The study of growth performance and some biochemical parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings fed on olive mill waste

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Abstract- Nile tilapia *(Oreochromis niloticus)* fingerlings (Average weight 34.50 ± 0.05g) were cultured in glass aquaria and fed two formulated (pelleted) diets for 12 weeks. The control diet containing wheat bran replaced with 30% olive mill waste. Each diet was fed to 3 groups of 15 fish / aquarium. At the end of the trial, growth performance and biochemical parameters values of serum glucose, total protein, cholesterol, triglyceride, and serum enzymes, aspartate aminotransferase (AST) and alanine aminotransferase(ALT) were measured. Results indicated, no significant ($P<0.05$) differences in average body weight, protein efficiency ratio and the survival rate between fish fed on treated and control diets, while there was significant differences in standard growth rate and feed conversion ratio. Additionally, the blood serum of glucose, total protein, and cholesterol were lower in the treated than the control group. While the values of triglycerides of fish fed on treated diet was twice that found in fish fed on control diets. For the serum enzymes (AST and ALT) evaluated in this experimental caused no significant differences in both groups of fish.The mean values of these biochemical parameters were within the acceptable range for normal metabolism of Nile tilapia. The results of this study seem to indicate that olive mill waste improved its nutritional value in practical feeds for Nile tilapia fingerlings and without any effect on biochemical parameters.

Index Terms- Olive mill waste, Nile tilapia, growth, feed utilization, biochemical parameters.

I. Introduction

Olive oils are considered as one of the most important food in many countries around the world, especially in the Mediterranean countries which produces 95% of the world's olives (Boskou, 1996, Al-Malah *et al*, 2000). Libya, as a Mediterranean country, has millions of olive trees, which are produced 180,000 tonnes of olive oil in 2009 (FAO, 2009). In addition, the olive oil production industry generates a large amount of waste, known as olive mill wastes (i.e. raw materials), which represents a major environmental problem in Mediterranean countries (Luísa *et al*, 2004). For this reason, sometimes used as fuel due to their high-energy content (Encinar, 2008 and 2009), and its use as a component of fertilizers or animal feed is limited (Kavdir and Killi, 2008 and Aliakbarian et al 2011). A number of studies have been conducted on the use of olive mill waste for animal feed such as, broilers (Abo-Omar, 2005), rabbits (Carraro et al., 2005), goats (Ben-Salem et al, 2003). and also used as medicine for some diseases, as reported in traditional medicine (Sanarya et al, 2011). Olive mill waste (solid matter) represent up to 20 g/L from olive mill waste water. It is contains about, 10.41 - 12.2 % lipid, 5.80 -7.2% protein, 39.62 - 57.5% fiber and 16.1 - 20.7% % nitrogen free extract (NFE) depending on olive species and varieties (Nasser et al, 2011). About 33.7 % of NFE in the olive mill is mainly cellulose (Froig et al., 2006; Borja et al., 2006). This situation created need for usage of alternative sources in fish feed, such as, gilthead sea bream (*Sparus aurata*) by Sioriki *et al*, 2013., Nile tilapia (*Oreochromis niloticus*) by (Nasser *et al*., 2011). Although, the used of olive waste in fish feed have been studied. However, only limited information is available on the use of olive mill waste (OM) in Nile tilapia (*Oreochromis niloticus)* feed and correlated with blood biochemical parameters. Thus, The purpose of this experiment was to study the utilization of olive mill by Nile tilapia (*Oreochromis niloticus*) fingerlings and their effect on some biochemical parameters, because biochemical profile of these fish has not been reported.

II. Materials and Methods

The research was conducted at Aquatic laboratory of Faculty of Science Al-Marghib University, Libya. Experimental diets result was analyzed at Factory of biscuit, Zliten , Libya.

Experimental design

The experiment design was utilized six glass aquaria, (40 W X 80 L X 40 H cm) for each aquarium. Each aquarium was supplied with none-chlorinated water from a deep tube well. Water quality parameters were: temperature 24-26°C; pH, 7.2-7.8 and dissolved oxygen, 5.5-6.7 mg/L. All glass aquaria were provided with continuous aeration from an air compressor. About fifty percent of the water in the system was replaced biweekly to avoid accumulation of excretory products.

Experimental fish

Nile tilapia (*Oreochromis niloticus*) fingerlings obtained from Ain Kiam Fish Farm near Zliten, Libya, and then transferred to indoor culture systems where they were acclimated to laboratory conditions for two weeks, during this period the experimental fish are fed on commercial pellets. After acclimation, 90 fishes were selected and fasted for one day, with average body weight, 34±0.05g/ fish and randomly distributed among 6 glass aquaria at a density of 15 fishes per aquarium. The

used fish were apparently healthy and free from any external parasites. Fish was hand-fed to visual overfeed twice daily at 8.00 a.m. and 5.00 p.m. for 12 weeks.

Feed formulation and pellet preparation

The basal experimental diets were formulated with the commonly available ingredients (Table 1). The diets contained fish meal (FM), wheat flour (WF), wheat bran (WB) and olive mill wastes (OMW). FM, WE, and WB were obtained from a local supplier, while olive mill wastes were obtained from olive oil Squeezer at Zliten, Libya. All ingredients were ground to a fine powder using a laboratory grinder mill and the powder was sieved through a 250 µm sieve. The dry ingredients were hand mixed and homogenised separately before loaded into mechanical mixer. Oil was slowly added until over mixing. When homogenous mixture was obtained, 30 ml water was slowly added to the dry mixture and blended until it became dough like paste according to Arunlertaree and Moolthongnoi (2008)., then the pellets of 2.5 mm diameters were produced using meat machine. The pellets were dried then broken into small crumble. Dried pellet diets were collected, labeled and stored in refrigerator at 4°C until used.

Nutrition analysis of experimental diets

The proximate analysis of all ingredients and experimental diets given in Table 2 and Table 3. And analysed in triplicate following the standard methods of AOAC (1990). Crude protein by Kjeldahl Nitrogen. Crude lipid by petroleum extraction. Total ash by Muffle, Furnace Combustion. Crude fiber based on Tecator Technology MT Technology. Carbohydrate (NFE %) and gross energy was determined by calculated according to NRC (1993):

Carbohydrate (% NFE) = 100 – (% protein + % lipid + % fiber + % ash + moisture)

Gross Energy (GE) = (% NFE x 4.11) + (% protein x 5.64) + (% lipid x 9.44).

Growth performance analysis of fish

The growth performance parameters were calculated according to the following equations:

Weight gain (WG, kg) = final weight (kg) – initial weight (kg);

Average daily gain(ADG) = [average final weight (g) – average initial weight (g)] / time (days) ;

Weight gain percent = (final body weight - initial body weight / initial body weight × 100

standard growth rate (SGR) = (In final weight - In initial weight)x l00 / number of days) ;

Survival rate (%) = (final number of fish / initial number of fish) × 100

Protein consumed (kg) = food consumed (kg) x percentage of protein in diet;

Protein efficiency ratio (PER) = live weight gain (g) / crude protein fed (g dry weight);

Food conversion ratio (FCR) = food consumed (kg) / weight gain (kg).

Blood samples and biochemical analysis

At the end of experiment (12 weeks), the blood samples collected from the caudal vein of three randomly chosen fish from each aquarium by using a medical syringe. Each sample was centrifuged at 3000 rpm for 10 minutes to obtain serum for biochemical studies. Blood serum glucose were measured by Glucose Oxidase method. Total serum protein was estimated by the Biuret method. Serum cholesterol by Enzymatic Colorimetric test. Serum triglycerides by Colorimetric method. All these parameters follow procedures according to (Chawla, 2003). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to the method described by BergMeyer and Bernt(1974).

III. STATISTIC ANALYSIS

Results from all calculations were subjected to analyses of variance (ANOVA) and Ducan's multiple test (Duncan, 1955) was further used to evaluate the mean differences at 0.05 significant levels.

Table 1. Experimental diet formula of Nile tilapia *(Oreochromius niloticus)*

Ingredient (%)	Diets	
	Wheat bran (Control)	Olive mill wastes
Fish meal	40	40
Wheat flour	20	20
Wheat bran	30	-
Olive mill	-	30
Cod liver oil	3	3
Corn oil	2	2
Mineral premix	2	2
Vitamin premix	3	3
Total	100	100

Table 2: The chemical analysis of items used in the experimental diets:

Ingredients	Dry matter	Crude protein	Crude lipid	Crude fiber	Ash	NFE	Gross energy kcal/g
Fish meal	92.50	71.70	04.15	00.54	08.51	07.60	519
Wheat flour	95.00	10.80	01.50	01.30	01.20	80.20	405
Wheat bran	90.50	16.91	03.76	07.06	07.60	55.17	377
Olive mill	87.00	13.80	11.70	18.70	06.90	35.90	376

NFE ( )

Table 3. Proximate composition of Nile tilapia experimental diets (on % dry matter basis).

Proximate analyses	Wheat bran (Control)	Olive mill wastes
Crude protein %	35.9	34.98
Crude lipid %	8.09	10.47
Total carbohydrate (NFE) %	36.88	30.65
Ash %	20.51	18.81
Gross energy (kcal/g)	4.34	4.25
Protein / NFE ratio	0.97	1.14
NFE / lipid ratio*	4.55	0.75
Protein / lipid ratio**	4.44	3.34

* Carbohydrate to lipid ratio on a weight basis.
** Crude protein to lipid ratio.

IV. Results and Discussion

Chemical composition of the experimental diets .

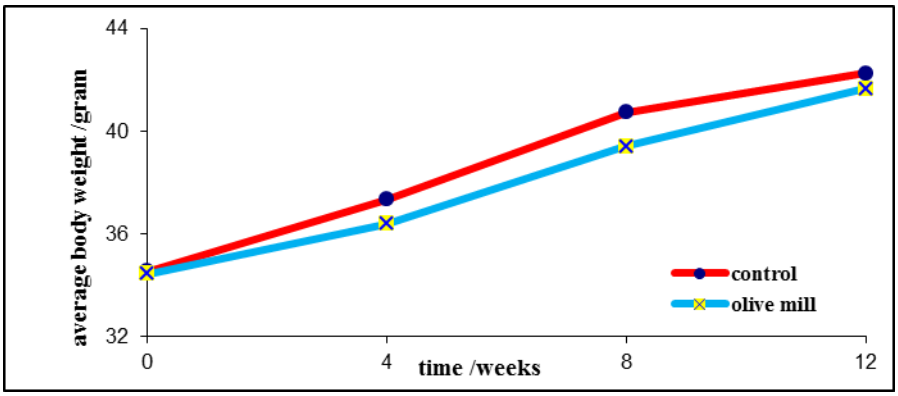
Chemical composition and calculated energy of different diets are presented in Table (3). The chemical analysis shown that no differences were observed between both diets in dry matter and crude protein, while there was some differences observed between different diets of ash, carbohydrates (NFE) and crude lipid, this differences may due to ingredients contains in different diets. Furthermore, the crude protein content was around 35% on dry matter basis. Such level was within the range suggested by Balogun et al, 2004 and NRC (1993). The calculated energy were similar in the tested diets, where the energy values was about 4 kcal/g , it was higher than that suggested by El-Sayed (1987) for the practical diets for Nile tilapia (2.90 kcal/g). However, it was nearly similar to that used by Ahamad Ali (1982).

Growth performance and feed utilization

There was no feed rejection during the experiment, and the acceptability of the diets looked similar. Fish mortality wasnot recorded in all replicates of the treatments during the entire period of the experiment. Table 3 shows values for initial body weight, final body weight , mean weight gain, daily weight gain, weight gain percent, standard growth rate, protein efficiency ratio, and feed conversion ratio. At the beginning, average initial body weight ranged from 34.45 to 34.55g. The difference of average weight was begun significantly at the week 8th of the feeding period. At the end of the experimental period (week 12th), there was no difference in final body weight between both groups of fish. In addition, the average final weight were 41.65 g for fish fed on olive mill compared with fish fed on control 42.26 g. This result agrees with studies of Nasser *et al* (2011). The average body weight of Nile tilapia for every two weeks was illustrated in Fig.1. Furthermore, the values of protein consumed and protein efficiency ratio did not differ significantly ($P < 0.05$) between the feeding groups, but there is significant differences were found in weight gain percent, standard growth rate (SGR) and feed conversion ratio (FCR). The values of FCR and PER were observed high level compared to the diets contained castor seed used by Balogun *et al*, 2004. In the present experiment, FCR was 1.79 for fish group fed on olive mill, this value was better than that found by previous studies on tilapia. Iluyemi *et al* (2010) recorded an FCR that ranged from 2. 86 to 5.34 for red tilapia fed on 33% crude protein. Omoregie *et al* (2009) noted an FCR that ranged 1.71- 2.96 for Nile tilapia fed 32 crude protein. In our trial diet, we had used a 40% level of fishmeal, hence the better performance when compared to the diets used by Wee and Wang (1987). The mean individual weight gain was not significantly different ($P < 0.05$) and averaged 41.65g in treatment fish group. This result are agreement with previous findings of Bahnasawy *et al*, 2003, and Catalina *et al*, 2013 on Nile tilapia *(Oreochromis niloticus).*

Table 4. Growth performance of Nile tilapia *(O. niloticus)* fed experimental diet for 12 weeks

Parameters	Treatments		ANOVA Sig. (*P* value)
	Control (wheat bran)	Olive mill wastes	
Initial number of fish	45	45	NS
Initial total weight (kg)	1.554	1.550	NS
Initial mean individual weight (g)	34.55	34.45	NS
Final number of fish	45	45	NS
Final total weight (kg)	1.914	1.874	NS
Final mean individual weight (g)	42.26	41.65	NS
Total weight gain (WG, kg)	0.360	0.324	NS
weight gain (g)	7.71	7.2	S
Individual daily weight gain (g d^{-1})	0.09	0.08	Ns
weight gain percent	22.32a	20.89b	S
Standard growth rate (SGR)	3.87	3.37	S
Survival ratio (%)	100	100	NS
food consumed (kg)	7.000	7.000	NS
Protein consumed (kg)	2.513	2.449	NS
Protein efficiency ratio (PER)	1.668	1.592	NS
Feed conversion ratio (FCR)	1.67a	1.79b	S



Biochemical parameters

Results of biochemical parameters assessments of fish blood at termination of the experiment are presented in Table 4. Blood glucose, total protein, and cholesterol in blood serum were lower than that of fish fed on control diet. Blood biochemistry of tilapia has been examined previously Hrubec and Smith (1999) reported blood chemistry in hybrid tilapias; they showed lower blood glucose (50 mg/dl), higher total protein (3.1g/dl) and cholesterol (216mg/dl) values than those measured in our study. Chen et al (2003) reported another study on Nile tilapia *(Oreochromis niloticus)*, which showed similar blood glucose (88.3 mg/dl), higher total protein (4.29 g/dl), and cholesterol (307.1g/dl). While the triglyceride of trial group highly significant and twice that found in fish fed on control diets. Similar results was in Liza klunzingeri obtained by Mohammadizadeh *et al* (2012), and lower than in red lionfish (298 mg/dl) reported by Anderson and Stoskopf, (2010), then higher than in Oreochromis mossambicus (55 mg/dl) reported by Demir et al (2014), however in normal range (59–661 mg/dl) .

The elevation of the aminotransferases activities in blood has been considered as an indicator of tissue damage. The data obtained of both ALT and AST activities did not significantly differ between trial group and control group, and similar results were reported by Hoseinifar *et al.* (2011) in serum of beluga *(Huso huso)*, and lower than with the results of Zaki *et al* (2010), they were (81.00 U/L) for AST and (22.00 U/L) for ALT in blood serum of tilapia Zilli. In both studies, the species of fish were different those in our study. This could explain the differences in the results. In addition, blood parameters among fish species may be affected by sampling technique, analyses methods, age, and diet (Sakamoto et al., 2001). Therefore, values reported here will be useful for the early detection, identification and monitoring of diseases and sublethal conditions in this species.

Table 5. blood chemistry parameters of Nile tilapia *(Oreochromis niloticus)* fed experimental diet for 12 weeks

Parameters	Treatment		ANOVA Sig. (*P* value)
	Control (wheat bran)	**Olive mill wastes**	
Blood glucose (mg/dl)	127±1.77a	109.50±1.73b	*
Total protein g/dl	2.45±0.06a	2.10±0.03b	*
Cholesterol (mg/dl)	134.33±1.36a	100.83±2.97b	*
Triglycerides (mg/dl)	70.67±1.36b	149.67±2.74a	*
AST U/L	34.05±0.08a	33.04±0.1a	NS
ALT U/L	17.72±0.2a	17.83±0.2a	NS

V. Conclusion

From the results of this study, the assimilation of olive mill waste (for the level studied) in the diet of *Oreochromis niloticus* has supported good growth performance nor deleterious effect on health of the fish. Therefore, the reference values obtained in the current study may provide overall plans for the interpretation of laboratory data for this species of fish, which are very important for aquaculture.

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