are involved during the energy production process which eventually is carried out in the inner mitochondria by the process of β -oxidation. The final stage of the process is the contribution of the acetyl Coenzyme A (CoA) which is involved in the Krebs's cycle for energy production (William, 2002).

The long chain fatty acid oxidizing effect of L-carnitine is shown by Chatzifotis *et al.* (1995) when they experimented on the lipid composition of L-carnitine supplemented Red Sea bream, *Pagrus major* fingerlings where they recorded reduced amount of free fatty acids in the liver which is an indication of increased fatty acid utilization.

2.1.1 Fatty acid metabolism and β -oxidation

Energy production in the body of animals involves various enzymatic actions where triglycerides, the major sources of energy, in the adipose tissues are catabolized by hormone-sensitive lipase to produce free fatty acids (FFA) and glycerol. The FFA are then released to the blood plasma and bound with albumin becoming ready to be transported into the muscle cells while the glycerol component is transported to the liver for further metabolism. The first step of fatty acid oxidation takes place at the muscle cells, where the FFA are activated to fatty acyl CoA by the action of the enzyme fatty acyl CoA synthetase (William, 2002). This enzymatic action during the fatty acyl CoA synthesis is carried out in the outer mitochondrial membrane which necessitates for the acyl CoA to be transported into the inner site of the mitochondria to produce the required energy. However, fatty acyl CoA cannot penetrate through the inner membrane of the mitochondria into the site of β -oxidation, which is the process by which long chain fatty acids are catabolized to release the required energy by the body cells (Montgomery, *et al.*, 1980). After synthesized in the endoplasmic reticulum or in the outer mitochondrial membrane, acyl CoA group is transported into the site of β -oxidation in the mitochondria by the help of L-carnitine. The enzyme

carnitine palmitoyl transferase (CPT-1) synthesizes an acyl-carnitine complex which is further activated by the enzyme acylcarnitine transferase in order to penetrate across the inner mitochondrial membrane. Another enzymatic action by CPT-2 is carried out opposite to CPT-1 exchanging carnitine to CoA. Eventually, the acyl CoA is formed and enters into the site of β -oxidation reaction resulting in the synthesis of the Acetyl-CoA needed for the citric acid cycle (Morin, *et al.*, 2001).

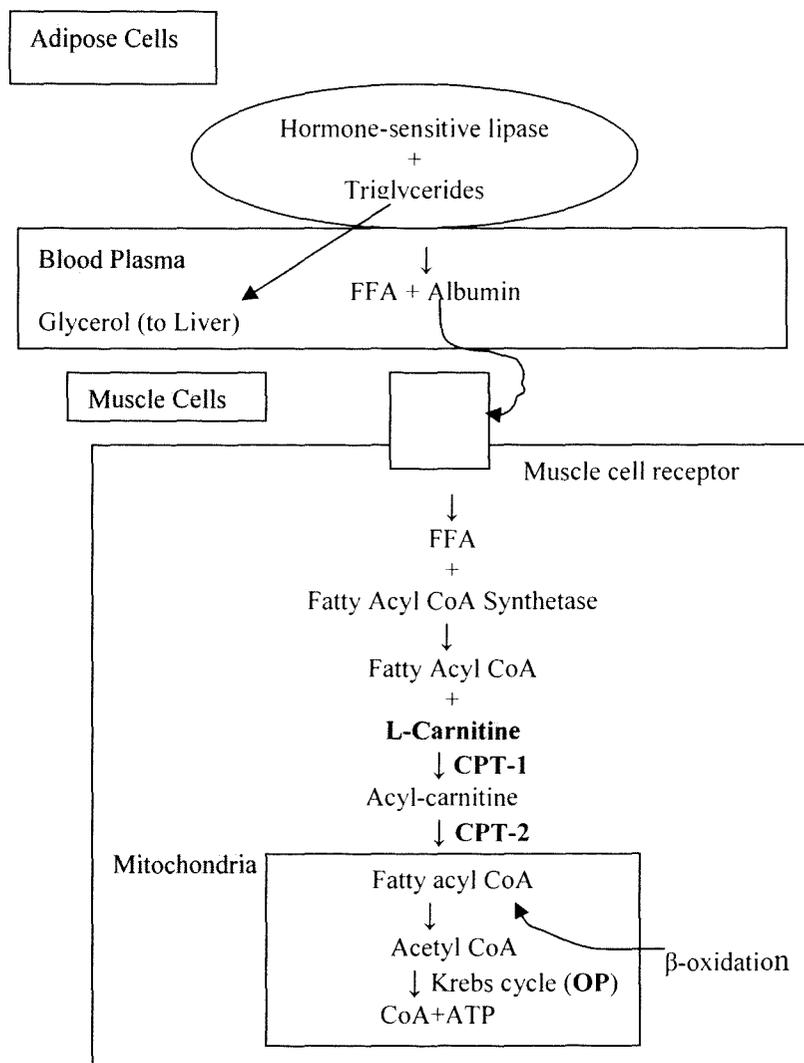


Figure 2.1 The process of free fatty acid metabolism and β -oxidation (Williams, 2002; Eder *et al.*, 2002): CPT-1=carnitine palmitoyl transferase type 1, CPT-2=carnitine palmitoyl transferase type 2, OP=oxidative phosphorylation.

In the process of free fatty acid metabolism, FFA may be released by the adipose tissue triglycerides or by the muscle cell triglycerides. However, during the process of fat breakdown, the Acyl CoA groups cannot penetrate through the inner mitochondrial

membrane and hence the importance of L-carnitine to carry these groups into the site of β -oxidation by creating a carnitine-enzyme complex.

2.1.2 Physiological role of L-carnitine

In addition to the facilitating role in the transport of long chain fatty acids into the mitochondria, L-carnitine is essential in the removal of excess mitochondrial acetyl-CoA that limit efficient fatty acid utilization by the cells which is also a mechanism of regulating the acetyl-CoA:CoA ratio that influences the enzymatic activity of the mitochondrial pyruvate dehydrogenase (PDH) (Rebounche and Seim 1998). It also prevents glycolysis which inhibits absorption of free fatty acids (FFA) by the muscle cells (Dambrova *et al.*, 2002).

Another metabolic function of L-carnitine as indicated by Neuman *et al.* (2002) while experimenting on Leghorn Roosters is that the antioxidant properties which may protect sperm membranes from toxic oxygen metabolites thereby extending the longevity of the sperm cells.

The physiological role of L-carnitine in humans and other animals can be summarized on the basis of reports by Arduini *et al.* (1990), Chatzifotis *et al.* (1995), Harpaz *et al.* (1999), Neuman *et al.* (2002), Dambrova *et al.* (2002), and Eder *et al.* (2002) as:

- Oxidation of long chain fatty acids
- Activation of aerobic glycolysis and stimulation of pyruvate dehydrogenase complex
- Regulation of the mitochondrial acyl CoA/CoA group ratios
- Decrease levels of toxic FFA metabolites by the removal of excessive acyl groups
- Improving myocardium functioning, and maintaining healthy heartbeat
- Maintains healthy cholesterol and triglyceride levels
- Membrane stabilization of the red blood cells
- Antioxidant property for sperm cells and enhancement of sperm production
- Improving feed conversion efficiency and growth rate in fish and other livestock
- In fishes detoxification of total ammonia nitrogen and tolerance to environmental stresses such as exposure to extreme cold shock

- Improving the reproductive performance of livestock by enhancing the number of off springs, number of early spawners and weight gain of the off springs at birth

To summarize the metabolic role of L-carnitine, its deficiency leads to an impaired energy metabolism, membrane malfunctioning and poor animal performance in terms of growth, reproduction, tolerance to environmental and physiological stresses, etc.

2.2 The use of L-carnitine in animal nutrition

Since its discovery, L-carnitine has been studied extensively for the better understanding of its physiological importance in the body of animals including human. As Swart *et al.* (1997) observed L-carnitine supplementation to male marathon athletes proved positive influence of aerobic capacity: increased peak treadmill running speed by 5.68% and decreased oxygen consumption and heart rate was observed.

Nowadays, animal nutrition researches are giving more attention to the application of L-carnitine as dietary supplement so as to enhance optimal utilization of feed in the livestock industry. Researches are continuous regarding the effect of L-carnitine supplementation to the feed of poultry, swine, fish, pets and ranch animals. It is also administered to human as it is becoming very essential for various physiological control, weight management and wellness of organ-functioning such as the heart.

2.2.1 Human

L-carnitine plays an important role in many therapeutic processes of the heart, liver and other organs. Benvenga *et al.* (2001) indicated the role of L-carnitine in preventing or reducing the symptoms associated with hyperthyroidism. The authors also indicated that primary and secondary deficiencies of L-carnitine result in coronary heart disease and heart failure and recommended oral administration of 1-4g of L-carnitine per day to prevent such deficiencies. Arduini *et al.* (1990) showed the positive role of L-carnitine in increasing membrane stability of human erythrocytes when they are subject to high shear stress.

Clinical data indicate that L-Carnitine supplementation can positively support healthy heart functioning by favouring healthy heart muscle, increasing heart muscle viability and by supporting a healthy heartbeat. Other clinical research data indicate that L-Carnitine supplementation is helpful in maintaining healthy cholesterol and triglyceride levels. The heart obtains 70% of its energy from fat breakdown. The fundamental role that L-carnitine plays in energy metabolism together with the dependence of the heart on fatty acid breakdown for energy production makes L-carnitine a crucial energy provider for the heart. In fact, a number of scientific evidences have shown that L-Carnitine supplementation has beneficial effects in maintaining cardiovascular wellness and proper functioning of the heart (Dambrova *et al.*, 2002).

L-carnitine plays an important role in the developing foetus for the processes underlying foetal maturation. It also performs a crucial role in the energy supply of the tissues during foetal life and in the neonatal period by regulating the influx of fatty acids into mitochondria (Arenas *et al.*, 1998).

2.2.2 Swine

In their study on the reproductive performance for sows, Eder *et al.* (2002) proved that sows treated with L-carnitine supplemented feed were observed significantly undergoing lowered still-born piglets, increased weight of piglets, increased litter mass and more weight gain of piglets during the suckling period. On an optimal dosage between 49 and 64mg L-carnitine per kilogram of feed supplemented to nursery and growing-finishing pigs, significant increase in percentage lean and percentage of carcass (quadratic, $P < 0.05$) is reported by Owen *et al.* (2001).

2.2.3 Poultry

A number of studies on the effect of L-carnitine supplementation to the diets of poultry have been conducted by various authors. Growth performance, egg hatchability and fertility, sperm yield of roosters have been investigated by a number of authors. A study on young and aged white Leghorn roosters by Neuman *et al.* (2002) revealed that a greater sperm concentration when administered a diet supplemented with L-carnitine at an inclusion level of 500mg/kg. Furthermore, the authors concluded that L-carnitine may improve the longevity of sperm cells as sperm

cells from L-carnitine treated roosters were less susceptible to oxidative stress. The positive effect of L-carnitine on the egg fertility and hatchability of broiler breeders was also investigated by Sachy *et al.* (2002). However, no significant differences in the live weight of the treatment groups of both sexes were observed.

2.2.4 Effects of supplementary L-carnitine to aquafeed

2.2.4.1 Effects on animal growth performance, feed intake and FCR

Several studies have been conducted on the effect of dietary L-carnitine supplementation for dozens of fish species and crustaceans concerning their growth, feed conversion efficiency, reproductive performance, body composition for proteins and lipids, tolerance to environmental stress such as cold shock and certain body metabolic and physiological activities like oxygen consumption, feed consumption, nitrogen excretion and reduction of ammonia toxicity. Blinski and Jonas (1970) administered L-carnitine to the diet of trout and isolation of mitochondria from the red muscle proved that the transport and oxidation of long chain fatty acids was enhanced. According to Becker *et al.* (1999), dietary supplementation of L-carnitine improved the growth rate and feed conversion efficiency of *Oreochromis niloticus* × *O. aureus* hybrids. Growth experiment by Becker and Focken (1995) on carp *Cyprinus carpio* also showed better growth rate and lowered feed consumption by 10% when fed L-carnitine supplemented diet. Further studies on the effect of L-carnitine supplementation to the diets of the African catfish *Clarias gariepinus* (Torreele *et al.*, 1993), rohu *Labeo rohita* (Keshavanath and Renuka, 1988), Red Sea bream *Pagrus major* (Chatizifotis *et al.*, 1995), hybrid striped bass *Morone saxatilis* (Twibell and Brown, 2000) and white prawn *Penaeus indicus* showed significant changes in growth and feed conversion efficiency at levels inclusion levels of 500mg/kg, 500mg/kg, 2088mg/kg, 369.7mg/kg, and 500mg/kg feed respectively.

On the contrary, several studies show that L-carnitine supplementation to diets at the respective inclusion levels has no effect on the growth performance of the experimental fish. Gaylord and Gatlin (2000) on hybrid striped bass *Morone chrysops* × *M. saxatilis*, Bradley and Tremblay (1996) on Atlantic salmon *Salmo salar* and Harpaz *et al.* (1999) on the ornamental cichlid *Pelvicachromis pulcher* proved that no significant difference between the L-carnitine supplemented and the basal diets.

2.2.4.2 Effects on body composition

Though researches by different authors showed results that seem contradictory, L-carnitine when supplemented to the diets of fish generally is expected to enhance the protein sparing efficiency and also lowers the body lipid content. Santulli *et al.* (1988), Torreele *et al.* (1993), Burtle and Liu (1994) and Jayaprakas *et al.* (1996) indicated that L-carnitine supplementation to different species of fish lowered the body lipid content. A study on the Atlantic salmon *Salmo salar* also showed reduced body fat content and increased body protein when treated with L-carnitine supplemented diets (Ji *et al.*, 1996). According to Torreele *et al.* (1993), protein efficiency ratio, protein retention and energy retention improved significantly ($P < 0.01$) in fingerlings of the African catfish, *Clarias gariepinus*, when fed L-carnitine supplemented diets. Although the studies available on the effects of L-carnitine on crustaceans is limited, Jayaprakas and Sambhu (1996) investigated increased total dry matter and protein content of the body in the white prawn, *Penaeus indicus*, fed diets with L-carnitine. The lipid content also decreased in all the carnitine fed groups indicating enhanced lipid catabolism. Several authors show that no significant effect on the body composition of fish when L-carnitine is supplemented to the diets at various levels. As Twibell and Brown (2000) suggested, growth rate but not body composition of the hybrid striped bass *Morone chrysops* × *M. saxatilis* can be improved with relatively low concentration of dietary carnitine. Gaylord and Gatlin (2000) experimented on the same species and found that dietary lipid but not L-carnitine affects the growth performance, liver lipid level and body condition. Moreover, Becker and Focken (1995) reported no significant changes on the whole body composition for proteins and lipids of tilapia *Oreochromis niloticus* × *O. aureus* hybrid when supplemented dietary L-carnitine.

2.2.4.3 Tolerance to physiological and environmental stress

Several studies have suggested that L-carnitine is supposed to react against various physiological, metabolic and environmental stresses such as reducing oxygen consumption, promote ammonia detoxification and nitrogen excretion and tolerance to cold shock.

A study by Becker and Focken (1995) on the effect of L-carnitine supplemented diet when treated to carp, *Cyprinus carpio*, was observed to lower the oxygen consumption by 10% and the nitrogen excretion by 15% as compared to the untreated control groups. The effect of L-carnitine on the reduction of ammonia toxicity has been investigated when Tremblay and Bradley (1992) administered a subsequent intraperitoneal injection to juvenile Chinook salmon, *Onchorhynchus tshawitscha*. Similar experiment by Groth *et al.* (1998) on juvenile tiger shrimp, *Penaeus monodon* FABRICTUS, predicted improved stability of cell membrane against xenobiotics and reduced toxicity of ammonia when supplemented L-carnitine treated diets. In the African catfish, *Clarias gariepinus*, total ammonia nitrogen (TAN) excretion levels decreased significantly ($P < 0.01$) when the feed was supplemented with 660mg/kg L-carnitine in combination with 175g fat suggesting more energy utilization of ingested protein by fish fed high fat and high carnitine diets (Ozario *et al.*, 2001).

Treatment of L-carnitine supplemented diet prior or during cold phase overcomes cold stress to fish. As Harpaz *et al.* (1999) investigated administration of elevated L-carnitine concentration up to 1000mg/kg feed to the diet of the ornamental cichlid *Pelvicachromis pulcher* proved best protection against exposure to cold shock.

2.2.4.4 Effects of L-carnitine on reproductive performance of fish

Supplementation of L-carnitine to diets of fish is believed to improve the reproduction performances of the animal such as promoting spermatogenesis, increasing total number of offspring and increasing the survival rate of larvae/fry. In their study on female guppies, *Poecilia reticulata*, Dzinkowski *et al.* (2001) recorded an increased number of offspring and high number of early spawning females when the diet is treated with L-carnitine. Increasing the number of off springs, promoting spermatogenesis and speeding up smolt stage which all lead to enhanced reproductive performance are also observed as a result of L-carnitine supplementation to fish diets.

2.2.4.5 Effects of L-carnitine on survival rate of fish larvae/fingerlings

Researches in many species of fish give evidence that often fish diets do not fulfil the requirements for larvae or fingerlings of species of commercial interest. The supplementation of dietary L-carnitine is therefore essential as the biosynthesis of L-

carnitine in fish larvae is still not well developed. Several studies have shown that survival rate of larvae can be increased by enriching zooplankton such as *Artemia*, with L-carnitine at a dosage of 250mg L-carnitine per kilogram of *Artemia* (C.A. Fernandez-Pato and C Martinez-Tapia, 1991, *unpublished result*).

Generally, the benefit of supplementing dietary L-carnitine to fish can be summarized as follows (Tremblay and Bradley, 1992; Chatzifotis *et al.*, 1996; Schreiber *et al.*, 1997; Becker *et al.*, 1999; Ozario *et al.*, 2001):

- Enhance the utilization of the fat components in fish feed
- Promotes weight gain and growth rate
- Exerts a protein-sparing effect
- Improves reproductive performance by promoting spermatogenesis, increasing total number of off springs, lowering larval mortality and increasing the number of early spawners
- Promotes the body's ammonia detoxification
- Enhances tolerance to extreme cold exposure (e.g. over wintering)
- Species such as Salmon require less smoltification period when supplemented with dietary L-carnitine.
- Lowering fry/larval mortalities
- Promote cell membrane stability against xenobiotics

2.2.4.6 Effect of L-carnitine on the dress out weight of fish

The economic success of any aquaculture production operation is dependent on the maximization of live harvest and overall production efficiency which includes reducing feed conversion efficiency and cost of production as well as maximizing carcass yield or dress out weight of fish which is usually expressed as a percentage of fish weight with head and viscera removed per unit live body weight (D. Allen Davis, 2001, unpublished report) (see equation 4). Excess lipid in fish decreases the dress out yield (Gatlin, 2001) and this will definitely lead to the economic inefficiency of fish culture. Sealey *et al.* (1998) also mentioned that fish diets with high level of protein content (41% protein) and lower protein to energy ratio result in poor dress out percentage of fish because the excessive protein will accumulate as abdominal fat and as lipid in the carcass which results in less weight gain in the carcass. Although no

previous works are conducted on the effect of L-carnitine on the dress out weight of fish, it can be postulated that its fatty acid oxidizing effect plays an important role in improving the carcass quality and dress out yield of fish products.

The relative percent weight of the head, which is expressed as the percentage of the head to the gutted body weight and usually indicated by the cephalosomatic index (CSI), also affects the product depending on whether the fish is sold whole or processed. For products sold whole, large head is not a disadvantage especially when it is desired for the preparation of soup. For processed products however, the large head is a disadvantage as it contributes to relatively poor dress out percentage (Stone *et al.*, 2000). Lower CSI indicates relatively smaller head weight and better dress out weight (or filets) which is an indication of the positive effect of L-carnitine on the flesh quality and dress out weight.

Somatic indices such as the viscerosomatic index (VSI) and hepatosomatic index (HSI) are used as indicators of body composition and condition of fish which affects the dress out weight and the meat quality. Higher VSI indicates large amount of fat/lipid deposition in the abdominal cavity and in the muscle (Twibell and Brown, 2000) and this reduces the dress out weight of fish. In fish liver, triglycerides serve as energy storage form of lipids and results on liver composition of Red Sea breams show that L-carnitine administration to the diets enhances energy and triglyceride formation by the liver favouring higher HSI (Chatzifotis *et al.*, 1995).

2.3 Recommended inclusion levels of L-carnitine to aquafeed

As can be observed in numerous works concerning the effect of L-carnitine on fish, the levels of inclusion administered to the diet vary according to species and the area of specific study and the results are variable accordingly. For example, for rohu *Labeo rohita* fingerlings, best growth was observed at 500mg L-carnitine per kilogram feed (Keshavanath and Renuka, 1998) while 2088mg L-carnitine per kilogram feed was optimum level for the highest growth rate of the Red Sea bream *Pagrus major* fingerlings (Chatzifotis *et al.*, 1995).

L-carnitine levels higher than the optimal could lead to an impaired growth and less weight gain. Keshavanath and Renuka (1998) mentioning from an unpublished report by Gunther showed that Nile tilapia, *Oreochromis nilotica*, performed better growth

when supplemented 300mg/kg than 900mg/kg. This reduction in weight is attributed to the energy loss through the excretion of excess acyl-carnitine and this will lead to an impaired growth. Especially in low-fat diets, low carnitine level is recommended (Torreele *et al.*, 1993).

In general, the recommended levels of L-carnitine to the diets of fish vary according to the species feeding behaviour, developmental stage and reproductive status of the animal and environmental conditions. Lonza Group deducing from various research results recommends the following levels according to the species feeding behaviour (Website: <http://www.karniking.com/karniking/en/animal/aquaculture/recommendation.html>).

Herbivores:	100-200 mg L-carnitine per kilogram feed
Carnivores:	up to 500 mg L-carnitine per kilogram feed
Shrimps:	500-1000 mg L-carnitine per kilogram feed

Furthermore, recommendations at specific level of fish group and developmental stages are shown in table 2.1.

Table 2.1 Recommended L-carnitine levels to aquafeed according to fish species and developmental stages

Fish group	L-carnitine inclusion level (mg/kg feed)
Tilapia	100-400
Carp (overwintering)	100-400
Catfish	300
Trout (overwintering)	500
Eel	100-500
Salmon, trout (fry, fingerling, smoltification)	500-1000
Guppy (reproduction)	500-1000
Shrimp ²	500-1000

Source:

Website: <http://www.karniking.com/karniking/en/animal/aquaculture/recommendation.html>

² For shrimp larvae, L-carnitine can be supplied indirectly via zooplankton such as Artemia at concentrations 250mg/kg Artemia.

3 Materials and Methods

3.1 Culture system and experimental fish

The experiment was conducted at Welgevallen Research facility of the Division of Aquaculture of the University of Stellenbosch in the Western Cape Province of the Republic of South Africa. The feeding trial was conducted in an outdoor recirculating system comprising five concrete ponds. The dimension of each pond is 8m x 3m x 1.5m for length, width and depth respectively. Each pond has its own inlet, drainage and aeration system. 40 experimental hapas³ were submerged in the ponds each pond housing 8 hapas. Each hapa has a dimension of 1m³. Approximately 140 Tilapia fry with a total biomass of 200g and average weight of $1.40\text{g} \pm 0.71\text{g}$ were allocated into each of the 40 experimental hapas and acclimatized for 24 hours prior to the start of the feeding trial. Figure 3.1 shows the arrangement of the experimental hapas in each of the five ponds.



Figure 3.1 Experimental 1m³ hapas submerged in the experimental pond water

³ Hapa is a fine-meshed nylon net cage used for the rearing and growing of fish in aquaculture.

3.2 Data recording

Water Quality Parameters: Water temperature was recorded at 8:00am and at 2:00pm respectively from which a daily average was calculated. Dissolved Oxygen, ammonia, nitrite, nitrate and pH were also measured every two weeks and compared for the tolerable range according to guidelines in Boyd (1990).

Growth: Mortality from the respective hapas was recorded both in terms of the number and weight of dead fish through out the experimental period. All the fish from each hapa were weighed every 14 days for the determination of the growth curve. Data for growth and feed intake were recorded over two separate trial periods. At the end of the first 30 days, all fish from each replicate were weighed as a group and the average was calculated. The total amount of feed consumed over the 30 days period was determined for the purpose of calculating the feed conversion ratio (FCR). Similarly, all the fish from each replicate were weighed at the end of the second 30 day growth/trial period. Average weight of fish and the total amount of feed consumed for the whole experimental period (60 days) was recorded. The average relative daily feed intake (equation 6) was calculated as the percentage of the initial body weight (Sanchez *et al.*, 2001).

24 hours after the last feeding, 8 fish from each replicate were slaughtered and the following parameters were recorded: total weight of fish, dress out weight (weight of fish with head and viscera removed), head weight, visceral weight, liver weight and standard length. From these parameters the percentage dress out weight, the viscerosomatic index, the hepatosomatic index, and percentage of the head weight were calculated for further statistical data analyses.

The above indices and parameters were calculated based on the following equations (Cunningham *et al.*, 1985; Keshevanath and Renuka, 1998; D. Allen Davis, 2001; Sanchez, *et al.*, 2001)

$$\text{Viscerosomatic index (VSI)} = \frac{\text{weight of viscera (g)}}{\text{total weight of fish (g)}} \times 100 \quad (1)$$

$$\text{Hepatosomatic index (HSI)} = \frac{\text{weight of liver (g)}}{\text{total weight of fish (g)}} \times 100 \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{total feed consumed (g)}}{\text{weight gain of fish (g)}} \quad (3)$$

$$\text{Percentage dress out} = \frac{\text{dress out weight}}{\text{total body weight}} \times 100 \quad (4)$$

$$\text{Specific growth rate (SGR)} = \frac{\log_e W_2 + \log_e W_1}{\text{days}} \times 100 \quad (5)$$

where W_2 is the final body weight at the end of 60 days and W_1 is the initial body weight.

$$\text{Fee Intake (FI)} = \frac{\text{Total feed consumed/day}}{\text{Initial body weight}} \times 100 \quad (6)$$

$$\text{Cephalosomatic index (CSI)} = \frac{\text{Head weight(g)}}{\text{gutted body weight(g)}} \times 100 \quad (7)$$

3.3 Preparation of the experimental diet

Commercial pre-starter feed of tilapia was obtained from AquaNutro (Pty) Ltd., a local aquafeed processing company⁴. The protein content of the diet is 48.6%. Two types of crumbles of tilapia pellet, 3mm and 5mm were used according to the size of the fish at the different growth stages. At the first stage of the experiment the 3mm crumble was used for 1 month thereafter the 5mm was used for the following one month. L-carnitine (Carniking® 500g/kg active ingredient) was supplemented at different inclusion levels to prepare 5 diets accordingly: C₀, C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀ representing for the control 0, 250, 500, 750 and 1000 milligram of L-carnitine per kilogram of feed respectively.

In preparing the diets, a definite amount of L-carnitine was added to a portion of the basal diet of tilapia at a proportion of 0.1% and the whole content was mixed thoroughly. The five levels of inclusion were prepared by mixing portion of the L-carnitine-supplemented feed with the appropriate proportion of the basal diet. The experimental feeds were then stored at a temperature of 5°C.

⁴ 5 Aqua Crescent, PO Box 45, Malmesbury 7299, South Africa

3.4 Feeding method

At the first stage of the experiment, 900g of feed from each diet were weighed and filled in 40 airtight bottles to feed the fish in the respective 40 hapas. The diets were randomly assigned to the experimental ponds and hapas so as to limit the extent of environmental variance, thus experimental error. Fish were fed 6 times a day on an *ad lib* basis.

3.5 Experimental design and data analysis

The experiment was conducted in accordance to a randomized design where the five treatments (diets) were assigned at random to the 40 experimental units (hapas) each treatment comprising 8 replicates. The design of the randomization is shown in Table 3.1.

Table 3.1 Completely randomized experimental design. Numbers 1-40 represent the treatment classes and the connotations in brackets are the treatments/diets showing their respective inclusion levels.

37 (C ₂₅₀)	38 (C ₅₀₀)	39 (C ₇₅₀)	40 (C ₁₀₀₀)
33 (C ₅₀₀)	34 (C ₇₅₀)	35 (C ₁₀₀₀)	36 (C ₀)
29 (C ₇₅₀)	30 (C ₁₀₀₀)	31 (C ₀)	32 (C ₂₅₀)
25 (C ₁₀₀₀)	26 (C ₀)	27 (C ₂₅₀)	28 (C ₅₀₀)
21 (C ₀)	22 (C ₂₅₀)	23 (C ₅₀₀)	24 (C ₇₅₀)
17 (C ₂₅₀)	18 (C ₅₀₀)	19 (C ₇₅₀)	20 (C ₁₀₀₀)
13 (C ₅₀₀)	14 (C ₇₅₀)	15 (C ₁₀₀₀)	16 (C ₀)
9 (C ₇₅₀)	10 (C ₁₀₀₀)	11 (C ₀)	12 (C ₂₅₀)
5 (C ₁₀₀₀)	6 (C ₀)	7 (C ₂₅₀)	8 (C ₅₀₀)
1 (C ₀)	2 (C ₂₅₀)	3 (C ₅₀₀)	4 (C ₇₅₀)

Statistical analysis was performed using the statistical package MINITAB 13 for Windows (MINITAB, 2000). To test the significant differences between the various levels of the L-carnitine, data are analyzed by one-way ANOVA and Tukey's pairwise comparison test at a five percent level of significance ($P < 0.05$).

4 RESULTS

4.1 Water quality and temperature

Water quality parameters were measured for pH, dissolved oxygen, ammonia, nitrite and nitrate and all were within the tolerable range for fish throughout the experimental period (see Table 4.1). However, water temperature fluctuated between 16 and 23°C. The fluctuation average daily water temperature is presented in Figure 4.1.

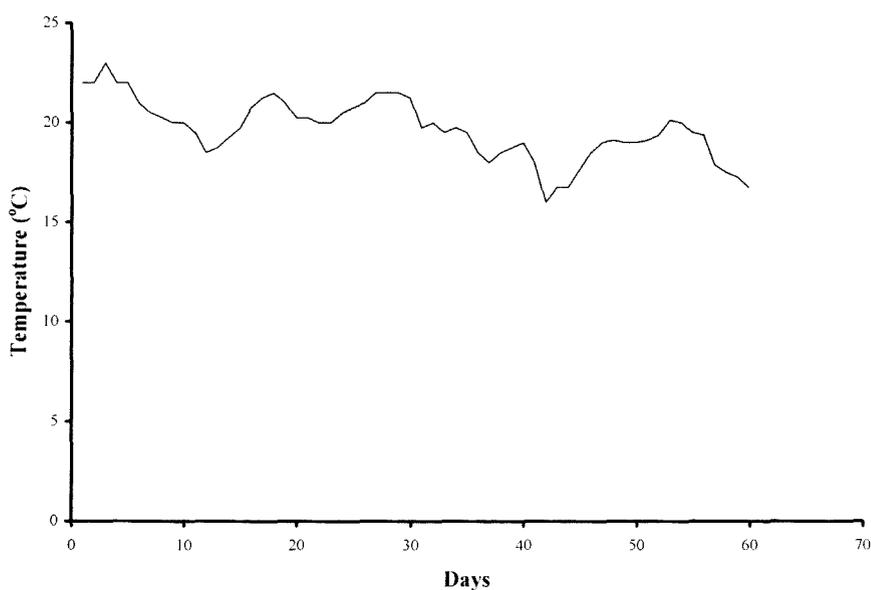


Figure 4.1 Daily average water temperature during the whole experimental period

First 30 days: average = 20.5°C, minimum = 18.5°C, maximum = 23°C

Second 30 days: average = 18.5°C, minimum = 16°C, maximum = 20°C

Table 4.1 Measurements of water quality parameters and their respective tolerable limits for fish

Parameters	Measured values (mg/l)			Tolerable limits (mg/l) (Boyd, 1990)
	System 1	System 2	System 3	
Dissolved oxygen	7.0	7.0	7.5	5 and above
pH	6.7	6.9	6.88	6.5-9.0
Ammonia (NH ₃)	0.17	0.11	0.01	0.7-2.4
Nitrite (NO ₂)	0.043	0.036	0.04	0.66-200
Nitrate (NO ₃)	2.42	2.42	1.14	Not serious

4.2 Growth study results

Data collected after the first 30 days and at the end of the 60 days period were analyzed separately. In both cases L-carnitine showed no significant effect ($P > 0.05$) on the growth of fish. However, from all the treatment levels the C₂₅₀ showed slightly better growth than the higher levels in both cases.

Survival was high in relation to all the treatments (99-99.6%) and differences were not statistically significant ($P > 0.05$).

Table 4.2 Effect of different levels of dietary L-carnitine on production performance parameters of Mozambique tilapia over the 60 days experimental period¹

Parameters	Diet				
	C ₀	C ₂₅₀	C ₅₀₀	C ₇₅₀	C ₁₀₀₀
Initial weight (g)	1.40 ± 0.22	1.40±0.25	1.40±0.10	1.40±0.13	1.40±0.25
Final weight (g)	6.02 ± 0.96	5.93±0.84	5.49±0.56	5.56±0.63	5.68±0.81
Average weight gain (g)	4.51 ± 0.76	4.45±0.63	4.13±0.48	4.22±0.59	4.26±0.65
Av specific growth rate (%)	3.67± 0.50	3.62± 0.50	3.36 ± 0.27	3.40 ± 0.26	3.45 ± 0.46
Feed conversion ratio (FCR)	2.30 ± 0.17	2.22±0.10	2.25±0.11	2.27±0.28	2.29±0.21
Total feed intake (g)	1354 ± 52.1	1307±50.1	1335 ± 36.6	1364 ± 61.2	1355 ± 77.1
Feed intake (%initial weight)	11.29± 0.43	10.89±0.42	11.12± 0.30	11.37± 0.51	11.29± 0.65
Survival (%)	99.55 ± 0.73	99.08±1.16	99.06±1.16	99.20±0.99	99.21±1.32

¹Values are means (± s.d) of eight replicates.

The feed intake of the fish was affected by temperature. During the first 30 days trial period, growth increased exponentially where after the growth rate declined over the next 30 day period (Figure 4.2) correlated to the decrease in water temperature as reflected in Figure 3.1. Feed intake was observed to be lowest in the C₂₅₀ fed fish and highest in the C₇₅₀ though differences are not statistically significant ($P > 0.05$). The feed consumption (intake) of the C₂₅₀ fed fish was 4% less than either the control or the C₇₅₀.

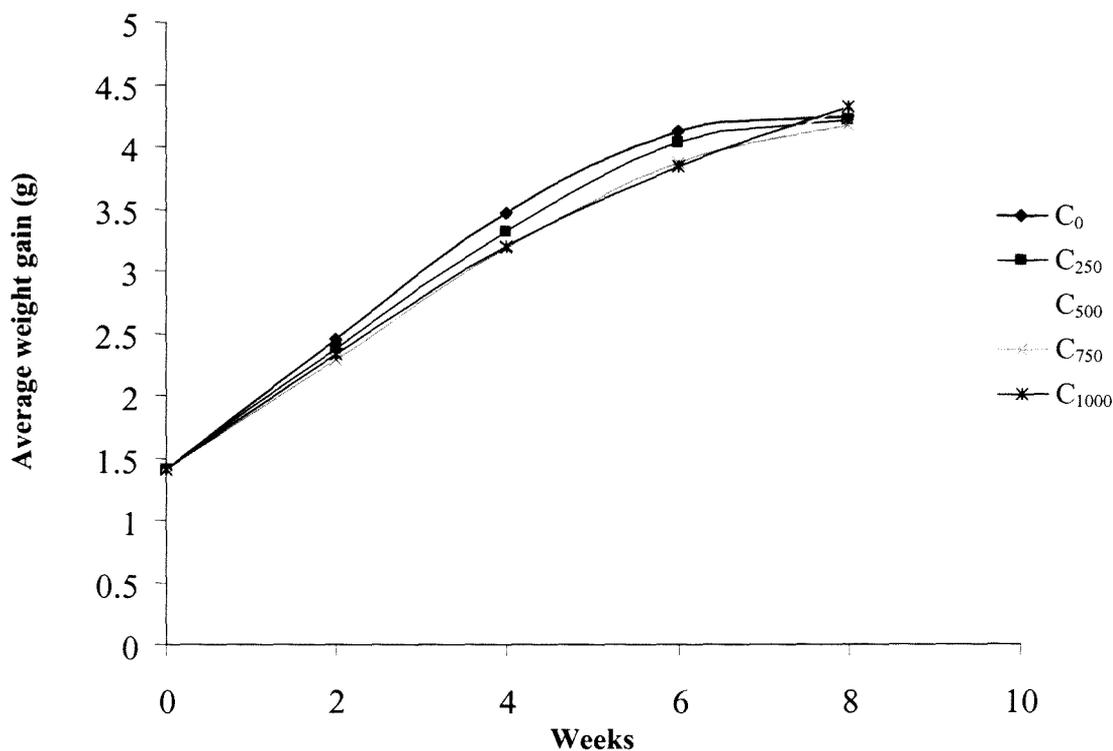


Figure 4.2 Average weights of tilapia fry at varying levels of L-carnitine; Values are means of eight replicates.

There was no significant difference ($P > 0.05$) in feed conversion ratio (FCR) between any of the treatment levels. However, the C₂₅₀ treatment group performed slightly better in terms of FCR and showed a trend especially during the whole trial period (after 60 days). Despite the insignificance of statistical differences, better FCR values were observed in the first 30 day trial (1.56 ± 0.13 , 1.52 ± 0.07 , 1.52 ± 0.07 , 1.53 ± 0.14 , 1.57 ± 0.16 for C₀, C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀ respectively) as compared to the whole trial period after 60 days (2.31 ± 0.17 , 2.22 ± 0.10 , 2.25 ± 0.11 , 2.27 ± 0.28 and 2.29 ± 0.21 for C₀, C₂₅₀, C₅₀₀, C₇₅₀ and C₁₀₀₀ respectively).

4.3 Results on body characteristics and Percentage dress out

Results on the effect of varying levels of L-carnitine on the somatic characteristics and dress out weight of Mozambique tilapia are presented in table 4.3.

One way ANOVA and Tukey's pairwise comparison test showed no significant difference ($P > 0.05$) between the treatment levels on the effect of the percentage dress out weight of fish. However, fish receiving the diet with the dosage 250mg/kg

L-carnitine exhibited better dress out weight either than the control or the other treatment levels. Moreover, the percentage of head (CSI) for fish fed the C₂₅₀ dietary levels of L-carnitine has showed significant difference ($P < 0.05$) from all the other treatment levels but not from the control.

Viscerosomatic index (VSI) showed an increasing trend from the control to the highest treatment level but differences are not statistically significant ($P > 0.05$). The highest VSI value and lowest dress out weight was observed in fish receiving the C₇₅₀. Hepatosomatic index showed a decreasing trend but no significant differences between the treatment levels.

Table 4.3 Effect of different levels of dietary L-carnitine on body characteristics of Mozambique tilapia over the 60 days experimental period¹

Parameters	Diet				
	C ₀	C ₂₅₀	C ₅₀₀	C ₇₅₀	C ₁₀₀₀
Dress out weight (%)	63.54 ± 2.61	63.76 ± 3.27	63.20 ± 2.20	62.91±2.23	63.43±2.15
Dress out weight	9.30 ± 1.66 ^a	9.40 ± 2.02 ^a	8.90 ± 1.60 ^{ab}	8.50 ± 1.2 ^b	8.80 ± 2.3 ^{ab}
VSI (%)	16.24 ± 3.03	16.64 ± 3.24	17.27 ± 2.38	17.18±2.30	17.05 ± 2.45
HSI (%)	5.80 ± 1.50	5.75 ± 1.32	5.74 ± 1.31	5.57 ± 1.32	5.53 ± 1.12
CSI (%)	13.92 ± 2.15 ^{ab}	13.38 ± 2.82 ^b	14.45±1.04 ^{ac}	14.67±1.0 ^a	14.24±1.1 ^{abc}

¹Values are means of eight replicates. Values with different superscripts in the same row show significant differences ($P < 0.05$)

As can be observed in Figure 4.3, the dress out weight and the cephalosomatic index are inversely correlated. Although the regression plot in Figure 4.4 showed that the correlation between dress out and CSI is not that much powerful ($R\text{-Sq} = 31.3\%$), highest dress out and lowest CSI were observed in the groups received C₂₅₀.

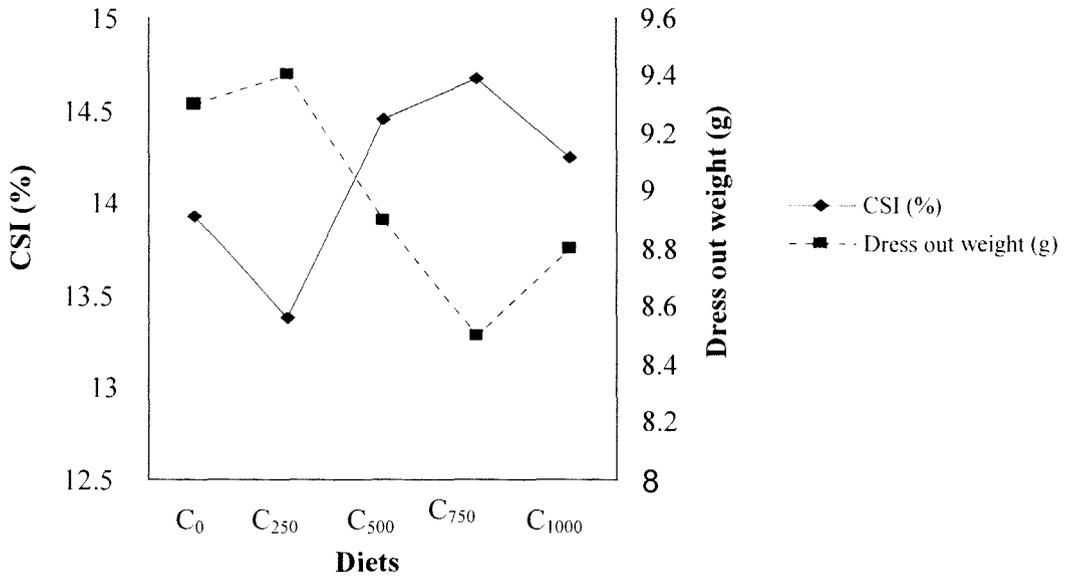


Figure 4.3 Dress out weight (g) and Cephalosomatic index (%) of samples of fish after 60 days in response to varying level of L-carnitine supplementation to the diet; the values are means of eight replicates.

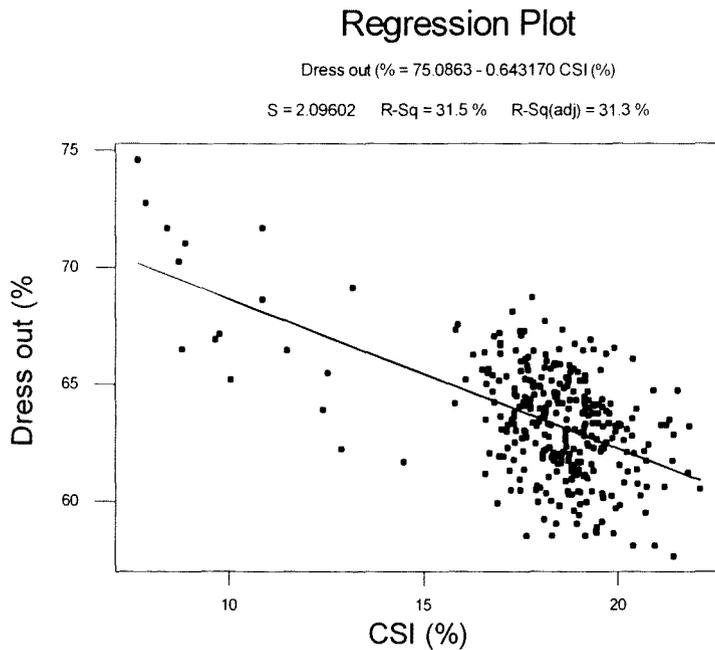


Figure 4.4 Regression plot showing the correlation between CSI (%) and dress out (%)

5 DISCUSSION

5.1 Growth

A number of studies (Santulli and D'Amelio, 1986; Torreele, *et al.*, 1993; Chatzifotis, *et al.*, 1995; Becker and Focken, 1993; Becker *et al.*, 1999) revealed that L-carnitine supplementation to the diets of fish significantly improves growth performances and feed conversion efficiency of fish. On the contrary, some other studies show the insignificance of the supplementation of the ingredient to the diet (Rodehutsord, 1995; Chatzifotis *et al.*, 1997; Gaylord and Gatlin, 2000; Bradley and Tremblay, 1996). The results of the current study are in agreement to the findings by the latter authors where no significant differences ($P > 0.05$) were observed between the carnitine treated diets and the control. Nevertheless, there could be possible reasons for the negative results. Quite often, discrepancies may occur with regard to the results of the same species under different experimental conditions and various L-carnitine supplementation levels. For instance, results on the effect of L-carnitine supplementation to diets of hybrid striped bass (*Morone saxatilis* male \times *M. chrysops* female) by Twibell and Brown (1999) show improved growth and feed intake at 369.7mg L-carnitine/kg feed while Gaylord and Gatlin (2000) experimenting on the same species reported no significant effect of L-carnitine supplementation at a dosage of 3000mg/kg.

Most of the previous works on the effect of L-carnitine supplementation to fish diets show improved growth rate, better feed conversion efficiency, improved metabolic efficiency, improved body composition and in general improved life performances. However, certain discrepancies occur in the results found by different authors that could be attributed to variable experimental conditions such as control of environmental temperature, the level of crude protein and lipid content of the experimental diet, L-carnitine content of the commercial feed of the fish and feeding status of the fish, i.e. whether it is starved or fed to satiation (Chatzifotis *et al.*, 1996). Temperature affects the feed intake of fish and hence affects the growth rate and the body composition of fish (Lovell, 1989). Peres and Roche (1983) on their studies on the effect of L-carnitine when supplemented to Sea bass, *D. labrax* L., under constant

temperature showed an increase in body lipid content while Santulli and D'Amelio (1986) demonstrated a continuous decrease of total lipid content in plasma, liver and skeletal muscle while experimenting under variable temperatures.

The recommended level of L-carnitine supplementation to tilapia feed is 100-400 mg/kg of feed (Lonza Group, 2003). However, the natural content of L-carnitine in the ingredients of fish diets affects the level of inclusion. For instance, fish meal based diets contain 60-120 mg of L-carnitine per kilogram of feed (Baumgartner, 2003) and this probably will lead to the impairment of the experimental levels of L-carnitine if not taken into account. Most fish diets contain considerable amount of fish meal and in this particular experiment, the diet used comprises 62% fish meal. The natural concentration of L-carnitine in fish meal which comprises 60-120 mg/kg will therefore affect the various experimental inclusion levels. Keshavanath and Renuka (1998) indicated that as the level of L-carnitine increases energy loss through excretion of excess acyl-carnitine will occur eventually leading to reduced weight gain. The results from this experiment are also revealing similar phenomenon where fish received the dosage C₂₅₀ performed slightly better growth than the higher levels. However, the C₂₅₀ did not show better performance than the untreated diet indicating that the lower recommended level could be by far less than 250 mg/kg L-carnitine. In agreement to this hypothesis, Becker *et al.* (1999) when studying the effect of L-carnitine supplemented diet on the growth performance of *Oreochromis niloticus* × *O. aureus* hybrid the L-carnitine level 150mg/kg was more effective than the higher levels.

Comparing the L-carnitine supplemented diets, fish receiving the C₂₅₀ showed lowest feed intake and slightly better FCR while the weights are also slightly higher than the higher treatments. Thus it can be hypothesized that lower L-carnitine levels (<250mg/kg) could favour better growth and FCR as higher doses react negatively. Becker *et al.* (1999) in their studies on *O. niloticus* analyzed the economic utilization of the carnitine supplemented diet at a level of 150mg/kg and proved more economical either than the control or the higher levels. Moreover, in the current experiment, feed consumption was found to be 4% less either than the control or the higher levels. In agreement to the result of this study, Becker and Focken (1995) on carp, *Cyprinus carpio*, also showed better growth rate and lowered feed consumption

by 10% when fed L-carnitine supplemented diet. This could be an implication that supplementation of fish diets with L-carnitine can improve FCR as a result of lower feed consumption and increased weight gain eventually minimizing the cost of feed.

The hepatosomatic index was decreasing steadily with the increase in the levels of L-carnitine. This is an indication of starvation which led to weight loss of fish (Sanchez-Paz *et al.*, 2003) due to high energy utilization in the liver which is the storage of the major source of energy - the triglycerides (Chatzifotis *et al.*, 1995). In contrast to the results of this study, Chatzifotis *et al.* (1995) found higher HSI in fish receiving

L-carnitine. The contrast could be attributed to the water temperatures where in their case was kept within the optimal range but in this study some times it was below the critical value for feeding activities which led fish to refuse feeding. Visual observations of the liver from the sample fishes indicate pale colour and easily disintegrated. It is speculated that this could suggest depletion of much energy as a result of starvation due to low feed intake.

5.2 Water quality and water temperature

Water temperature specifically during the second 30 day trial period was recorded as low as 16⁰C at times and this could not favour an appropriate growth. Becker *et al.* (1999) mentioned that growth performances of L-carnitine supplemented diet fed *Oreochromis niloticus* × *O. aureus* hybrid were negatively affected by the fall in water temperature where after optimal level was maintained favouring optimal growth. Growth rate of sea bass, *Dicentrarchus labrax* L, was also strictly related to water temperature variations (Santulli and D'Amelio, 1986). Even though tilapias can survive temperatures as low as 8⁰C to 12⁰C, their feeding activities and growth becomes reduced below 20⁰C and stop feeding around 16⁰C (Lovell, 1989).

Oreochromis mossambicus can survive a lower temperature limit of 11-14⁰C and the upper lethal limit is 38⁰C. However, the optimum water temperature for growth is above 20⁰C and feeding activities stop at temperatures below 15⁰C (http://cdserver2.ru.ac.za/cd/011120_1/Aqua/SSA/omossam.htm). In the current study, the drop in water temperature negatively affected the feed intake of the fish. During the second 30 days feeding trial period, fish were observed to regurgitate the

feed though they swallow it greedily. This led to the misestimating of the appetite of the fish that influences the results of FCR. Consequently, the FCR for the whole trial period (60 days) was found to be very high as compared to that of the first 30 days.

Although water quality parameters such as dissolved oxygen, pH, ammonia, nitrite and nitrate were within the tolerable range for fish there were differences between the systems/the different ponds (Table 4.1). Data were therefore analyzed for separate ponds to see if the differences could be attributable to a particular pond condition. Weight gain, FCR and Feed intake were all performing better in the fifth pond (system 3) where it exhibited better water quality (see Table 4.1) and slightly higher temperature ($2^{\circ}\text{C}+$ thorough out the experimental period). The difference in temperature is due to the exposure of the fifth pond to higher levels of direct sun light. The exposure could favour growth of phytoplankton that is attributable to the higher weight gain of the fish in that particular pond eventually leading to an improved FCR.

5.3 Percent dress out

One of the major problems with farmed fish is the accumulation of lipid in the visceral cavity and muscle (Twibell and Brown, 1999) as a result of high energy to protein ratio and this leads to reduced weight gain and poor product in terms of the dress out weight and carcass quality. Although little or no single work has been done on the effect of L-carnitine supplemented diets in relation to the dress out weight of fish, the results of the current study showed a trend on the effect of the ingredient on the product quality. This can be supported by previous works on the body composition of fish (Burtle, 1994) where 1000mg L-carnitine/kg feed significantly reduced the visceral fat quantities and muscle fat along the lateral line of channel catfish. However, in this study the C_{250} showed lowest VSI and performed better dress out weight. Highest VSI value and lowest dress out weight was observed in fish receiving the C_{750} . This could be attributed to the high feed intake in this group of fish which led to visceral fat accumulation.

The inverse correlation between the dress out weight and CSI (Figure 4.3) reveals the contribution of a relatively smaller head to the improved dress out weight. The highest dress out weight and lowest CSI were observed in fishes received the C_{250} differences being statistically significant ($P < 0.05$) between C_{250} and C_{500} , C_{250} and C_{750} for CSI

and between C₂₅₀ and C₇₅₀ for dress out weight. This is probably attributable to the fact that L-carnitine has a visceral fat reducing effect (Twibell and Brown, 2002) that led to better gutted weight, thus smaller CSI, eventually resulting in better dress out weight.

CONCLUSION

In conclusion of this study, although L-carnitine did not show significant difference in the weight gain of fish, the trends in the somatic indices, in the dress out weight and the significant difference in the CSI suggest the possibility of improving production performance parameters of fish with supplementation of the ingredient. The trend, in FCR and in feed intake with the supplementation of L-carnitine is also an indication of the economic utilization of feed and reduced production cost of fish culture.

In the current study, the sub-optimal water temperature was a major factor that affected the results as the fish were observed to refuse feeding especially during the second 30 day trial period when temperature dropped as low as 16°C. Furthermore, the tilapia diet used in this study comprises 60% fish meal with a natural L-carnitine content of 37-74mg/kg feed. This amount of fishmeal inclusion could potentially add to the various treatment levels of L-carnitine thereby impairing with the recommended levels for tilapia. Therefore, it is suggested that further study should be conducted at lower levels of L-carnitine and controlled optimal water temperature. The author also recommends further research on the effect of L-carnitine on cold tolerance of *Oreochromis mossambicus* as the temperature fluctuation is one of the major problems in obtaining optimal growth in the temperate regions.

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