INVESTIGATIONS INTO THE POTENTIAL OF FEED ADDITIVES TO INCREASE RESISTANCE TO DISEASE AMONG FARMED FISH

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Introduction

There has been considerable amount of research in the last 10 years on L-carnitine (LC), a water soluble vitamin-like compound that is readily synthesised in the body from lysine and methionine, due to its important role in fat metabolism and society's infatuation with weight loss. The biological functions of LC were first recognised in lipid metabolism, such as the transport of long chain fatty acids through the inner mitochondrial membrane under the form of acyl-carnitine compounds, as an essential phase of fatty acid oxidation. As such, LC plays a vital role in fat combustion. It has been suggested that LC supplementation may stimulate protein-sparing action by increasing energy derived from lipids. In animal production, LC has been shown to enhance animal productivity and is now recommended for use in swine mainly to induce a better growth ratio, i.e. to obtain more muscle and less fat in the resulting carcass. In fish, LC has also been recognised as a growth enhancer in a number of species, including sea bass[1], African catfish [2] and red seabream [3]. These results have not been confirmed in Rainbow trout [4,5].

CEFAS Weymouth controlled experimental facilities: 401 tanks.

LC has only recently been noted as an important substance essential to our health. It has been approved by the US FDA for use in humans as a therapy for low energy levels, congestive heart disease and other diseases associated with low cellular energy levels. Numerous published studies have either suggested or shown that LC can also be effective in treating a wide range of disorders including immune dysfunction [6,7,8]. Indeed, the use of acetyl L-carnitine as a therapeutical method of treating patients with impaired immune system is now patented in the US. LC deficiency has been shown to result in a weakening of the immune system and an increased risk of infection. On the other hand, the body's requirement for LC is increased during physical stress or infections and orally administered LC has been shown to promote the development and preservation of immunocompetence.

We report here the first investigations into the potential of L-carnitine supplementation to reduce disease susceptibility among farmed fish, using Rainbow trout and experimental enteric redmouth (ERM) disease as references. We tested the potential of 8 different regimen of LC oral supplementation in Rainbow trout fingerlings subjected to a cohabitation challenge with *Yersinia ruckeri*.

Material & Methods

Seven groups of 50 rainbow trout fingerlings averaging 11.3 +/- 1.5g were allowed to acclimate for at least 7 days in 401 tanks filled with dechlorinated freshwater at 15°C before being offered LC supplemented diets at three different dosages for either 5 or 12 days (see Table 1). L-carnitine (98% synthetic, Sigma-Poole) was coated onto standard immunostimulant-free trout diet using vegetable oil. The incorporation rate of LC to the diet was calculated so that the fish received either 20, 50 or 100 mg L-carnitine/kg BW/day when fed at 3.5% feeding rate. Control fish were fed the un-coated diet at the same feeding rate. Each group of fish comprised three replicates.

Table 1: Experimental design

Group	L-carnitine dosage (mg/kg BW/day)	Duration of treatment (days)	No fish per tank*	Total No tanks	No ERM challenged tanks
Control	N/A	N/A	50	3	2
L-carnitine	20	5	50	3	2
	50	5	50	3	2
	100	5	50	3	2
	20	12	50	3	2
	50	12	50	3	2
	100	12	50	3	2

* *Excluding ip injected fish*

At the end of the experimental feeding period, the fish were starved for 24h prior to introduction to two tanks for each group of 20 control fish that had been intra-peritoneally injected with 0.2 ml of a suspension of *Y. ruckeri* (FDL 2179/93) resulting in an infective dose of 6×10^6 CFU per ip fish. The last tanks of each group were kept as unchallenged controls in which 20 saline ip injected fish were introduced.

All tanks were observed for 35 days post challenge, period during which the fish were offered the standard diet. Dead fish were removed daily from the tanks and all sampled for confirmation of the cause of the death. At the end of the experiment all surviving fish were sacrificed and their kidney swabbed onto ROD plates to determine the carrier status of each group/tank. Fish were bulk weighed at their introduction into the experimental tanks and at the end of the experiment.

The protective effect of L-carnitine against ERM was assessed by comparing cumulative mortality rates, carrier rates amongst the survivors and overall protection against *Y. ruckeri* conferred by LC within and between groups using Chi square test at $\propto = 0.01$. Additionally final mean weights were compared using ANOVA.

Summary of results

- The cause of death was confirmed as ERM in all the fish that died in the challenged groups. No fish died of ERM in the control unchallenged groups.

- **Cumulative mortality rates:** Fig 1

Results within replicates were not significantly different, except for the group receiving LC at 20 mg/kg BW/d for 12 days.

The 5 days supplementation at 100 mg L-carnitine/kg BW/d was the only treatment for which a significantly reduction of mortality was observed when compared to the control group fed standard trout diet. None of the 12 days supplementation regime improved the survival of the fish.

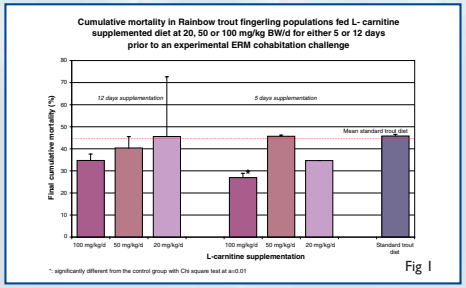

Fig 1

- **Carrier status:** Fig 2

Results within replicates were not significantly different.

A significant lower proportion of survivors were found to be carriers of *Y. ruckeri* in the groups receiving either 50 or 100 mg L-carnitine/kg BW/d for 5 days when compared to the group supplemented at 20 mg/kg BW/d for 5 days or the control unsupplemented group. There was no significant benefit obtained from the 12 days treatment when compared to the control unsupplemented group.

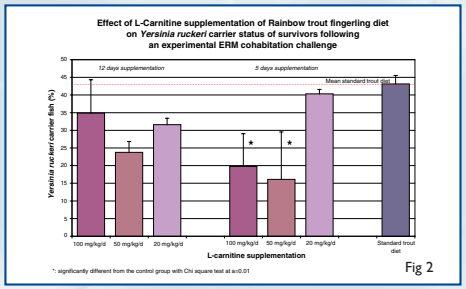

Fig 2

- **Overall protection:** Fig 3

Results within replicates were not significantly different, except for the group receiving LC at 20 mg/kg BW/d for 12 days.

Preventive LC supplementation of standard trout diet at 100 mg/kg BW/d for 5 days before experimental ERM challenge was the only tested treatment that offered a significant protection against either the disease or *Y. ruckeri* carriage in challenged fish, when compared to fish fed standard trout diet.

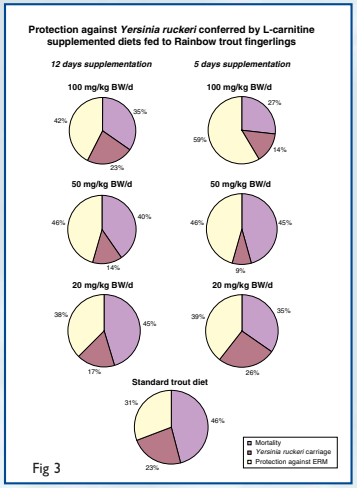

Fig 3

- **Growth performance:**

Comparisons of final mean weights between the groups (data not shown) did not show any significant improvement of growth performance in any of the groups fed LC supplemented diet, when compared to fish fed standard trout diet.

References

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Discussion

This is the first report on the health benefit of L-carnitine supplementation in fish. Our results clearly showed that LC supplemented diets could help reducing losses due to ERM and decrease the risk of asymptomatic carriage of *Yersinia ruckeri* by fish surviving a disease outbreak. The benefit of the diet supplementation with LC is however clearly dose and duration dependent, the maximum protection being offered in this study to fish fed 100 mg L-carnitine/kg BW/d for 5 days.

The mechanism of the protection offered by LC is still to be investigated. The fish used in this experiment had never been exposed to any fish pathogen prior to the trial. Following exposure to ERM, the non-specific immune response was the first mechanism of disease protection to be stimulated, relying on phagocytosis and the activity of serum factors before activation of the specific humoral and cellular immune response.

Immunological processes are characterised by high cell division and synthesis rate, all requiring a high-energy supply rate. L-carnitine primarily acts in the body as a receptor for activated short- and long-chain fatty acids and is thus directly involved in cellular energy metabolism and the modulation and synthesis of membranes.

L-carnitine is present in high concentration in white blood cells of healthy patients [9], suggesting a link with their immune function. Conversely, reduced levels of plasma or lymphocyte carnitine have been observed in immunodeprived patients suffering from AIDS [10]. It has been demonstrated that administration of LC to immunodeprived patients strongly ameliorated the immune response of these individuals [7,10], by promoting the proliferation of lymphocytes and monocytes [10]. L-carnitine has also been shown to *in vitro* increase the phagocytic activity of human granulocytes but not that of monocytes [11].

It has been suggested that LC acts on the immune system by induction of a mitogenic agent [12]. Famularo *et al.*, 1993 [7] also ruled out the possibility that L-carnitine had a direct immunoenhancing effect but suggested that L-carnitine acts via a metabolic pathway.

We now intend to investigate the mechanisms of protection offered by L-carnitine against ERM and to extend the trials to other fish diseases such as RTFS in rainbow trout fry for which efficient means of control and prevention are still needed.

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