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A Preliminary Study on African Catfish (*Clarias gariepinus*) Larvae Fed with Diets Containing Different E/P Ratios and L-carnitine Supplementation

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Abstract: In this study, the effects of dietary energy/protein ratios and L-carnitine on growth and chemical composition of the whole body of African catfish (*Clarias gariepinus*) larvae, were investigated. Diets containing different E/P ratios were prepared by adding 0, 5, 10% (w/w) olive pomace oil to the trout starter diet (300-500 μm) and supplemented with L-carnitine at either 0 or 2 g kg^{-1}. A 3 (E/P ratio) x 2 (L-carnitine supplementation) factorial desing was used with three replicates. African catfish larvae were obtained by the artificial reproduction method and grown under laboratory conditions until 7 days old. 120 African catfish larvae (7 days old) were randomly stocked into each aquarium (80x40x40cm) and fed ad libitum three times a day for 18 days. Weight gain was significantly affected by only E/P ratios, but specific growth rates of larvae were influenced by both E/P ratios and L-carnitine supplementation ($p<0.05$). No statistical differences was observed with respect to survival rate of the larvae fed different diets ($p>0.05$). E/P ratios and carnitine supplementation had no significant effect on proximate composition of whole body of the African catfish larvae except protein contents. It was determined that total free fatty acid contents of the larvae fed the diets had the same E/P ratio with L-carnitine supplementation were lower than those of the larvae fed diets without L-carnitine. The current study has shown that the trout starter diet supplemented with 5% olive pomace oil and L-carnitine provided the best growth for African catfish larvae.

Key words: African catfish, larvae, olive pomace oil, L-carnitine, fatty acids

INTRODUCTION

Recently, the lipid contents of aquafeeds have significantly increased and different kinds of lipid sources were begun to be used in fish diets[1-2]. In most studies focussing on high lipid inclusion fish oil has been used; however, the demand for fish oil by the aquafeed industry has been predicted to exceed available sources in near future[3]. Also, vegetable oils obviously represents more sustainable sources in most countries. High energy diets have been considered beneficial for growth, feed utilization, protein sparing, effectively reducing nitrogen losses and therefore, facilitate more cheaper and environmental friendly production[4]. However, high lipid diets may cause some potential problems such as fat accumulation and oxidation in fish fillets[5]. To prevent these problems, use of L-carnitine with high lipid diets may be a useful tool as a transporter of long chain fatty acids into the mitochondria for â-oxidation[6-7]. To meet the increased L-carnitine requirement in high lipid diets, fish have either to synthesise it from the endegenous aminoacids lysine and methionine or obtain it from their diets[8]. L-carnitine has been used together with different dietary lipid sources in the various grow-out diets[9-13]. however, studies on growth and body composition of fish larvae fed with L-carnitine and lipid sources are still scarce. Therefore, in the present study, the effects of dietary olive pomace oil as a new energy source and L-carnitine with different Energy/Protein (E/P) ratios on the growth and chemical composition of the African catfish (*Clarias gariepinus)* larvae were investigated.

MATERIALS AND METHODS

Study was conducted at the Aquaria Unit of the Faculty of Fisheries at Mustafa Kemal University located in Hatay, Turkey. African catfish larvae were obtained by the artificial reproduction method described by Hogendoorn[14] and were grown until seven days old with artemia and trout starter diet.One hundred and twenty African catfish larvae were stocked into each aquarium (80x40x40 cm). Experimental diets (trout starter, 300-500 μm) containing 0, 5 and 10% (w/w) olive pomace oil supplemented with L-carnitine at either 0 or 2 g kg^{-1} were tested in a 3x2 factorial desing (triplicate aquaria per dietary treatment). Olive pomace oil was obtained from a local factory in Hatay, Turkey and L-carnitine from Germany (Schuchardt, 85662 Hohenbrunn, Germany).

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Table 1: Chemical composition of the experimental diets

	Diets					
	D_1	D_2	D_3	D_4	D_5	D_6
Carnitine Levels (g kg^{-1})	0	2	0	2	0	2
Lipid levels (%)	0	0	5	5	10	10
Dry matter	91.00	89.00	94.80	95.30	93.30	95.10
Lipid	14.34	14.34	17.90	17.90	23.09	23.09
Protein	56.00	56.0	53.20	53.20	50.40	50.40
Ash	10.51	9.80	10.34	10.91	9.53	10.18
NFE	9.00	8.86	12.36	12.29	9.28	10.43
Gross energy (kcal/100g)	487.90		519.20		544.68	
E/P ratio	8.71		9.76		10.75	

Experimental diets were prepared by spraying the olive pomace oil and L-carnitine on trout starter diet and then drying them in a shaded area (Table 1).

The larvae were fed ad *libitum* three times a day for 18 days. Aquaria were siphoned each day to remove feaces and uneaten feeds. Water quality parameters such as pH, oxygen (biweekly) and temperature (daily) were measured with a pH meter (Orion, Model: 420A) and an oxygenmeter (YSI, Model: 52).

Whole body lipid contents of the larvae were determined by choloroform/methanol extraction[15]. The whole body fatty acid compositions of the larvae were esterified according to Garces and Mancha[16]. Analyses of the fatty acid methylesters were carried out using GC-MS equipped with an SP-2330 fused capillary column (30 x 0.25 mm) at the labaratory of the Scientific and Technical Research Council of Turkey located in Kocaeli, Turkey using hydrogen as carrier gas and a temperature gradient programmed from 120 to 220 °C (5 °C min^{-1}). The temperature of the injector and of the detector was 240 and 250°C, respectively. Methylesters were identified by comparison with a known standard mixture of the fatty acids.

The statistical comparisons of the treatments were performed using a two-way ANOVA with a significance level of 0.05. SPSS statistical software(v9.0) was used to conduct all statistical analyses.

RESULTS

It was observed that the growth performance of African catfish larvae was primarily affected by the E/P ratios ($p<0.05$). The best weight gain was obtained from larvae fed with diets supplemented with 5% olive pomace oil and 2 g kg^{-1} L-carnitine. L-carnitine supplementation improved the growth; however, its effect was not significant ($p>0.05$). Both E/P ratio and L-carnitine supplementation changed the specific growth of the larvae ($p<0.05$). No statistical differences were observed in the survival rates of the experimental groups (Table 2). The whole body lipid content of the larvae, at the end of the study, increased compared to the initial content ($p<0.05$). Protein contents of the larvae tended to decrease with dietary carnitine supplementation ($p<0.05$). Dietary carnitine had no significant effect on the proximate composition of the whole body of the larvae except protein contents (Table 3). Total free fatty acid contents of the larvae decreased compared to the initial content. In addition, L-carnitine supplementation caused a reduction in the total free fatty acid contents for all groups. Although arachidonic acid (20:4n-6) was not detected at the begining of the study, it was found in the larvae only fed with diets without olive pomace oil at the end of the experiment (Table 4).

Water quality parameters for oxygen, pH and temperature were 5.7-6.1 mg L^{-1}, 7.7-8.2 and 26-27°C, respectively.

DISCUSSION

Although E/P ratios affected the growth significantly, L-carnitine supplementation with the same E/P ratios improved the growth performance of the African catfish larvae. Also, this growth-promoting effect of L-carnitine has been reported in several marine and fresh water fish species[11,12,17]. We found that the same growth effect of L-carnitine was also valid for the African catfish larvae as well as the juvenile and fingerling stages of fish[12-18]. L-carnitine supplementation with the increased E/P ratio improved the specific growth of the larvae. The current result of this study was also supported by Torreele *et al.*[18], who clearly reported a faster and more efficient growth with increased levels of dietary carnitine.

Decreased fatty acid contents with L-carnitine supplementation in the same E/P ratio indicated that L-carnitine is a good transporter of long chain fatty acids into the mitochondria for β-oxidation[6,7]. The reduction in the lipid contents of the larvae might be attributed to the decrease of the fatty acid contents for the larvae fed with diet containing olive pomace oil and L-carnitine. Similar to our findings, a decrease in the long chain fatty acid concentration in African catfish fed with high carnitine diets was observed by Ozorio[19]. It is interesting to note that arachidonic acid (20:4n-6) was not detected at the beginning of the experiment, but it was found in the larvae only fed with diets without olive pomace oil at the end of the study. This might be explained by the conversion of the fatty acids from 18 carbon atoms to 20-22 ones[20].

Also, the lipid lowering effect of L-carnitine was well documented by Rodehutscond[21] who used L-carnitine in

Table 2: Growth performance and survival data of African catfish (*Clarias gariepinus*)larvae fed diets at three energy/protein ratios with or without carnitinesupplementation for 18 days.

E/P ratio	Carnitine Level(g kg^{-1})	Initial Weight(mg)	Final Weight(mg)	Weight Gain (mg)	Specific Growth Rate	Survival r a t e (%)
8.71	0	26.19^{aA}	149.46^{aA}	123.27^{aA}	9.53^{aA}	82.22^{aA}
	2	26.45^{aA}	294.46^{aA}	268.00^{aA}	13.35^{aB}	84.44^{aA}
9.76	0	23.84^{aA}	348.03^{bA}	324.18^{bA}	14.84^{bA}	95.00^{aA}
	2	26.33^{aA}	454.90^{bA}	428.56^{bA}	15.71^{bB}	83.88^{aA}
10.75	0	29.05^{aA}	293.70^{bA}	264.64^{bA}	12.84^{cA}	90.00^{aA}
	2	27.83^{aA}	355.80^{bA}	327.96^{bA}	14.09^{cB}	92.22^{aA}
ANOVA(Pr>F)[6]						
E/P		0.21	0.03	0.03	0.02	0.19
Carnitine level		0.73	0.09	0.08	0.01	0.53
(E/P) x Carnitine level		0.60	0.61	0.61	0.19	0.23
Pooled s.e		0.99	22.37	22.16	0.44	0.94

a, b, c ; refers to difference between E/P ratios, A, B; refers to difference between carnitine levels, [1,2] Values are means of ten fish from each of three replicates groups, [3] Differences between initial and final body weight, [4] SGR=[ln final weight- ln initial weight]x100/time (days), [5] Values are refer to survival rates between treatmments, [6] Significance probability associated with the F statistic

Table 3: The proximate composition of African catfish, *Clarias gariepinus,* larvae fed diets at three energy/protein ratios with or without carnitine supplementation (%wet matter basis)

E/P ratio	Carnitine level (g kg^{-1})	Ash	Lipid	Protein
8.71	0	1.24^{aA}	4.67^{aA}	19.36^{bA}
	2	1.23^{aA}	5.32^{aA}	9.91^{bB}
9.76	0	1.59^{aA}	3.86^{aA}	21.01^{cA}
	2	1.87^{aA}	3.71^{aA}	18.26^{cB}
10.75	0	1.09^{aA}	6.10^{aA}	16.32^{bA}
	2	1.61^{aA}	4.71^{aA}	15.70^{bB}
Initial		2.05^{a}	3.19^{a}	11.06^{a}
ANOVA (Pr>F)				
E/P		0.104	0.121	0.000
Carnitine level		0.162	0.674	0.000
(E/P) x Carnitine leve		l0.465	0.498	0.002
Pooled s.e		0.123	0.719	0.404

Table 4: The fatty acid contents of African catfish *(Clarias gariepinus)* larvae fed at three energy/protein ratios with or without carnitine supplementation (wet matter basis; mg/100mg).

		E/P Ratio					
		8.71		9.76		10.75	
Fatty acids	Initial	0	2	0	2	0	2
C8:0	- 0.464	0.177	0.04	-	-	-	
C10:0	- 0.103	0.085	-	-	-	-	
C12:0	- 0.111	0.105	-	-	-	-	
C13:0	- 0.016	-	-	-	-	-	
C14:0	0.715	0.493	0.315	0.099	-	0.227	0.163
C15:0	0.456	0.133	0.052	0.086	-	-	0.104
C15:1	- 0.078	0.067	-	-	-	0.058	
C16:0	9.594	5.122	3.981	4.172	3.882	5.685	3.912
C16:1	0.751	0.423	0.252	0.031	-	0.066	0.156
C18:0	4.723	2.429	2.158	2.517	2.636	3.493	2.367
C18:1n9	5.736	2.426	2.102	2.999	1.827	5.883	3.661
C18:2n6	1.142	0.081	0.061	0.538	0.175	0.922	0.530
C18:2n6	4.45 2.429	1.221	1.187	1.07	2.466	1.28	
C18:3n6	- 0.02	-	-	-	-	-	
C18:3n3	0.511	0.252	0.165	-	-	-	0.036
C20:0	0.177	0.098	0.074	-	-	-	-
C20:1n9	1.306	0.916	0.708	0.82	-	0.566	0.288
C20:2	0.268	0.102	0.092	-	-	-	-
C20:3n3	- 0.1	0.074	-	-	-	-	
C20:4n6	- 0.405	0.248	-	-	-	-	
C20:5n3	2.706	1.295	0.961	1.079	0.994	1.267	0.876
C21:0	0.106	0.125	0.053	-	-	-	-
C22:0	0.364	0.071	0.024	-	-	-	0.053
C22:1n9	0.771	0.064	0.043	0.074	-	0.37	0.129
C22:2	1.12 0.616	0.325	0.219	-	0.282	0.186	
C22:6n3	7.604	3.247	2.65	2.774	1.86	2.779	2.333
C23:0	0.143	0.041	0.038	-	-	-	-
C24:0	- 0.056	0.022	-	-	-	-	
C24:1n9	0.888	0.748	0.528	0.264	0.542	0.143	0.192
∑ FA	43.531 16.324	22.464	16.581		16.899	12.986	2.149

(-) ; Not detected

rainbow trout diet with high-lipid level (26%). Similarly, Ji *et al.*[22] found that Atlantic salmon fed with a 10% lipid diet with carnitine resulted in a decrease in the lipid content compared to the diet without carnitine. In addition, similar effects of L-carnitine on the growth performance and whole body composition have been reported in salmonids and hybrid stripted bass (*Morone chrysops* x M. saxatilis)[21,23,24]. According to our findings, decreases in both lipid and free fatty acid contents without reducing growth have showed that L-carnitine promoted oxidation of the fatty acids to meet the energy requirement of the larvae. Although L-carnitine supplementation increased the oxidation of the free fatty acid to meet the energy requirement, it is very difficult to conclude that the protein degradation was reduced (increased protein-sparing action) due to the decreased protein content of whole body with L-carnitine supplementation. However, the present findings showed that the protein degradation in larvae fed with L-carnitine supplementation and 10% olive pomace oil was lower than that of the fish fed with L-carnitine supplementation and 5% olive pomace oil, supporting the decrease in whole body protein content.

In conclusion, the use of olive pomace oil (5%) could be used in the diets of the African catfish larvae to improve the growth performance. Present outcomes suggested that different inclusion levels of olive pomace oil and L-carnitine should be studied in both warm and cold fish diets to understand the mechanism between this lipid source and L-carnitine.

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