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The Effects of Different Oils Sources on the Growth Performance and Body Composition of Juvenile Nile Tilapia (*Oreochromis niloticus*, L.)

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Abstract: A total of 600 juvenile fish with average initial weight of 2.54±0.07 g were enrolled to this study to investigate the effects of different oil sources (fish oil, soybean oil, linseed oil and beef tallow) on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*, L.) during 14 weeks. Experimental diets were prepared as isonitrogenous (30.76% CP) and isocaloric (13.09 MJ DE kg^{-1}). At the end of the study, it was observed that there were no significant differences among the groups for average live weight, live weight gain, feed consumption, Condition Factor (CF), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Specific Growth Rate (SGR) and survival rate ($p>0.05$) but carcass composition ($p<0.05$).

Key words: Tilapia, oil sources, feeding performance, feed consumption, carcass composition, soybean oil

INTRODUCTION

Dietary lipids are one of important sources of highly digestible energy and are the only source of essential fatty acids required for normal growth rate and development. They are also carriers and assist in the absorption of fat-soluble nutrients such as sterols and Vitamins A, D, E and K serve as a source of phospholipids which are important for cellular structure and maintenance of membrane flexibility and permeability, participate in the synthesis of hormones, prostaglandins and other metabolically active compounds and improve the flavour of diets and affect the diet texture and fatty acid composition of fish (Shiou, 2002).

Stickney and Wurts (1986) compared the growth of blue tilapia fed diets containing graded levels of catfish and menhaden oil. The growth of *Oreochromis aureus* can be substantially improved when menhaden oil or fish oil is provided at 7.5-10.0% of the diet as compared with lower levels.

These studies gave contradictory results as to the requirement of tilapia for dietary ω3 and ω6 PUFA. The optimal dietary lipid level for tilapia was quantified by Chou and Shiau (1996). Five isoenergetic and isonitrogenous purified diets containing 0-20.0% lipid (corn oil, cod-liver oil and pork lard at a 1:1:1 ratio) in 5% increments were fed to the juvenile tilapia hybrid, *O. niloticus* x *O. aureus*. Results indicated that 5% dietary lipid appeared to be sufficient to meet the minimal requirement of juvenile tilapia but a level of 12% was needed for maximal growth. Stickney and McGeachin (1985) reported that growth of blue tilapia, *O. aureus* was not affected by dietary linoleic acid levels as high as 0.2%. When blue tilapias were fed diets containing soybean oil, growth improved as the percentage of linoleic acid increased (Stickney *et al*., 1982). Takeuchi *et al*. (1983) found that the growth of *O. niloticus* was significantly reduced with a fish oil containing diet (pollock liver oil) as compared with the diets containing corn oil or soybean oil. However, Santiago and Reyes (1993) indicated that although, a fish oil (cod liver oil) high in 22:6ω3 promoted the highest weight gain in *O. niloticus*, this same lipid resulted in the poorest reproductive performance of this species.

Blue tilapia have been reported to grow well on practical diets containing either 1% soybean oil which is high in 18:2ω6 or 1% menhaden oil which is high in 20:5ω3 and 22:6ω3 (Stickney and McGeachin, 1983). Stickney and Hardy (1989) however, reported that *O. aureus* have a requirement for a relatively high level of ω6 fatty acids, though the requirement can be reduced when ω3 fatty acids are present. Chou and Shiau (1999) demonstrated that both ω3 and ω6 HUFA are essential for maximum growth of hybrid tilapia (*O. niloticus* x *O. aureus*). Recently, a study was conducted by Lim *et al*. (2008) to evaluate the effects of different lipid sources namely: Corn

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Oil (CO), Beef Tallow (BT), menhaden Fish Oil (FO), Linseed Oil (LO) and combinations of equal levels of FO+CO+BT or LO+CO+BT on the growth performance and whole body proximate composition of Nile tilapia. Results showed that tilapia appear to have dietary requirements for both linoleic (ω6) and linolenic (ω3) series of fatty acids. The present study was carried out to evaluate the effects of dietary lipid source on growth performance of Nile tilapia.

MATERIALS AND METHODS

Fish and feeds: A total of 600 Nile tilapia (*O. niloticus*) were enrolled to the study. The study was performed in application unit of Aquaculture and Fishery Faculty, Mersin University. Fish were obtained from Cukurova University. They were counted, weighted and stocked randomly chosen into tanks at a rate of 50 fish/tank with 3 repetitions for each the diets containing different oil sources. Triplicate groups of juvenile *O. niloticus* (2.54±0.02 g) were fed with isonitrogenous (30.76% CP) and isocaloric (13.10 MJ DE kg^{-1}) feeds (Table 1).

All feeds used in the experiment contained about 15% fish meal, 5% meat-bone meal, 31.5% soybean meal, 38.8% wheat bran and 8.2% four different oil, Soybean Oil (SBO), Linseed Oil (LSO), Beef Tallow (BTO) and Fish Oil (FO). Fish meal and soybean meal are extracted with diethyl ether to obtain defatted fish meal and soybean meal for better observation of the effect of different oils. The whole feedstuffs are grinded to medium fine size (0.3 mm) before pelleting. Pellet size was 3 mm diameter and 6 mm in length. After pelleting, they were crumbled suitably for juvenile fish.

Table 1: Composition of experimental fish diets

	Test diets (%)			
Feedstuffs	SBO	LSO	BTO	FO
Fish meal	15.00[a]	15.00[a]	15.00[a]	16.80[b]
Bone-meat meal	5.00	5.00	5.00	5.00
Soybean meal	31.50	31.50	31.50	31.50
Wheat bran	38.80	38.80	38.80	38.80
Soybean oil	8.20	-	-	-
Linseed oil	-	8.20	-	-
Beef tallow	-	-	8.20	-
Fish oil	-	-	-	6.40
Vitamin premix[c]	0.60	0.60	0.60	0.60
Mineral premix[d]	0.15	0.15	0.15	0.15
Lignobond[e]	0.65	0.65	0.65	0.65
Butil hydroxi toluen[f]	0.10	0.10	0.10	0.10
Total (%)	100.00	100.00	100.00	100.00

[a]Fish oil is extracted totally by the help of soxleth and it does not include oil (74% CP); [b]Natural fish meal (65% CP); [c]Vitamin premix (mg kg^{-1} or IU kg^{-1} of DM): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, inositol 600 mg, ascorbic acid 5000 mg, α-tocopherol 300 mg, menadione 20 mg, cholecalciferol 2000 IU, retinol acetate 5000 IU and α-cellulose was used as a carrier; [d]Mineral premix (g kg^{-1} of DM): calcium orthophosphate 1.80 g, calcium carbonate 5 g, ferrous sulphate 1.7 g, magnesium sulphate 1.8 g, potassium phosphate 3.0 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g. α-cellulose was used as carrier; [e]This commercial product is used as pellet binder; [f]Antioxidant powder; [e, f]These additives were obtained by Sigma

Feeding trial: The research was conducted in plastic tanks sized 200×50×60 cm. Water was distributed with PVC pipes for each tank. The water flow rate was fixed at 0.09 L min^{-1} for all treatments thus, 25% of total water volume was changed with fresh water daily. Values of pH (Hanna HI 8314), dissolved oxygen and water temperature (Schott Gerate CG 867) measured periodically are shown in Table 2. Fish was fed twice a day, morning (9 am) and evening (5 pm) according to free feeding (*ad libitum*) method. It is supposed that all given feed was consumed by the fish. The amount of consumed feed was calculated by determining weight of lacking total feed.

The whole fish starved before 24 h was taken from each tank then weighed as a group every 2 weeks. The experiment lasted for 14 weeks.

Chemical analysis and calculations: At the end of the experiment, fish weight gain, FCR, PER (Hepher, 1988), PPV (Wilson, 1989), SGR (Hepher, 1988), CF (Brown, 1957) and survival rate were estimated through the following equations:

$$PER = \frac{\text{Live weight gain in an identified period (g)}}{\text{Consumed protein with the diet in the same period (g)}}$$

$$PPV = \frac{\text{Final carcass protein content} - \text{Initial carcass protein content (g)}}{\text{Consumed protein with the diet (g)}} \times 100$$

$$SGR = \left[\frac{\ln W_t - \ln W_o}{t - t_o}\right] \times 100$$

$$CF = \frac{BW}{L^3}$$

Just before the start of the experiment, 20 fish were randomly collected for proximate carcass analysis and 7 fish from each treatment were sacrificed and pooled for total body and carcass composition analyses at the end of experiment (Hepher, 1988; Wilson, 1989; Brown, 1957).

The chemical compositions of carcass, complete eeds and feedstuffs were measured following standard AOAC Methods (AOAC, 1997). Table 3 and 4 show nutritional composition of these four trial feeds and fish fillets.

Table 2: Some quality parameters of artesian water used in the trial

Parameters	Values
Temperature (°C)	22.000
pH	7.190
EC (mS cm^{-1})	1080.000
DO_2 (mg L^{-1})	5.200
Na^{+1} (mg L^{-1})	118.100
K^{+1} (mg L^{-1})	3.240
Ca^{+2} (mg L^{-1})	68.500
Mg^{+2} (mg L^{-1})	19.000
CO_3^{-2} (mg L^{-1})	0.000
HCO_3^{-1} (mg L^{-1})	324.950
Cl^{-1} (mg L^{-1})	122.300
SO_4^{-2} (mg L^{-1})	65.210
NO_2^{-1} (mg L^{-1})	0.000
NO_3^{-1} (mg L^{-1})	52.761
NH_3	0.000
PO_4^{-3}	0.000
Σ Anion	10.134
Σ Cation	10.297

Table 3: Nutritional composition of experimental diets

	Test diets (as fed basis %)			
Ingredients	SBO	LSO	BTO	FO
Dry matter	93.64	92.86	94.11	93.88
Crude protein	30.77	30.62	30.77	30.88
Crude oil	10.12	9.98	10.15	10.02
Crude fibre	2.52	2.41	2.28	2.59
Nitrogen free extract	41.33	40.81	41.66	40.86
Ash	8.90	9.04	9.25	9.53
Digestible energy (MJ kg^{-1})	13.11	13.11	13.11	13.05
Fatty acid composition[a]				
14:0	0.13	0.13	2.38	6.27
14:1	TR	TR	1.07	0.41
15:0	TR	TR	0.33	5.3
15:1	TR	TR	0.29	TR
16:0	9.83	6.03	20.82	15.45
16:1	2.78	2.83	6.44	14.79
17:0	TR	TR	0.6	TR
17:1	0.15	TR	0.64	0.4
18:0	4.19	2.65	11.35	3.88
18:1ω9	23.77	20.08	43.61	21.32
18:2ω6	51.71	28.69	10.91	9.93
18:3ω3	6.66	38.84	0.24	0.66
18:3ω6	0.92	1.16	1.07	0.92
18:4ω3	ND	ND	ND	0.16
20:0	0.38	0.51	0.22	0.25
20:1ω9	0.20	TR	0.44	1.33
20:4ω6	TR	TR	TR	0.01
20:5ω3	ND	ND	ND	14.01
22:0	0.18	0.1	0.1	0.8
22:5ω3	ND	ND	ND	1.31
22:6ω3	ND	ND	ND	7.21
24:1ω9	0.1	ND	ND	0.41
Total ω3	6.66	38.84	0.29	0.67
Total ω6	59.28	68.70	12.31	11.52
ω3/ω6	8.90	1.77	42.45	17.19

[a]Each fatty acids are presented as percentage and TR = Trace (<0.1% fatty acids); ND = Non-Detectable (<0.01% fatty acids)

Lipids for fatty acid analysis were extracted from diets and fillets with chloroform and methanol, methylated and transesterified with boron trifluoride in methanol (Bligh and Dyer, 1959). Fatty acid methyl esters were then resolved and analyzed by a gas-liquid chromatograph (Shimadzu GC-14A) equipped with a flame ionization detector and a Shimadzu C-R6A Chromato-Integrator. The esters were separated on an OmegawaxTM 320 fused silica capillary column (30 m×0.32 mm ID; Supelco, Bellafonte, PA). Separation conditions were used as previously described by Ng *et al*. (2003).

Fatty acids were identified by comparing retention time with those of known standards (Supelco, Bellafonte, PA) and areas beneath the identified chromatographic peaks were calculated by integration. Fish production cost was calculated with complete feed price multiplied by FCR. The price of feed ingredients is concerned in early 2009.

Statistical analyses: Random block experimental design was used to evaluate the differences between treatments. The mean final body weights in each treatment were subjected to statistical comparisons using ANOVA. All statistical analyses were carried out using the SPSS (Version 16) program (Anonymous, 2007). Results and mean differences between treatments were tested for significance ($p<0.05$) by the help of Tukey's multiple range test. Results shown in Table 5 and 6 are reported as means±SD (n = 3, 20 and 7, respectively).

Table 4: Fatty Acid (FA) compositions of Nile tilapia (*Oreochromis niloticus*) fillets at the end of the trial[*]

Fatty acids	SBO	LBO	BTO	FO
14:0	0.68±0.07^{ab}	0.69±0.06^{ab}	0.81±0.06^{a}	0.64±0.03^{b}
16:0	15.5±0.07	16.1±0.03	16.7±0.08	16.4±0.05
16:1	1.77±0.03^{a}	1.65±0.4^{ab}	1.68±0.8^{ab}	1.54±0.07^{b}
17:0	0.62±0.09	0.61±0.13	0.77±0.12	0.59±0.16
18:0	8.27±0.46	8.22±0.59	8.51±0.66	8.44±0.78
18:1ω7	2.32±0.11	2.22±0.26	2.45±0.15	2.53±0.19
18:1ω9	27.44±0.63^{b}	27.81±0.42^{b}	30.88±0.39^{a}	27.77±0.28^{b}
18:2ω6	34.24±0.21	32.56±0.44	31.46±0.28	30.14±0.17
18:3ω3	2.86±0.41^{b}	3.91±0.25^{a}	2.07±0.34^{c}	4.04±0.18^{a}
18:3ω6	0.94±0.08^{b}	0.88±0.05^{a}	0.82±0.03^{a}	0.75±0.09^{a}
20:0	TR	TR	TR	0.12±0.04
20:1ω9	0.49±0.02^{b}	0.37±0.06^{c}	TR	1.03±0.02^{a}
20:4ω6	2.95±0.24^{a}	3.02±0.17^{a}	2.26±0.42^{b}	3.18±0.16^{a}
20:5ω3	ND	0.09±0.04	ND	0.14±0.02
22:5ω3	0.49±0.08^{ab}	0.38±0.08^{b}	0.19±0.04^{c}	0.56±0.05^{a}
22:6ω3	1.38±0.15^{b}	1.46±0.19^{b}	1.36±0.13^{b}	1.98±0.06^{a}
24:1ω9	0.03±0.01^{b}	0.02±0.01^{b}	ND	0.15±0.04^{a}
Total ω3	4.73	5.84	3.62	6.72
Total ω6	38.13	36.46	34.54	34.07
ω3/ω6	0.12	0.16	0.11	0.20

[*]Results are expressed as a percentage of the total fatty acids. Averages followed by different letters in the same line are significantly different ($p<0.05$) by Tukey's test (n = 7)

Table 5: Average initial weight, final weight, weight gain, FCR, SGR, PER and survival rate for Nile tilapia fed different diets for 98 days*

	Test diets ($\bar{X} \pm S_{\bar{X}}$)			
Items	SBO	LSO	BTO	FO
Trial period (day)	98	98	98	98
Total fish number	150	150	150	150
Survival rate (%)	100	100	100	100
Initial weight (g)	2.56±0.12	2.56±0.15	2.52±0.12	2.54±0.21
Final weight (g)	16.7±1.14	15.96±2.24	16.40±1.24	21.14±3.57
Average live weight gain (g)	14.14±2.16	13.40±3.22	13.88±1.27	18.60±2.55
Average feed consumption (g)	16.81±1.98	17.75±2.96	16.30±1.70	19.62±2.21
Feed conversion rate (g)	1.19±0.06	1.33±0.21	1.17±0.11	1.09±0.18
Protein Efficiency Rate (PER)	2.74±0.13	2.47±0.38	2.77±0.04	3.07±0.28
Specific Growth Rate (SGR)	1.91±0.16	1.87±0.19	1.91±0.11	2.16±0.22
Protein Productive Value (PPV)	57.42±5.68^{ab}	45.81±3.58^{b}	67.60±4.51^{a}	51.67±3.29^{b}
Complete feed production cost (€ kg^{-1})	2.94	2.76	2.77	3.07
Fish production cost (€ kg^{-1})	3.50	3.67	3.24	3.35

*Results are means±SD (n = 3). Averages followed by different letters in the same line are significantly different ($p<0.05$) by Tukey's test

Table 6: Nutritional composition of carcass at the end of trial[a]

	Test diets (%) ($\bar{X} \pm S_{\bar{X}}$)			
Items	SBO	LSO	BTO	FO
Initial carcass[b]				
Moisture	80.06±0.11	-	-	-
Crude protein	13.62±0.21	-	-	-
Crude fat	2.47±0.27	-	-	-
Ash	2.06±0.12	-	-	-
Final carcass[c]				
Moisture	74.32±0.10	75.40±0.86	74.6±1.240	73.25±1.61
Crude protein	22.88±0.05^{a}	21.68±0.04^{b}	23.22±0.08^{a}	23.57±0.86^{a}
Crude fat	1.26±0.59	1.44±0.40^{a}	0.58±0.11^{b}	0.79±0.30
Ash	1.53±0.09	1.40±0.33	1.40±0.11	2.04±0.70

[a]Results are means±SD. Averages followed by different letters in the same line are significantly different ($p<0.05$) by Tukey's test; [b]n = 20, [c]n = 7

RESULTS AND DISCUSSION

The results obtained in this experiment are shown in Table 4. Important differences is observed among the groups for PPV ($p<0.05$). While the best results for PPV are obtained with BTO and SBO groups, there are no important differences among the groups for initial live weight, live weight gain, feed intake, FCR, PER and SGR ($p>0.05$). In other words, various fat sources did not affect the growth performance. Thus, BTO and SBO groups evaluated the protein more effectively. In addition, the feed containing beef tallow is produced with cheaper cost. Cold-water fish have a higher requirement for ω3 Polyunsaturated Fatty Acid (PUFA) whereas warm-water fish tend to require greater quantities of ω6 fatty acids. The results are in agreement with Takeuchi *et al.* (1983), Santiago and Reyes (1993), Stickney and McGeachin (1983), Stickney and Hardy (1989) and Kanazawa *et al.* (1980) findings. While arachidonic acid (20:4ω6) can be detected as trace amounts in the feeds, it can be found detectable amounts in the carcass (Table 3 and 4). Thus, it is concluded that Nile tilapias can convert some fatty acids to another one. However, Kanazawa *et al.* (1980) suggested that this same species probably does convert dietary 18:2ω6-20:4ω6 and they also stated that tilapia requires ω6 fatty acids rather than ω3 fatty acids. Moreover, both 20:4ω6 and ω3 HUFA were also found to have no EFA value for fish by Takeuchi *et al.* (1983). They also determined that the best weight gain was obtained in the fish receiving a diet containing 0.05 or 0.1% 18:2ω6. On the contrary, there are other studies reported tilapia appear to have dietary requirements for both linoleic ω6 and linolenic ω3 series of fatty acids (Stickney and Wurts, 1986; Chou and Shiau, 1999; Lim *et al.*, 2008). When researchers look at Table 2, researchers can see that the amount of 18:2ω6 is enough for a good growth performance.

Although, fish oil is often used in production aquafeeds, it also generally increases the cost of the feed. Furthermore, fish oil is not commonly available and it is difficult to keep it stable. It is also known that the oil sources vary widely in different countries. Thus, researchers believe that the results provide useful information to the feed manufacturers on the more effective use of available oil sources. This is especially important for aquaculture studies which uses high amount of oil as a source of energy in coldwater fish feeds in order to provide economic use of proteins.

In general, there is no consensus on which fatty acids are essential for fish and the necessary level of these essential fatty acids in fish feed as well. On the other hand, an important ingredient of freshwater fish feed ω6 HUFA presents enough amounts in most plant oil. As a results, researchers suggest that any oil type containing essential fatty acids in minimal concentrations can successfully be used in fish feed and thus cheaper feeds can be produced.

CONCLUSION

This study shows that there is no an important effect of different oil sources in fish feeds when they have sufficient essential fatty acids in their feeds.

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