Effect of L-carnitine on reproductive performance and fry performance of Nile tilapia broodstock

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L-carnitine feed additive can be applied in different fish species for improving reproductive performance due to effect on energy utilization. This study aimed to investigate the effect of L-carnitine on reproductive performance and fry performance of Nile tilapia Oreochromis niloticus broodstock. Females (48) and males (24) of Nile tilapia broodstock were fed commercial pelleted diet (35% crude protein (CP), 4586 Kcalkg⁻¹ diet Gross Energy (GE) supplemented with different concentrations of L-carnitine (0.0, 300 and 500 mgkg⁻¹ diet) for 105 days feeding trial in 6 breeding hapas (2x2x1m³) with feeding rate 2% then offspring’s collected to study reproductive performance. For fry feeding in nursery hapas. A commercial powder diet (40.5% CP and 4897 GE Kcalkg⁻¹ diet) were fed for 60 days. The diet was fed at rate 10% of the total fry biomass at the first 30 days started from the 2nd day of collecting fry from the broodstock (spawning) hapas and decreased to 5% in the 2nd growth interval which extended for 30 days. Results indicated that a significant improvement in seeds production with 500 mgkg⁻¹ L-carnitine. The data also indicated that the inclusion of L-carnitine in tilapia broodstock diets have a beneficial effect on growth performance, energy utilization with no significant effect on body chemical composition of Nile tilapia fry.

Keywords: Nile tilapia, L-Carnitine, seeds production, fry growth performance.

INTRODUCTION

Intensification and diversification of aquaculture operations has become the global trend of commercial aquaculture all over the world. Aquaculture production should increase five folds during the next five decades to cope with increasing population, which is not an impossible goal if aquaculture shift to intensive or super intensive practices (FAO, 2016). Application of new technologies that increase efficiency of production and avoids competition resources are often required (Naglaa, 2017). This intensification has created an increasing demand for artificial feed. However, some ingredient in artificial feed had anti nutrients which retard fish growth performance. Plentiful variety of certain compounds has been broadly applied worldwide with reasonable success (Yang et al, 2015). Addition of L-carnitine with these ingredients can enhance the nutritive value for fish and feed costs are reduced (El-Sayed et al, 2010). The multi-physiological, bioactive and pollution free additive L-carnitine known as growth enhancer and powerful attractant for crustaceans and fish (Harpaz, 2005) through increased feed intake and/or better conversion of feed (Mohseni et al, 2008). L-carnitine is synthesized from essential amino acids (lysine and methionine) via the assistance of vitamin C and other secondary body compounds (Harpaz, 2005). It has been evaluated in several fish species with conflicting results (Ma et al, 2008). Improved growth performance and feed conversion ratios is the result of L-carnitine supplementation in hybrid tilapia (Oreochromis
niloticus × Oreochromis aureus), Becker et al. (1999) and Nile tilapia (Oreochromis niloticus) El-Sayed et al. (2010). In hybrid tilapia (O. niloticus×O. aureus), Schlechtriem et al. (2004) and common carp (Cyprinus carpio) Soltan et al. (2016) application of L-carnitine have not improve their growth. The mechanism explaining the growth promotion effects of L-carnitine supplemented to feeds is by increasing energy utilization resulting from increasing fatty acid oxidation (Becker et al, 1999). Consequently, it appears that the response of fish to L-carnitine as an additive is specific to the species. This response may be influenced by different dietary components (level of L-carnitine, culture conditions, fish age and quality of water), Li et al, (2017).

Feeding regime and its protein content is one of the factors affecting fish reproductive performance (Abdelhamid et al, 2003), feeding rate (Abou-Zied, 2006), and hatchery management (Abou-Zied and Ali, 2007). Other conditions of the environment may affect the photoperiod, water temperature (Tekle, 2004) and water depth (Salem et al, 2005). Therefore, to verify the effect of L-carnitine as a feed additive supplement on some reproductive performance parameters.

This study aimed to investigate the effects of treatments with graded levels of L-carnitine on reproductive performance and fry production performance, feed utilization and chemical body composition of Nile tilapia Oreochromis niloticus broodstock.

**MATERIALS AND METHODS**

**Fish and culture facilities**

An over-wintered Nile tilapia O. niloticus broodstock were obtained from a commercial tilapia farm located in Kafr El-Sheikh Governorate. Broodstock were netted from earthen ponds, manually selected, sexed and transferred to conditioning hapas, where they were held and kept separately for 20 days for acclimatization to the new environment until starting the experiment. A total number of 48 females and 24 males of Nile tilapia broodstock were counted, batch weight and stocked in each hapa at a rate of 6 (2♂: 4 ♀) fish/m³. Body weight was recorded at the beginning, subsequent spawning and at the end of experiment, which lasted for 105 days. Natural mating was practiced by placing the broodstock in six breeding hapas (2×2×1m³). Before mating, the breeders were conditioned and given better space and feed, to improve and increase sexual maturation. Every hapa was inspected once every week for seed. Seed out were recorded and representative sample swim-up fry from the third clutch were collected from each hapa and transferred to nursery hapas at a stocking density of 100 fry (One nursing hapa for each group of fry). Different water quality parameters were monitored.

**Test diets and feeding regime**

Commercial diet were formulated for tilapia broodstock containing ( 35 % CP and 4586 Kcal kg⁻¹ diet Gross Energy (GE) in pelleted form with graded level of feed additive L-Carnitine supplemented to the diets at three treatments (T₁: 0.0, T₂: 300 and T₃: 500 mgkg⁻¹, respectively). Broodstock fed the experimental diets at a feeding rate of 2% from the total broodstock biomass in each hapa daily (6 days/week) for 105 days. The feed was introduced to broodstock in spawning hapas at 9.00 am) six days per week with amounts adjusted at approximately 15 days interval in response to their new weight gain. For fry feeding in nursery experiment a commercial diet (40.50 % CP and 4897 Kcal kg⁻¹ diet (GE). The diet was in powder form and rate of feeding was 10% of the total fry biomass at the first 30 days started from the 2nd day of collecting fry from the broodstock (spawning) hapas and decreased to 5 % in the 2nd growth interval which extended for 30 days with a whole experimental period of 60 days. In the fry growth trial, feed was introduced to fry at a feeding frequency of 5 times daily with amounts adjusted every week interval in response to fry weight gain each nursing hapa.

**Body composition analysis**

At the end of the fry growth trial, fry in each hapa were netted, weighed and finally frozen for final body composition analysis. Representative samples of the experimental fish were randomly taken at the end of the experiments. Fish samples were killed and kept frozen (-18°C) until performing the body chemical analysis. All proximate analyses of fish were determined according to AOAC (2006).

**Spawning performance and seed output.**

Variables were estimated according to Mair et al. (2004) from the data included:-

Mean weight = (final weight for female brood stock (g)-initial weight of brood stock (g))/2

Total seed number= summation of seeds for four clutches.
Seeds/g female= Total seed number/mean weight (g).
Seeds/female/day= Total seed number for each female/days of the experiment (105 day).
Seeds/m³/day = total seed number for all females in m³/days of the experiment.

Calculations of fish performance for fry *O. niloticus*

The growth performance and feed utilization efficiency were calculated as following:

Weight gain (WG) = final weight (g) – initial weight (g).
Specific growth rate (SGR) % = 100 (ln W₂ – ln W₁)/T

Where W₁ and W₂ are the initial and final weight, respectively, In is represent Natural logarithm and T is the number of days in the feeding period.

Survival rate (SR) %= (No. of fish survived at the end of the experiment/ whole number of fish at the beginning) ×100.

Feed conversion ratio (FCR) = feed intake (g)/fish weight gain (g).
Protein efficiency ratio (PER) = weight gain (g)/protein intake (g).
Protein productive value (PPV) % = 100 [protein gain (g)/protein fed (g)].

Energy utilization (EU) % = Retained energy in carcass (Kcal)/energy intake (Kcal) ×100.

**Statistical analysis**

All data were subjected to one-way analysis of variance (ANOVA) at a 95% confidence limit, using SPSS software, version 16 (2007). Differences among means were tested using Duncan's Multiple Range (Duncan, 1955).

**RESULTS AND DISCUSSION**

The different water quality parameters were in tolerable ranges for Nile tilapia broodstock culture in all treatments which in parallel with the results of Abdelhamid (2009).

**Reproductive performance of broodstock**

The data of the present study (Table 1) showed that the highest mean weight for the female broodstock was recorded in treatment T₃ (161.14 g) with no significant difference with treatment T₂ (156.82 g), the lowest mean weight noticed at treatment with no supplementation T₁ (155.19 g). Concerning the total seed number, the results declared that the highest significant differences (p<0.05) was found at treatment T₃ (1664 seeds) compared with the other two treatments T₁ and T₂ (1428 and 1326 seeds) respectively with no significant difference (p<0.05) between them. The same trend was observed at all other reproductive performance parameters of female broodstock. The highest seeds/g female (10.28), seeds/female/day (16.64) and seeds/m³/day (66.57) were found in T₃. There were no significant differences between the other two treatments T₂ (9.11, 13.60 and 54.38) and T₁ (8.55, 12.63 and 50.52) in the same parameters although there were numerical differences between them.

**Table 1: Effect of dietary graded levels of L-Carnitine on seed production of female Nile tilapia (*Oreochromis niloticus* L.) broodstock.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean weight (g)</th>
<th>Total seed number</th>
<th>S/g female</th>
<th>Seed/female/day</th>
<th>Seeds/m³/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>155.19±1.94ᵃ</td>
<td>1326±1.85ᵇ</td>
<td>8.55±0.23ᵃ</td>
<td>12.63±0.18ᵇ</td>
<td>50.52±0.71ᵇ</td>
</tr>
<tr>
<td>T₂</td>
<td>156.82±1.19ᵇᵃ</td>
<td>1428±4.75ᵇ</td>
<td>9.11±0.24ᵇ</td>
<td>13.60±0.46ᵇ</td>
<td>54.38±1.81ᵇ</td>
</tr>
<tr>
<td>T₃</td>
<td>161.14±0.81ᵃ</td>
<td>1664±2.52ᵃ</td>
<td>10.28±0.21ᵃ</td>
<td>16.64±0.25ᵃ</td>
<td>66.57±1.01ᵃ</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at P<0.05.
Where (T₁: 0.0, T₂: 300 and T₃: 500 mgkg⁻¹, respectively).
Mean weight= (final weight for female brood stock-initial weight of brood stock)/2
Total seed number = summation of seeds for four clutches.
Seeds/g female= Total seed number for each female/mean weight (g).
Seeds/female/day= Total seed number/days of the experiment (105 day).
Seeds/m³/day = total seed number for all females in m³/days of the experiment.
L-carnitine is greatly associated with lipid metabolism due to fatty acid oxidation. It is an essential cofactor by transporting long and medium fatty acid to fuel in mitochondria. So, energy utilization is expected to be improved when fish is administrated on L-carnitine. The present study deals with investigation of the response of Nile tilapia broodstock to two levels of carnitine (300 and 500mg kg\(^{-1}\)) supplemented to their diets. The results clearly revealed that the seeds production and (seeds/m\(^3\)/day) were improved for female broodstock after supplemented with L-carnitine for 105 day, which in agreement with the studies of Izquierdo et al. (2001) who found that there was greatly improvement in seeds production when broodstock nutrition and feeding have been improved. In this connection (Dzinkowski et al. 2001) found that supplementation of carnitine to fish diets improved the reproduction performance via increasing total number of offspring, promoting spermatogenesis and enhancing the survival rate of larvae/fry. Like female guppies, Poecilia reticulate which recorded an enhancement in numbers of spawning and offspring of females when their diets with L-carnitine. Another study found that enhanced performance of reproduction have been evaluated via speeding up smolt stage and promoting spermatogenesis, also increasing the number of offspring's as clearly found in (Abdelhamid et al, 2010) who found improvement in reproductive performance of Nile tilapia broodstock when using 700 mg kg\(^{-1}\)diet of L-carnitine for males and females which be economically.

Since, the industry of fish farming focused on the quality of eggs and larvae more than sperm, however, the quality of both gametes can affect fertilization and larval survival. Fertilization success depends mainly on motility of sperms (Rurangwa et al, 2004). In this connection the substrate of the spermatozoa is the energetic compound (L-carnitine). It influences directly the maturation of the sperm. The mechanism of that is via lowers triglyceride increases the high density of lipoprotein when supplemented by L-carnitine (Chatzifotis et al, 1995). Parallel with our results Teklea (2004) found that L-carnitine show significant difference in the Gonado somatic index in Oreochromis mossambicus suggest that there is a possibility of improving fish production performance when it fed on L-carnitine as a supplement.

Growth performance, feed intake and feed utilization parameters of Nile tilapia fry:
Average values of initial and final body weight (IW, FW), weight gain (WG), specific growth rate (SGR) and survival rate (SR) of Nile tilapia fry produced from broodstock fed different levels of feed additive L-carnitine are given in Table (2). The data showed that initial weight was nearly similar in all treatment groups with no significant differences (P<0.05). Concerning with final body weight, body weight gain, specific growth rate and survival rate the results declared that there were significant difference among the T\(_2\) and T\(_3\) for FW (12.80 and 12.30 g); WG (12.69 and 12.19g); SGR (7.85 and 7.87); SR (97 and 99%) and the T\(_1\) with no supplementation in FW, WG, SGR and SR (10.54g, 10.42g, 7.39 and 93%) respectively. Feed intake , protein and energy utilization parameters expressed as feed intake (FI) , feed conversion ratio (FCR), protein efficiency ratio (PER) , protein productive value (PPV%) and energy utilization (EU%) were illustrated in Table (3). Data revealed that no significant differences (P<0.05) were found among different treatments in feed intake and different feed utilization parameters also there were numerical differences among treatments except in energy utilization. The data showed that the highest EU was recorded in T\(_2\) (22.55%) compared with the two other treatments T\(_1\) and T\(_3\) (18.06 and 19.22%) respectively with no significant difference between them. The maternal effect of Nile tilapia broodstock after feeding L-carnitine for 105 days has been investigated in the present study on the offsprings performance and utilization of feed. The data indicated that growth of fish increased and utilization of energy improved in treatments supplemented with Carnitine (T\(_2\) and T\(_3\)) than the group which not supplemented with L-carnitine (T\(_1\)). In a similar studies on Mozambique tilapia (Oreochromis mossambicus), Jayaprakas et al. (1996), hybrid tilapia (Oreochromis niloticus × O. aureus) Becker et al. (1999), the results indicated that dietary L-carnitine improved growth rates and efficiency of feed. Similar data reported with other species grown on diets supplemented with L-carnitine, involving African catfish (Clarias gariepinus), Ozorio et al. (2001a,b) and beluga Huso huso L. (Linnaeus, 1758), Mohseni and Ozorio (2014).
Soltan et al. (2015) found that L-carnitine improve survival rate during overwintering period for Nile tilapia.

These improvements in growth performance and survival rate due to L-carnitine feeding may be attributed to more efficient utilization of energy from fatty acids, Ji et al. (1996) found that dietary L-carnitine stimulating fatty acid oxidation and alter intermediates of metabolism in Atlantic salmon. The results revealed an induction of pyruvate carboxylase and synthesis of protein as the mechanism for L-carnitine-induced changes in nitrogen metabolism and gluconeogenesis. In contrast with our results, on Common carp (Cyprinus carpio) Soltan and Abou Zead (2016) and Hybrid tilapia (O. niloticus × O. aureas), Schlechtriem et al. (2004). They found no improvement in growth performance or feed utilization when fish fed diets supplemented with L-carnitine. These results may be due to increased feed consumption and not through improved feed utilization as there were no significant differences among treatments in utilization parameters. The cause is a small quantity of uneaten food remained in the course of feeding and the higher protein content of fish in the diet were supposed to be the two main factors resulting in relatively lower feed efficiency and protein efficiency ratio (Ma et al., 2008).

Effects on body composition

Concerning to body composition in (Table 4), the statistical analysis of these data showed no significant difference (p<0.05) among all treatments in (Dry matter (DM), Ether extract (EE), Crude protein (CP) and Ash. Although, there were numerically difference between different treatments but no significant differences were reordered. Several investigators indicated that fish feed on diets supplemented with L-carnitine at different levels showed no significant differences on the body composition in Juvenile black Sea bream (Sparus macrocephalus), Ma et al. (2008). With low concentration of L-carnitine Twibell and Brown (2000) reported that no improve in body composition in hybrid Striped bass (Marone chrysops × M. saxantilis). Gaylord and Gatlin (2000 a, b) experimented on the same species; they found that dietary lipid affects growth performance, liver lipid level and body condition, but not L-carnitine. Moreover, Beacker and Focken (1995) proved that tilapia (O.niloticus × O.aureus) feed with L-carnitine have no significant changes on the whole body composition for lipids and protein.

On the contrary with pervious results in another study by El-Sayed et al. (2010) found enhancement in protein sparing efficiency and also lower the body lipid content. Suggesting that carnitine enhanced energy utilization in Nile tilapia fingerlings via enhancement of oxidation of fatty acids, consequently sparing dietary protein for somatic growth, which in agreement with the results of (Ozorio et al, 2001b) in African catfish. Jayaprakas et al. (1996) investigated that 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. From aforementioned studies, among various studies, it is difficult to compare effects of dietary L-carnitine on the growth performance and metabolism of lipid because the beneficial effect differ among species, developmental stage, diet composition and environmental condition (Harpaz, 2005) quantity, duration and route of carnitine delivery.

Table 2: Growth performance parameters of Nile tilapia fry fed the tested diet.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IBW(g)</th>
<th>FBW(g)</th>
<th>WG(g)</th>
<th>SGR</th>
<th>SR%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0.125±0.01</td>
<td>10.54±0.04</td>
<td>10.42±0.29</td>
<td>39.06±0.06</td>
<td>93.5±0.50b</td>
</tr>
<tr>
<td>T2</td>
<td>0.115±0.01</td>
<td>12.80±0.20a</td>
<td>12.69±0.20a</td>
<td>85.04±0.04a</td>
<td>97.50±0.50a</td>
</tr>
<tr>
<td>T3</td>
<td>0.11±0.01</td>
<td>12.30±0.15a</td>
<td>12.19±0.14a</td>
<td>87.01±0.13a</td>
<td>99.00±0.00a</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at P<0.05.

IBW, Initial body weight; FBW, Final body weight; SGR, Specific growth rate and SR, Survival rate.

Weight gain (WG) = Final weight (g) – initial weight (g).

Specific growth rate (SGR) = 100 (ln Wt – ln W1)/T

Survival rate (SR) % = (No of fish survived at the end of the experiment/ whole number of fish at the beginning) ×100
Table 3: Feed intake and Feed utilization parameters of Nile tilapia fry fed tested diets.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed intake (g)</th>
<th>FCR</th>
<th>PER</th>
<th>PPV%</th>
<th>EU%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>15.59±0.28</td>
<td>1.46±0.01</td>
<td>1.69±0.02</td>
<td>23.60±0.10</td>
<td>18.06±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15.82±0.27</td>
<td>1.26±0.05</td>
<td>1.97±0.08</td>
<td>28.28±0.15</td>
<td>22.55±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>15.74±0.38</td>
<td>1.29±0.05</td>
<td>1.91±0.09</td>
<td>24.28±1.68</td>
<td>19.22±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at P<0.05.

Feed conversion ratio (FCR) = feed intake (g)/fish weight gain (g).
Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)
Protein productive value (PPV) % = 100 (protein gain (g)/protein fed (g)).
Energy Utilization (EU) % = Retained energy in carcass (Kcal)/energy intake (Kcal)×100.

Table 4: Body composition (%) on dry matter basis of Nile tilapia fed the tested diets.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DM</th>
<th>Crude Protein</th>
<th>Ether Extract</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>24.64±0.71</td>
<td>57.95±0.60</td>
<td>22.13±1.22</td>
<td>18.75±0.75</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>25.15±1.2</td>
<td>57.20±0.60</td>
<td>24.19±1.4</td>
<td>19.50±1.03</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>25.43±0.75</td>
<td>54.38±3.1</td>
<td>22.62±0.01</td>
<td>19.75±0.25</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at P<0.05.

CONCLUSION
Significant improvements in seed production for female Nile tilapia have been indicated in the present study, when diets supplemented with 500 mg kg<sup>-1</sup> L-carnitine. Also data revealed a positive effect in case of inclusion of L-Carnitine in tilapia broodstock diets and have positive maternal effect on growth performance, energy utilization with no significant effect on body chemical composition of Nile tilapia fry, consequently suggesting to a beneficial effect we must supplemented the diets of fry with L-carnitine in range from 300 to 500 mg kg<sup>-1</sup> diet.

CONFLICT OF INTEREST
The present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
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AUTHOR CONTRIBUTIONS
Dr. Hanan: participated in designed the experiments, Laboratory analysis, statistical analysis and wrote the manuscript of the paper.
Dr Al-Azab: participated in practical work in field experiment. All authors read and approved the final version.

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