Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(3): 589 – 598 (2019)
www.ejabf.journals.ekb.eg

Role of dietary L-carnitine supplements on improving of reproductive performance of Nile tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article History:
Received: Aug. 21, 2019
Accepted: Sept. 18, 2019
Online: Sept. 20, 2019

Keywords:
L-carnitine
reproductive performance
growth performance
Nile tilapia
O. niloticus

ABSTRACT

Studies on the effect of dietary L-carnitine supplements on reproductive performance for *Oreochromis niloticus* are scarce. A Seven months feeding trial was undertaken to examine four L-carnitine levels (control, 300, 600 and 900 mg kg 1 diet) on the reproductive performance for *Oreochromis niloticus* females and their fries during whole hatching season. A 120 females for were randomly distributed into 4 ponds (30 females per pond) represent four treatments and 4 ponds for the fry produced. During of this study, the reproductive traits for females and growth performance for fries were recorded. It was found that the dietary L-carnitine supplementation at all levels (300, 600 and 900 $mg^{-1}kg$) significantly ($P<0.01$) increased all reproductive performance parameters (females weight (FW), total egg weight per female (EW/F), egg weight (g) per gm of body weight (EW/GF), Absolute and relative fecundity (ABS) and (REL), The ratio of number of eggs / one gram of egg (NE/GE), Hatchability percentage (Hat%), Fry number per fish (FN/F) and Fry number per gram of female body weight (FN/GF)), also, increased all growth performance parameters for fries produced (body weight, daily weight gain and specific growth rate). and improved feed conversion ratio (FCR) and protein efficiency ratio (PER).

INTRODUCTION

One of the basic requirements of intensive farming of any fish species is a constant supply of good quality for eggs and fry from females. Commercial growers should be able to program the timing and the magnitude of spawning to fit with their hatchery and grow out fish requirements (Hebisha and Fathi, 2014).

The reproductive traits of Nile tilapia include many aspects like absolute fecundity, relative fecundity, gonads weight, egg weight, Ganado-somatic index (GSI), and fry performance (Bhujel, 2000; Gómez-Márquez *et al.*, 2003; Mohamed *et al.*, 2014).

As there are wide contrasts in the reproductive performance among species and individuals within the species (Kirpichnikov, 1981; Macaranas *et al.*, 1997), many studies had been conducted to investigate different factors involved in tilapia reproductive performance. Some studies reported different relationships between females' weight and absolute fecundity (Kariman *et al.*, 2008); relative fecundity

(Farag, 2003); ovaries weight; GSI (Mohamed *et al.*, 2014) and egg weight (Fath El-Bab *et al.*, 2011). Moreover, other studies reported some effects of female size on fry production performance of *Oreochromis* (Bhujel, 2000; El-Saidy and Gaber, 2005 and Mohamed *et al.*, 2014).

L-carnitine (4-N-trimethylammonium-3-hydroxybutyric acid) is one of the multi-physiological, bioactive and pollution-free additives known to work as a growth, reproduction and hatching rate improver as a powerful cute for fish and crustaceans through better feed conversion and/or increased feed intake (Mohseni *et al.*, 2008). L-carnitine is synthesized from two of the essential amino acids lysine (peptide-bound) and methionine with the assistance of ascorbic acid (vitamin c) and other secondary compounds produced in the body (Harpaz, 2005). as well, during lipid catabolism, L-carnitine is required for the transfer of medium and long-chain fatty acids from the cytosol to the mitochondria for energy production (Ozorio *et al*., 2002). inasmuch L-carnitine increasing lipids oxidation, it perhaps permits the use of high-fat diets by reducing the lipid accumulation in tissues. according to that, improvement in growth rates should be observed. Over the last years, evidence has been provided both to favor (Mohseni and Ozorio 2014). and reject this hypothesis (Rodehutscord, 1995; Ji *et al*., 1996). The improved energy production in mitochondria through â-oxidation of fatty acids may proposed that exogenous administration of L-carnitine could enhance the growth and reproductive traits of fish by improving energy utilization efficiency from lipid oxidation (Chatzifotis *et al.*, 1995). L-carnitine could catalyze some specific cell roles and may impact several biochemical and physiological process during reproduction periods (Chatzifotis *et al.*, 1995). as well, supplementation of dietary L-carnitine significantly improved survival rates and reproduction of Nile tilapia during overwintering period (Soltan *et al.*, 2015).

This study aimed to investigate the effects of L-carnitine different levels on different reproductive performance aspects and fry performance of Nile tilapia in order for determine the optimum breeding stocks management that may improve hatcheries production for the commercial scale.

MATERIALS AND METHODS

Location:

The present study was carried out on groups (males and females) of Nile tilapia (*Oreochromis niloticus*) at the Fish Hatchery of Al-Manzalah Integrated fish Farm, General Authority for Fish Resources Development, Ministry of Agriculture. Al-Manzalah Integrated fish Farm located in southern of Al-Manzalah city, Dakahlia governorate.

Experimental design:

Ten concrete ponds in this experiment ($2.5\times10\times1m^3$) were divided into four ponds for the four different treatments control, 300, 600 and 900 mg L- carnitine and two ponds for the replacement of females after the hatching and four ponds for their fry produced. The females and males were stocked in these tanks (30 females and 10 males 3:1, respectively per pond). Each pond was filled with 17.5 m^3 water from Bahr Hadous drain.

Preparation of diets and feeding practices:

The experimental fish were conducted to 4 treatment according to rate of L-Carnitine in diets (control, 300, 600 and 900mg L-carnitine) during hatching season. The fish were fed commercial floating diet to keep the diets available for fish contain

and about 3200 Kcal / Kg metabolizable energy (ME) for males and females (pellets 3 mm in diameter) at a daily rate of 5% of total biomass for 6 days / week twice daily at 9.00 am and 3.00 pm (Table 1).

Table 1: Composition of groups diets used during the experimental period.

Feed ingredients	Experimental diets			
	Diet1	Diet2	Diet3	Diet4
Fish meal (72%)	14	14	14	14
Soybean meal (44%)	40	40	40	40
Yellow corn	25.50	25.20	24.9	24.60
L-carnitine	0	0.30	0.60	0.90
Rice bran	15	15	15	15
Fish oil	2.5	2.5	2.5	2.5
Vit. & Min. mixture1	3	3	3	3
Sum	100	100	100	100
Proximate analysis (dry matter basis)				
Crude protein (CP)	31.11	31.1205	31.096	31.0715
Ether extract (EE)	8.09	7.92	7.86	8.83
Crude fiber (CF)	10.19	9.98	10.26	10.17
Ash	11.16	11.08	11.05	11
Digestible energy (Kcal/kg)	3216	3192	3181	3175

The larvae produced were abandoned to complete absorption of yolk-sac. After 2 days from hatching, the fries were stocked in the ponds corresponding to weight of females (each pond $3 \times 10 \times 1m^3$). And the fry granules diet 40 % crude protein and about 2900 Kcal / Kg (ME) (Table 2) was and offered at a daily rate 20% of total biomass put in dishes of floating (three times 9.00 am, 1.00 pm and 5.00 pm) for 30 days. Every 10 days, fry groups were randomly obtained from each pond then weighted and amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Table 2: Composition of fry diets used during the experimental period.

Feed ingredients	Experimental diets			
	Diet1	Diet2	Diet3	Diet4
Fish meal (72%)	26	26	26	26
Yellow corn	13.5	13.20	12.90	12.60
Soybean meal (44%)	40	40	40	40
L-carnitine	0	0.30	0.60	0.90
Rice bran	15	15	15	15
Fish oil	2.5	2.5	2.5	2.5
Vit. & Min. mixture1	3	3	3	3
Sum	100	100	100	100
Proximate analysis (dry matter basis)				
Crude protein (CP)	38.59	38.56	38.54	38.51
Ether extract (EE)	7.64	7.71	7.68	7.73
Crude fiber (CF)	9.89	9.82	9.81	9.75
Ash	10.87	10.68	10.62	10.63
Digestible energy (Kcal/kg)	2966	2948	2931	2922
Each 40g contains				
* vit A 200000 I. U.	vit D3 30000 I. U	vit E 250 mg	vit K3 50 mg	
vit B1 15 mg	vit B2 12mg	vit B12 250 mg	Niacin 15 mg	
Zn 1800 mg	Folic Acid 2 mg	vit B6 20 mg		
** Fe 1200mg	Bantothonic 80 mg	Mn. 2400 mg	Copper 200 mg	
Biotin 100 mg	Selenium 10 mg	Sodium 100 mg	Phosphorus 1000 mg	

The fish were grouped into four diets with L-carnitine (Arab Company for Pharmaceutical & Medical Plants-MEPACO- Egypt). The basal diet divided into four parts and L-carnitine and its precursors were added to formulate 4 diets as follow:
Group (1): control group (without supplementation of i.e. 0 L-carnitine).
Group (2): was supplemented with 300 mg L-carnitine.kg basal diet.
Group (3): was supplemented with 600 mg L-carnitine.kg basal diet.
Group (4): was supplemented with 900 mg L-carnitine.kg basal diet.

Eggs striping:

At Middle of April month when water temperature reached 28 ± 2ºC the fish were striping their eggs. The eggs produced were collected by decreasing the water column of each pond about 50 cm. after that, *O. niloticus* females were transported via small scoop into small plate containing water. As a consequence, to the fright stress, each mother threw its eggs into the plate. Then, the eggs of each female were collected, and their measurements were recorded. The eggs were collected from the mouth of females after fertilization and weighted and counted and then were put in jars (one jar per female) until hatching and allocated in its ponds of fry until end of treatment (30 days). The eggs were hatched using the available water system. The eggs production was repeated and continued throughout the hatching season.

Records maintained:

The measurements used in this study were females weight (FW), average of egg weight (EW/F) spawned per female of Nile tilapia was determined by gram. Number of eggs per gram eggs weight (NE/G) was determined by weighting one gram of eggs then all eggs presented in this gram weight were counted. Weight of eggs in gram per kg live body weight was calculated by dividing the weight of eggs spawned per female on its live body weight.

The absolute and relative fecundity was determined according to Bhujel (2000) as follows:

Absolute fecundity (ABS) = total weight of eggs per female (g) ×number of eggs in one gram.
Relative fecundity (REL) = absolute fecundity / body weight (g).
Fertilizability was determined by counting the number of fertile eggs in sample as a percentage of the total number in the same sample.

$$\text{Hatchability (HAT)} = \frac{\text{Number of fry obtained per female} \times 100}{\text{Total number of fertilized eggs spawned per female}}$$

Growth performance parameters *O. niloticus* fry:

Growth performance:

Records of live body weight (g) of fries were measured about 100 fry for each pond and registered every 14 days (two weeks) during the experimental period. Growth performance parameters were measured by using the following equations:

Specific Growth Rate (SGR):

$$\mathbf{SGR} = \frac{\text{LnW2} - \text{LnW1}}{\text{t}} \text{ x } 100$$

Where: -
Ln = the natural log
W1 = first fish weight
W2 = the following fish weight in “grams” and
t = period in days.

Daily Weight Gain (DWG):

$$DWG = \frac{\text{Total weight gain}}{\text{Period (day)}}$$

Where :

Total weight gain = final weight (g) – initial weight (g)

Feed Efficiency Parameters:

Feed Conversion Ratio (FCR):

A lower value indicates an improved outcome. Feed conversion ratio was calculated by the equation:

FCR = Feed intake (g)/Weight gain (g)

Protein Efficiency Ratio (PER):

PER is probably the most widely method used for valuating protein quality in fish. PER was measured by the following equation:

PER = Weight gain (g)/Protein intake (g).

Statistical analysis:

The statistical analysis of date was carried out by applying the computer program, **sas (1996)** by adopting the following model: -

$\mathbf{Y_{ijk} = \mu + R_i + E_{ik}}$

Where:

Y_{ik} = the K-th observation of the i-th treatment.

μ = overall mean

R_i = the effect of j-th treatment

E_{ik} = random error assumed to be independently randomly distributed (0, δ^2 e)

Differences among means were tested for significance according to Duncan's multiple range test (1955).

RESULTS AND DISCUSSION

Reproductive performance for *O. niloticus* females:

The results were indicated in table 3 for female weight (g) and total egg weight (g) per female (EW/F) as affected by L-carnitine different levels on hatching are presented in Table (3). The averages of FW were 285.56, 290.34, 293.58 and 287,49g and EW/F were 6.75, 7.67, 8.17 and 7.80 g for the four treatments control, 300, 600 and 900 mg L-carnitine per Kg diet, respectively.

These results indicate that, no significant differences recorded by effect of L-carnitine levels on body weight of females. while, EW/F for 600 mg /kg diet was higher than that obtained in control, 300 and 900 mg L- carnitine per kg diet. The differences between the means of each of EW/F were non-significant (Table 3).

Table 3: Effect of dietary L-carnitine supplements on reproductive performance for Nile tilapia.

Variable	No.	control	D1	D2	D3
(FW)	30	285.55±3.19	290.34±2.36	293.53±4.75	287.50±3.46
EW/F	30	6.75±0.21b	7.67±0.23b	8.17±0.22a	7.80±4.11b
Abs	30	532.70±16.62b	669.04±21.84a	706.99±20.97a	659.43±16.69a
Rel	30	1.83±0.065c	2.46±0.1b	2.53±0.091a	2.33±0.057b
NE/GF	30	1.83±0.065c	2.46±0.1b	2.54±0.091a	2.33±0.55b
Hat	30	73.72±1.54b	78.56±1.70a	78.77±1.82a	76.69±1.81a
FN/F	30	349.57±2.04bd	498.12±2.8b	521.41±3.90a	486.35±3.05c
FN/G	30	1.20±0.07c	1.85±0.050a	1.82±0.12a	1.72±0.16b

+ Means with the same letter in each row are not significantly differences ($P < 0.05$).

These results are agreement with Soltan *et al.*, (2016) found that, Dietary L-carnitine supplementation caused a significant increase in body weight of common carp indicating a higher utilization of the dietary energy and protein for growth. A growth-promoting effect of supplemental dietary L-carnitine has been reported in all L-carnitine different levels (300, 600 and 900 mg kg^{-1}) and have generally been explained by increasing utilization of dietary energy resulting from increased in fatty acids oxidation (Becker *et al.*,1999).

Results presented in Table (3) shows that, averages of absolute fecundity (ABS) as affected by the L-carnitine different levels were 532.71, 669.24, 706.99 and 659.44 for the four levels control, 300, 600 and 900 mg L-carnitine/ kg diet, respectively, and the differences between means were significant ($p<0.001$) and the same trend was also observed for relative fecundity (REL), where the averages were 1.83, 2.46, 2.53 and 2.33 for the four different levels, respectively.

Wahbi and Sangak (2017) found that, the incorporation of supplemented Spirulina diets in tilapia feed favorably influenced, fecundity, GSI, fry production and survival. Spirulina contain significant quantities of protein, lipids and fatty acids which are the main constituents of egg yolk. Also, its essential fatty acids content provide energy for spawning activities. the lipids, essential fatty acids, proteins, vitamins and food additives of fish diet influenced rate of absolute and relative fecundity.

Averages of hatchability percentage as affected by L-carnitine different levels were 73.72, 78.56, 78.77 and 76.69% for the four levels control, 300, 600 and 900mg L-carnitine, respectively. Guroy *et al*., (2012), James *et al.*, (2006), James *et al*., (2009), Vasudhevan and James (2011) and Meng-Umphan (2009) have reported that, Spirulina as food additives enhanced hatchability% in yellow tail cichlid, *Pseudotropheus acei*, in goldfish, *Carassius auratus*, in swordtail, Xiphophorus helleri and bassa fish, *Pangasius bocourti*, respectively.

Table (3) shows that, the average fry number per fish (FN/F) as affected by L-carnitine different levels and Number of hatching times are listed in Table (3). As shown in Table (3), the averages fry number per fish (FN/F) as affected by L-carnitine different levels were 349.57, 498.12, 521.41 and 486.35 fry/fish for the four levels control, 300, 600, and 900mg L-carnitine per kg diet, respectively, and the differences between these averages were significant ($P<0.001$). This indicated that, the fry number produced per fish for diet contained 600 mg L-carnitine/kg diet was better than those of other treatments. Soltan *et al.*, (2012) reported that, Dietary protein and L-carnitine has been found to influence seed production in tilapia. An increase in egg production with an increase in L-carnitine levels has been reported for Nile tilapia, *O. niloticus* (Santiago, *et al*., 1985) and Taiwanese red tilapia (Chang *et al*., 1988).

The averages of fry number per gram fish (FN/GF) as affected by L-carnitine different levels and Number of hatching times are presented in Table (3). As shown in table (3) the averages (FN/GF) were 1.20, 1.85, 1.82 and 1.72 for the four levels control, 300, 600 and 900 mg /kg diet, respectively, and the differences between these averages were significant ($P<0.001$). In a study carried out by Wahbi and Sangak (2017) found that, the numbers of fries produced were greater in the Spirulina-fed fish could be attributed to the fatty acids contain of Spirulina. Santiago and Reyes (1993) also stated the important of n-6 fatty acids group specially ARA acid in improving spawning efficiency and fry morphometric characteristics.

Growth performance for fry.

As shown in Table (4), the effect of L-carnitine different levels after 2 days the averages of fry body weight were 0.0251, 0.0274, 0.0257 and 0.0265g for 4 L-carnitine different levels. While, the averages of body weight of fry after 30 days were 0.389, 0.406, 0.407 and 0.401g, respectively, and the differences between averages were significant (P<0.001) (Tables 4). These results indicated that, the body weight affected by L-carnitine, this may be due to increased lipid oxidation in fry body weight or the growth performance affected by diets with dietary L-carnitine supplements this is probably due to influenced by other factors in addition to diet composition, such as species differences, developmental stage and husbandry conditions.

Wahbi and Sangak (2017) found that, the weight and length of produced fries were greater in the Spirulina-fed fish could be attributed to the fatty acids contain of Spirulina.

Table 4: Effect of dietary L-carnitine supplements on growth performance for Nile tilapia fry.

Variable	No.	control	D1	D2	D3
Initial weight		0.0251±0.001	0.0274±0.001	0.0257±0.001	0.0265±0.001
Final weight	30	0.389±0.001c	0.406±0.002a	0.407±0.001a	0.401±0.001b
DWG (2-30 D)	30	0.0129±0.0001b	0.0136±0.0001a	0.0137±0.0001a	0.0133±0.0001a
SGR (2-30 D)	30	9.75±0.31b	9.96±0.02a	9.98±0.24a	9.95±0.31a
FCR (2-30 D)	30	1.27±0.003c	1.29±0.003b	1.31±0.03a	1.31±0.03a
PER (2-30 D)	30	0.12±0.001b	0.14±0.01a	0.14±0.01a	0.14±0.012a

+ Means with the same letter in each row are not significantly differences (P < 0.05).

Results of Daily Weight Gain for fry (DWG) for Nile tilapia fry as affected by L-carnitine different levels during the whole experimental period are illustrated in (Table 4). Averages of DWG during the whole period 2-30 days (Table, 4) as affected by L-carnitine levels were 0.0129, 0.0136, 0.0137 and 0.0133g for the four treatments. Soltan *et al*., (2015) found that, the averages of DWG during the period 20-30 days were 0.025, 0.026, 0.027 and 0.027g for the different levels while the averages of DWG during the same period were 0.03, 0.03g for the all hatching times and the analysis of variance between averages were significant (P<0.05) for L-carnitine different levels.

As shown in Table (4), averages of SGR during the whole experimental period 2-30 days as affected by L-carnitine levels were 9.75, 9.96, 9.98 and 9.95 for the four treatments, control, 300, 600 and 900 mg L-carnitine, respectively.

Wahbi and Sangak (2017) stated that, although there were no detected differences in egg morphometric characters, the specific growth rate of fries from fish fed on the Spirulina supplemented diets was higher compared to control that may be attributed to high Spirulina content of essential amino acids, fatty acids and vitamins that influence the growth performance of fish.

As shown in Table (4), averages of FCR during the whole experimental period 2-30 days as affected by L-carnitine levels were 1.27, 1.29, 1.31 and 1.31 for the four L-carnitine levels control, 300, 600 and 900 mg L-carnitine, respectively. Vasudhevan and James (2011) reported that, L-carnitine as food additives enhanced Feed Conversion Ratio for fry produced in goldfish *Carassius auratus.*

As presented in Table (4), averages of PER during the whole experimental period 2-30 days as affected by the L-carnitine different levels were 0.12, 0.14, 0.14 and 0.14 for the four treatments control, 300, 600 and 900 mg L-carnitine, respectively. These results were indicated that, L-carnitine at levels 300, 600 and 900mg L-carnitine were improved for PER in Nile tilapia. Schlechtriem *et al*., (2004)

he found that, the diets contained the lower L-carnitine level (300 mg kg-1 diet) released the best growth and feed utilization indices PER which did not significantly different from the higher L-carnitine levels (600 and 900 mg kg-1 diet) and these results indicated that, dietary L-carnitine at the lower level (300 mg kg^{-1}) is effective for improving of growth and feed utilization of common carp.

CONCLUSION

Based on results obtained in this study, it could be concluded that, the L-carnitine at levels 300 and 600 mg L-carnitine were improved each of reproductive performance aspects and fry performance for Nile tilapia under optimum breeding stocks management that may improve hatcheries production for the commercial scale.

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