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EFFECT OF DIETARY OLIVE POMACE OIL AND L-CARNITINE ON GROWTH AND CHEMICAL COMPOSITION OF AFRICAN CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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Abstract

The effects of dietary olive pomace oil and L-carnitine on growth performance, body indices and chemical composition of African catfish, *Clarias gariepinus*, were investigated. A 3x2 factorial design was used by offering diets having three different energy/protein ratios and supplemental L-carnitine at either 0 or 2000 mg/kg diet. Ten juvenile African catfish (12 g/fish) were stocked into 80-l glass aquaria and fed an experimental diet for seven weeks. The L-carnitine supplementation did not affect body indices. However, growth and chemical composition were influenced by both dietary lipid and L-carnitine. The increase in muscle lipid was greater than in the liver for all treatments. Fish fed the high energy diet (9% olive pomace oil) had a low appetite and feed intake, while fish fed the low energy diet (3% olive pomace oil) and supplemental L-carnitine had better feed intake and growth. On the other hand, fish fed the high energy diet (9% olive pomace oil) with or without L-carnitine had better feed conversion.

Introduction

Lipid nutrition and its protein-sparing capability have been studied by many researchers on both cold and warmwater fishes (Hutchinson et al., 1998; Hardy, 1999). While, the carbohydrate and lipid utilizing capabilities of some species such as sunshine bass, *Morone chrysops x M. saxatilis* (Nematipour et al., 1992; Hutchinson et al., 1998); striped bass, *Morone saxatilis* (Rawles and Gatlin, 1998) have been studied and are well-known, studies on African catfish are still inadequate. The mechanism between L-carnitine and lipid

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sources is not completely understood except in some species such as tilapia, *Oreochromis niloticus x O. aureus* (Becker et al., 1999), red sea bream, *Pagrus major* (Chatzifotis et al., 1995) and sea bass, *Dicentrarchus labrax* (Santulli and D'Amelio, 1986).

L-carnitine, in general, is known as a methyl donor and for its lipotropic effect (Jayaprakas et al., 1996). L-carnitine has been used with different dietary lipid sources in various experiments (Gaylord and Gatlin, 2000a; Ronald and Paul, 2000). In the present study, olive pomace oil was tested as a new energy source for African catfish. Olive pomace oil has been used in the soap industry, but not in animal and fish feeds or human food.

Materials and Methods

Diets containing 0, 3% or 9% (w/w) olive pomace oil, supplemented with L-carnitine at either 0 or 2000 mg/kg were tested in a 3x2 factorial design in the Faculty of Fisheries Science Laboratories at the Mustafa Kemal University. The lipid source was chosen to either meet or exceed the energy needs of the fish. Diets were formulated by mixing locally available ingredients to contain different energy/protein ratios (Table 1). Experimental diets were prepared in the laboratory as follows. First, the dry ingredients were mixed and minced trash fish were added to the dry mixture. Then, the mixture was autoclaved at 121ºC for 15 min. The dough was passed through a 2 mm mesh mincer to form pellets. Olive pomace oil and L-carnitine were sprayed, respectively, on the pellets, which were then were dried in a shaded area. Olive pomace oil was bought from a local olive oil factory in Hatay, Turkey, and L-carnitine from Germany (Schuchardt, 85662 Hohenbrunn, Germany).

Juvenile African catfish were obtained by the artificial reproduction method described by Hogendoorn (1980) and were grown to 10-15 g body weight under laboratory conditions. Ten African catfish, initial mean body weight 12 g, were stocked into 18 aquaria (80 x 40 x 40 cm). The experiment included six feeding treatments in three replicates. The fish were fed *ad libitum* twice a day for seven weeks. Aquaria were siphoned each day to remove feces and uneaten feed. Water quality parameters such as pH, oxygen (biweekly) and temperature (daily) were measured with an Orion 420A pH meter and YSI Model 52 Oxygenmeter.

Three fish per aquarium were removed for chemical composition analysis and body index measurements such as the hepatosomatic index (HSI), intraperitoneal fat (IPF), the muscle (MR) and head (HR) rates. Muscle and liver samples were taken and esterified for fatty acid analysis according to Garces and Mancha (1993). Analysis of the fatty acid methylesters were carried out on GC-MS equipped with a SP-2330 fused capillary column (30 x 0.25 mm; The Scientific and Technical Research Council of Turkey) using hydrogen as the carrier gas and a temperature gradient programmed from 120°C to 220°C (5°C/min). The temperature of the injector and the detector was 240°C and 250°C, respectively. Methylesters were identified by comparison with a known standard mixture of fatty acids. Muscle and liver lipid contents were determined by chloroform/methanol extraction (Bligh and Dyer, 1959).

Comparisons were made using a one-way ANOVA test, differences were considered significant at $p<0.05$. SPSS statistical software was used for statistical analyses (SPSS, 1993).

Results

The growth of the African catfish was affected mainly by the energy/protein ratio (Table 2). The weight gain was greater for fish fed diets containing a low (0 olive pomace oil) or intermediate (3%) energy level than for those given the diet with the highest energy level (9%). The feed conversion and protein efficiency ratios of the fish fed 3% or 9% olive pomace oil were better than those of the low energy group (0). Neither the energy/protein ratio nor L-carnitine supplementation affected the body condition indices (hepatosomatic index, intraperitoneal fat, muscle and head rates; $p>0.05$). The proximate analyses of the

Table 1. Composition of the experimental diets (g/kg dry mixture).

	Diet					
Ingredients	*R1*	*R2*	*R3*	*R4*	*R5*	*R6*
Minced fish	115.6	115.2	115	114.6	113.8	113.4
Corn meal	163.3	162.9	162.7	162.3	161.5	161.1
Barley meal	136	135.6	135.4	135	134.2	133.8
Wheat bran	217.7	217.3	217.1	216.7	215.9	215.5
Cotton seed cake	350	349.6	349.4	349	348.2	347.8
Vitamin and mineral mixture*	17.4	17.4	17.4	17.4	17.4	17.4
Olive pomace oil	0	0	3	3	9	9
L-carnitine	0	2	0	2	0	2
Energy (kcal/100 g)	382		413		454	
Protein	30.02		29.15		27.54	
Energy/protein	12.72		14.16		16.48	

* Vitamin premix supplied the diets with (mg/kg or IU/kg dry diet): vitamin A, 1.000.000 IU; vitamin D_3 , 200.000 IU; vitamin E, 5.000 mg; vitamin B_1, 50 mg; vitamin B_2 , 200 mg; calciumpantotenate, 360 mg; vitamin K_3 , 100 mg; niacin, 500 mg; vitamin B, 10 mg; vitamin C, 100 mg. Mineral premix consisted of (mg/kg of premix): Fe, 5.000 mg; Mn, 5.000 mg; Cu, 1.000 mg; Co, 20 mg; Zn, 5.000 mg; I, 80 mg; Na, 120.000 mg; P, 43.000 mg, Ca, 132.537 mg, antioxidant (Endoxdry), 5.000 mg.

fish are given in Table 3. The increase in lipid level was greater in the muscle than in the liver in all treatments. Lipid levels in the muscle increased with the increase in dietary olive pomace oil but decreased in the liver. In contrast to the muscle, total free fatty acid levels increased in the liver with the increase in dietary olive pomace oil. Although arachidonic acid (20:4n-6) was not detected at the start of the experiment, it was found in both muscle and liver samples from all treatments at the end (Table 4).

Water quality parameters for oxygen, pH and temperature varied 5.65-6.01 mg/l, 7.8-8.2 and 26-27°C, respectively.

Discussion

Dietary carnitine did not alter the weight gain but acted as an attractant for the fish. In contrast to our results, Torrelee et al. (1993) found a positive relation between carnitine and weight gain. However, changes in lipid levels were similar with his results. According to Gaylord and Gatlin (2000b), if a limited precursor pool had been available in the diet, supplemental L-carnitine might have had more dramatic influences on the chemical composition of the gain.

In contrast to the liver, the total lipid level in the muscle increased with the increase in dietary lipid in all treatments. This might be

Table 2. Growth, FCR and PER of African catfish (*Clarias gariepinus)* fed diets with two carnitine levels and three energy/protein ratios.

	Carnitine level (mg/kg)	*Average[1] weight (g)*	*Weight[2] gain (g)*	*FCR[3]*	*PER[4]*
Energy/protein ratio					
12.72	0	26.35^{aA}	143.5^{aA}	2.71^{aA}	1.23^{aA}
	2000	25.90^{aA}	118.5^{aA}	2.98^{aB}	1.12^{aB}
14.16	0	28.10^{bA}	144^{aA}	2.46^{bA}	1.39^{bA}
	2000	26.85^{bA}	142.5^{aA}	2.47^{bB}	1.4^{bB}
16.48	0	23.85^{cA}	99.5^{bA}	2.29^{bA}	1.59^{bA}
	2000	22.35^{cA}	93.5^{bA}	2.65^{bB}	1.37^{bB}
ANOVA (Pr>F)[5]					
Energy/protein ratio (E/P)		0.001	0.008	0.002	0.001
Carnitine level		0.065	0.231	0.008	0.019
(E/P) x carnitine level		0.659	0.498	0.078	0.107
Pooled S.E.		0.259	4.451	0.093	0.061

a, b, c refer to differences between E/P ratios.
A, B, C refer to differences between carnitine levels.
[1] Values are means of ten fish from each of three replicates groups.
[2] Difference between initial and final body weight
[3] g dry diet fed/g weight gain
[4] g weight gain/g protein fed
[5] Significance probability associated with the F statistic.

Table 3. The proximate composition of African catfish, *Clarias gariepinus*, fed diets containing differing amounts of olive pomace oil and L-carnitine (%, wet matter basis).

		Diet (E/P level, L-carnitine)					
		12.72		*14.16*		*16.48*	
	Initial	*0*	*2000*	*0*	*2000*	*0*	*2000*
Dry matter	25.28	27.98	25.47	27.71	25.46	27.22	25.33
Ash	1.63	2.41	2.3	3.23	3.42	1.88	1.89
Lipid	5.23	2.19	3.67	2.93	7.85	4.88	5.61
Protein	20.32	14.75	15.84	15.33	16.25	14.43	15.69

Table 4. The fatty acid contents of the muscle (fillet) and liver of African catfish, *Clarias gariepinus*, fed diets containing differing amounts of oil and L-carnitine (wet matter basis; mg/100 mg).

			Diet (E/P level, L-carnitine)											
			12.72				*14.16*				*16.48*			
	Initial		*0*		*2000*		*0*		*2000*		*0*		*2000*	
Fatty acid	*Muscle*	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*
C8:0	0.75	0.5	0.08	0.26	0.3	0.26	0.13	0.1	0.32	0.5	0.21	0.7	0.43	0.8
C10:0	0.11	-	-	-	0.1	0.08	0.01	-	-	0.1	0.09	0.2	0.01	-
C12:0	-	-	-	-	-	0.02	-	-	0.01	-	-	-	0.04	-
C13:0	-	-	0.11	-	0.2	-	-	-	0.01	-	0.01	-	0.18	-
C14:0	1.04	0.2	0.64	0.37	0.3	0.53	0.51	0.5	0.31	0.3	0.36	0.4	0.16	0.2
C15:0	0.14	-	0.08	-	0.2	-	0.07	-	0.01	-	0.02	-	0.14	-
C15:1	-	-	0.02	-	0.1	-	-	-	0.01	-	0.07	-	0.23	-
C16:0	12.3	2.1	14.1	9.75	8.8	14.2	11.4	12	8.81	9	7.9	7.5	7.36	9.1
C16:1	1.01	0.2	1.13	0.83	0.6	0.78	0.77	0.6	0.63	0.6	0.54	0.5	0.41	0.6
C18:0	5.37	1.3	5.34	5.35	3.7	10	4.28	8.4	2.9	4.6	2.2	3.9	2.78	4.6
C18:1n9	6.54	1.1	9.56	9.4	3.1	12.5	12.1	6.4	10.1	10	7.89	0.9	8.67	15
C18:2n6	0.19	0.2	0.09	-	-	0.01	0.11	-	0.02	-	0.15	0.1	0.08	-
C18:2n6	8.59	1.1	6.01	1.67	5.2	2.79	7.51	3	4.35	1.9	4.94	2	4.06	3.6

Table 4: cont'd

C18:3n6	-	-	0.14	0.17	0.1	0.17	0.28	0.2	0.18	0.1	0.63	0.5	0.18	0.4
C18:3n3	0.8	-	0.27	0.09	-	0.01	0.33	-	0.14	-	0.24	-	0.21	-
C20:0	0.1	-	0.08	0.02	-	0.02	0.14	0.1	0.04	-	0.16	0.1	0.09	-
C20:1n9	1.66	0.2	0.24	0.36	0.4	0.47	0.2	0.5	0.33	0.3	0.38	0.4	0.28	0.5
C20:2	0.39	-	0.31	0.31	0.2	0.37	0.24	0.3	0.12	0.2	0.13	0.3	0.15	0.3
C20:3n3	0.56	-	0.73	0.98	0.9	0.94	0.61	0.9	0.52	0.7	0.48	1.2	0.78	1.8
C20:4n6	-	-	0.39	0.75	0.6	0.67	0.33	0.9	0.22	0.6	0.4	1.9	0.84	2.5
C20:5n3	2.53	0.4	0.27	0.01	0.3	0.09	0.21	0.1	0.13	-	0.19	0.1	0.38	-
C21:0	0.16	-	0.07	0.06	-	0.07	0.06	0.1	0.04	0.1	0.02	0.1	-	-
C22:0	-	-	0.04	-	-	0.04	0.09	-	0.03	-	0.1	0.2	0.05	0.1
C22:1n9	0.77	0.2	-	-	-	-	0.01	-	0.01	-	-	-	0.04	-
C22:2	-	0.2	0.03	-	-	-	0.14	-	0.04	-	0.08	-	0.07	-
C22:6n3	8.61	0.9	1.25	1.15	2.3	0.91	1.1	1	0.89	0.8	0.87	1.6	0.83	2.8
C23:0	0.15	-	-	-	-	-	0.01	-	0.01	-	-	-	-	-
C24:1n9	0.74	1.1	0.3	0.39	0.3	0.2	0.26	0.3	0.06	0.2	0.2	0.4	0.09	0.3
∑ FA	52.5	9.7	41.3	32	28	45.1	40.9	36	30.2	30	28.3	23	28.5	43
∑ Saturated	20.1	4.2	20.6	15.8	14	25.2	16.9	22	12.5	15	11.2	13	11.3	15
∑ Unsaturated	32.4	5.5	20.8	16.1	14	19.9	24	14	17.7	15	17.1	9.9	17.2	28

attributed to the lack of lysine and/or methionine in the experimental diets. A significant increase in feed intake was observed in African catfish fed with low energy diets and carnitine supplementation. Increased growth of fish fed supplemental carnitine may be due to improved feed conversion via increased fatty acid oxidation and increased utilization of dietary energy or increased feed consumption (Ronald and Paul, 2000). The growth rates of the African catfish in the present study increased due to increased feed consumption, and not because of improved fatty acid oxidation since feed utilization decreased with supplemental L-carnitine within each energy level. Supporting the findings of Ji et al. (1996) and Ronald and Paul (2000), increased fatty acid oxidation was observed in muscle tissues of fish fed L-carnitine without significant improvement in weight gain or feed conversion.

Supplemental L-carnitine in all three dietary lipid treatments increased total lipids in the muscle and decreased total lipids in the liver, supporting the results of Gaylord and Gatlin (2000b). In contrast, the total fatty acid content in liver increased as the energy/protein ratio increased. Especially docosahexaenoic and eicosapentaenoic, initially at intermediate levels, decreased by the end of the study in both muscle and liver samples. Although arachidonic acid was not detected initially, this fatty acid was found in different levels in all treatments in both the muscle and the liver at the end of the experiment. This might be explained by the conversion of fatty acids from 18 carbon atoms to 20-22 (Cowey and Sargent, 1972).

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