Enhancingthe Utilizationof Olive Cake Treated with Different Probiotic ExogenousFibrolytic Enzymes (ZAD®) Concentrationsin Nile Tilapia Diets

Abu-Ayyana,A.K.A[1].;Srour,T.M.A[2].;Zaki,M.A.A[3].and Mansour,A.T[2]

[1]Desert Research Center, Ministry of Agriculture ,Egypt

[2]Faculty of agriculture (Saba Basha) Alexandria University

[3]Faculty of agriculture (El Shatby) Alexandria University

ABSTRACT: The increasing costs of feed ingredients is triggering the continuous search for cheap and high value supplements to reduce production cost of farmed fish. This study was conducted in order to improve the nutritional value of olive cake (source of energy) with different concentrations of probiotic exogenous fibrolytic enzymes ZAD® to partially replace yellow corn (25 and 50% respectively), and to investigate its effect on growth performance, feed utilization. Seven experimental diets containing 30% crude protein were formulated, diet 1 a control diet without olive cake, diet2 and diet No.3 containing 25 and 50 % dried olive cake (DOC), respectively. Correspondingly, diet4 and diet 5 containing 25 and 50 % DOC treated with low concentration of ZAD® (LZOC (10^4 cell per ml))respectively. However, diets 6 and diet 7 containing 25 and 50 % olive cake treated with high concentration of ZAD® (HCOC(10^4 cell per ml)) respectively. Twenty-one glass aquaria with dimensions of (100 x 30 x 40 cm) and 100 L capacity of water were used in this experiment, triplicate per treatment. Fifteen Nile tilapia fingerlings with average weight of (0.9 ± 0.02g / fish) were stocked in each aquarium in this experiment. Fish were fed 3 times daily (six days per week) at a rate 3 % of the live body weight per day (dry food / whole fish). The results showed that diets2 and 3 containing 25 % DOC and 25% LCOC, respectively were significantly different than the control diet on Nile tilapia growth performances. Increasing the concentration of ZAD® lead to the decrease of protein efficiency ratio, protein productive value, energy utilization percentage and did not improve feed conversion ratio. Moreover, increasing ZAD® concentration in tilapia diets from 4 to7 lead to the decrease of dry matter, fat content, crude protein content and ash content in fish, while energy content increased. Thus, it could be concluded that diets containing dried olive cake or dried olive cake treated with low concentration of ZAD® as an energy source instead of 25% Yellow corn have positive effects on growth performance, feed and nutrient utilization parameters of Nile tilapia *(O. niloticus)* fry.

Keywords:olive cake, fibrolytic enzymes, Nile tilapia, probiotics, growth performance, growth promoters

INTRODUCTION

The aquaculture industry has been globally recognized as the fastest growing food producing industry (FAO, 2014). Aquaculture contributes more than 77 % of one million tons of fish annually produced in Egypt (GAFRD,2014).The growth and intensification of aquaculture has raised several issues in the development of fish feed from high quality, inexpensive sources as well as methods for making the feed free from anti-nutritional factors (El-Sayed, 1999).Carbohydrates are included in tilapia feeds to provide a cheap source of energy and for improving pellet binding properties. Tilapia can efficiently utilize as much as 35-40 percent digestible carbohydrate. Carbohydrate utilization by tilapia is affected by carbohydrate source (El-Sayed, 2006). Carbohydrate utilization by tilapia species have been reviewed by Shiau and Liang (1995).

Nile tilapia are capable of utilizing high levels of various carbohydratesbetween30to70percentofthediet.Grainsorgrainproductsarethemain carbohydrate sources in diets for cultivated fish (Tacon, 1993). A reduction of feed cost by using cheaper feed ingredients based on locally available materials such as dates and its by-products would almost double the farmer's income allowing expansion of cultured fish production (Alam*et al*., 1996) . The potential of by-products as fish feed ingredients in Egypt have been investigated by several authors (Saleh*et al* . (2014); Azaza *et al.* (2009); El-Sayed*et al*. (2006); Nour*et al.* (2004) and date stone Gaber*et al.* (2014b) ;Zaki*et al.* (2012).

Olive pomace has fragments of skin, pulp pieces of kernel and some oil. The major ingredients in pomace are polysaccharides, proteins, fatty acids like oleic acid and other C2–C7 fatty acids, polyalcohols, polyphenols and other pigments (Karantinos*et al.,* 2008). Olive pomace was recomended as an alternative source for animal feed (Nasopoulou and Zabetakis, 2013). Olive pomace includes some oil. Oil is produced by treating olive pomace. After obtaining oil from olive pomace, dry olive cake is gained. A total of 50– 80 g kg^{-1} olive pomace oil (Göğüş and Maskan, 2006) and 60–70 kg dry olive cake are obtained from 100 kg olive pomace. Studies are conducted on the use of these by-products obtained from olive industry. Apart from the researches on the use of olive cake, olive pomace and olive leaves in feeds of goats, sheeps and rabbits (Martin Garcia *et al.,* 2003; Dal Bosco*et al.,* 2012; Shdaifat *et al.,* 2013), there are many researches are conducted on the use of crude olive cake in feeds of rabbits, lamps, goats and sheeps (Carraro *et al.,* 2005; Molinaand Yanez, 2008; Omar *et al.,* 2012). At aquaculture feeds, although there are many researches on the use of olive pomace and pomace oil as an oil source (Yılmaz *et al.,* 2004; Sicuro*et al.,* 2010; Nasopoulou *et al.,* 2011), there is no study conducted on the use of dry olive cake in the diets of fish.

Therefore, the aim of the present experiment is to evaluate the effects of different levels (25 and 50 %, respectively) of dried olive cake instead of yellow corn without or with Low and High of ZAD® respectively in tilapia diets and its effect on growth performance, nutrient utilization of tilapia fry.

MATERIAL AND METHODS

Fish culture facilities

This study was carried out in the Wet Fish Laboratory, Department of Animal and Fish Production, Faculty of Agriculture (Saba Basha) Alexandria University during summer season of 2016. Nile tilapia, *Oreochromis niloticus* fry were acclimated to the experimental condition for 20 days. After that, fry (0.9 ± 0.02g / fry) were distributed at a rate of 15 fry per 100-L glass aquarium. Fry in each aquarium were fed on the tested diets twice a day; six days a week at a rate of 3% of their live body weight for 84 days. Fifty percent (50%) of aquarium's water was siphoned daily with fish faces and replaced by de-chlorinated tap water. Every two weeks, fry per each aquarium were group-weighted by a digital scale (accurate to ± 0.001 g) and feed quantity was adjusted accordingly. Dead fish once appeared in any aquarium were recorded and removed. At the start of the experiment, 60 fry (0.9 g/fry)as sample and frozen and reserved for initial proximate body chemical analysis. At the end of the experiment, fish were collected from each aquarium, counted, and weighed. Then, five fish were taken from each aquarium for the proximate chemical analysis.

Experimental diets

a) Preparation of olive cake with probiotic of ZAD®

The probiotic of ZAD® was used as a growth promoters at different dietary. The ZAD® is exogenous enzymes extracted from anaerobic bacteria,598/* it contains celluloses, xylanases, α– amylase and proteases (patent No. 22155 of Egypt).Drying olive mill wastes (olive cake) for 2 days at 55^{o} C in oven to get rid of moisture and fungus, Grinding and fragmentation of olive cake to get product in powder. Olive cake was treated with two different concentrations of ZAD® (low ZAD® (10^4cell per ml)and high ZAD® (10^{12} cell per ml))and store in plastic bags for 45 days to analyze fiber.

Experimental design

This experiment consists of four group treatments designed in randomized complete block design (RCBD) as the following:-

1. Group1: one treatment diet contained corn meal without dried olive cake(D1).

2. Group 2: two treatment diets contained 25 and 50 % dried olive cake without treated with probiotic exogenous fibrolytic enzymes ZAD® instead of YCM, (D2 and D3, respectively.

3. Group 3: two treatment diets contained 25 and 50 % Dried Olive cake treated with low concentration with probiotic exogenous fibrolytic enzymes ZAD® (LCOC) instead of YCM, (D4 andD5, respectively).

4. Group 4: two treatment diets contained 25 and 50 % Dried Olive cake treated with high concentration with probiotic exogenous fibrolytic enzymes ZAD® (HCOC) instead of YCM, (D6 andD7, respectively).

The applied treatments are summarized in the following Table (1).

Table (1). Experimental diets

Diet	Dietary treatments
D1	Yellow corn meal,(YCM) as a control diet
D2	25 % Dried olive cake without treated,(DOC) instead of YCM
D3	50 % Dried olive cake without treated,(DOC) instead of YCM
D4	25 % Dried Olive cake treated with low concentration with probiotic exogenous fibrolytic enzymes ZAD® (LCOC))instead of YCM
D5	50 % LCOC instead of YCM
D6	25% Dried Olive cake treated with high concentration with probiotic exogenous fibrolytic enzymes ZAD® (HCOC))instead of YCM
D7	50 % HCOC instead of YCM

b) Preparation of diets

Seven diets are nitrogenous (30% crude protein) and are caloric diets (445 kcal/100g) diets were prepared for the present study. The seven experimental diets were formulated as described in Table 1. Diet ingredients were ground and thoroughly mixed and the oil was slowly added at the same time of mixing with warm water (45°C) until the diets began to clump. Diets were processed by a California pellet mill machine and dried for48 hours in a drying oven at 70°C. The pellet size was 0.6 mm in diameter and 2 mm in length.

Fish performance and feed utilization

Fish growth performance and feed utilization parameters were calculated according to Cho and Kaushik (1985) as following equations:

1) Average weight gain (AWG, g / fish) = [final body weight-initial bodyweight];
2) Specific growth rate (SGR,% / day) = [final weight - initial weight] × 100 /time(days);
3) Feed conversion ratio (FCR) =feed intake(g)/body weight gain(g);
4) Protein efficiency ratio (PER) = gain in weight (g)/protein intake in feed(g);
5) Protein productive value (PPV, %) = 100 [protein gain in fish (g)/protein intake in feed(g)];
6) Energy utilization (EU, %) =100 [energy gain in fish / energy intake in feed].

Water quality parameters

Water temperature and dissolved oxygen were measured daily using an oxygen meter (YSI Model 58, YSI Industries, and Yellow Spring Instruments, OH, USA). The pH- value was monitored twice weekly using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH, USA). Total ammonia, nitrite, and nitrate were measured weekly using spectrophotometer **(**Spectronic 601, Milton Roy Company, San Diego, CA, USA) according to APHA (1998).Total alkalinity was monitored twice weekly using the titration method of Golterman *et al.* (1978). During the 12-weeks feeding trial, the mean values of water quality parameter (±SD) were: water temperature 28.3±0.4°C; dissolved oxygen 4.8± 0.2 mg/L; pH 7.3±0.2; total ammonia 0.021 ± 0.01 mg/L; nitrite 0.022±0.01 mg/ L; nitrate 0.6 ±0.2 mg/L, and total alkalinity 161±2.0 mg/L as $CaCO_3$. All water quality parameters are within the acceptable range for rearing Nile tilapia according to Boyd (1984).

Proximate chemical analysis

Samples of the experimental diets and fish were chemically analyzed to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash contents according to the methods of AOAC (2000).Nitrogen free extract (NFE) was calculated by differences, by deducting the sum of percentages of moisture, CP, EE, CF and ash from 100. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.64, 9.44 and 4.12 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 2011). Proximate analysis composition of the experimental diets was presented in Table (2).

Statistical analysis

The obtained data were subjected to two-way analysis of variance (ANOVA) to test the effect of replacement of yellow corn (0, 25and 50 %) yellow corn without or with Low and High of ZAD® respectively in tilapia diets as the two factors. Least significant difference (LSD) was used as a post hoc test to compare between means at P≤0.05. Data were analyzed by analysis of variance (ANOVA) using the SAS procedure (Statistical Analysis System, version 9.1.3, 2007). Steel and Terrie (1980) tests was used to compare differences among individual means. Treatment effects were considered significant at P<0.05. All percentages and ratio were transformed to arcsine values prior to analysis (Zar,1984).

RESULTS AND DISCUSSION

Chemical Composition of Ingredients

The proximate chemical analysis (%) of the four tested dietary energy sources (Yellow corn meal, YCM; dried olive cake ,(DOC); dried olive cake with low concentration ZAD®,(LCOC)and dried olive cake with high concentration ZAD®,(HCOC) is shown in Table (2). The chemical composition of the tested YCM, DOC ,LCOC and HCOC showed the following: (on DM basis) 89.49 , 90.91,91.31and 91.1% Dry matter,(DM);7.49, 8.48, 9.4and 9.71crude protein,(CP); 4.80, 18.24, 20.03 and 20.51 % ether extract,(EE) ; 2.14, 14.15, 12.15 and 11.58% ash; 1.40, 14.78; 83.17, 44.35 ,14.34 and12.03 % crude fiber,(CF);83.17 , 44.35 , 44.08and 46.17 % nitrogen free extract,(NFE) and 429.38, 402.733, 423.71and 438.60 Kcal/100 g diet gross energy,(GE), respectively. Dry matter content, CP, EE, ash and crude fiber of DOC, LCOC and HCOC were higher than yellow corn meal .On dry matter basis, nitrogen free extract of DOC, LCOC and HCOC were less than yellow corn meal. Also, gross energy of DOC, LCOC were less than yellow corn meal except HCOC was higher than yellow corn meal . Similar results when compared corn meal, dried dropping dates, wet dates and date stone were obtained by Labib *et al.,* (2016); Gaber *et al.* (2014 a and b); Zaki*et al.* (2012).

Table (2). Proximate chemical analysis (%) of Yellow corn meal ,(YCM), dried olive cake,(DOC),dried olive cake without or with treated with low,(LCOC) and high concentration (HCOC) on DM basis.

Chemical analysis(%)	Yellow corn Meal (YCM)	Treatments		
		DOC[1]	LCOC[2]	HCOC[3]
Dry matter (DM)	89.49	90.91	91.31	91.1
Crude Protein (CP)	7.49	8.48	9.4	9.71
Ether Extract (EE)	4.80	18.24	20.03	20.51
Ash	2.14	14.15	12.15	11.58
Crude Fiber(CF)	1.40	14.78	14.34	12.03
NFE [4]	83.17	44.35	44.08	46.17
GE (kcal/100g DM)[5]	429.38	402.73	423.71	438.60

[1]DOC=Dried olive cake .

[2]LCOC=Dried Olive cake treated with low concentration of probiotic exogenous fibrolytic enzymes ZAD®

[3]HCOC= Dried Olive cake treated with high concentration of probiotic exogenous fibrolytic enzymes ZAD®

[4]Nitrogen free extract(NFE,%)=100-(CP+EE+Ash+CF)

[5]GE (Gross Energy): gross energy calculated as 5.64, 9.44 and 4.11Kcal per gram of protein, lipid and carbohydrate, respectively.

Experimental diets

The composition and proximate analysis (%) of the seven experimental diets are shown in Table 3. The experimental diets were almost is nitrogenous (30 %) and is caloric (445 Kcal/100 g diet).The mean value of protein to energy ratio was 70.45 mg protein/Kcal gross energy.

Table (3). Feed ingredients (g 100 g^{-1}) and proximate chemical analysis (%) of the experimental diets.

Item	Experimental diets[1]						
	D1	D2	D3	D4	D5	D6	D7
Feed ingredients (g 100 g-1)							
Fish meal	25	25	25	25	25	25	25
Soybean meal	25	25	25	25	25	25	25
Wheat bran	5	5	5	5	5	5	5
Yellow corn	40	30.92	21.84	31.81	23.61	32.07	24.14
DOC	0	9.08	18.16	0	0	0	0
LCOC	0	0	0	8.19	16.39	0	0
HCOC	0	0	0	0	0	7.93	15.86
Sunflower oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vit.[2] And Min.mix.[3]	1	1	1	1	1	1	1
Methionen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Llysein	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$CaCO_3$	0.5	0.5	0.5	25	25	0.5	0.5
Total	100	100	100	100	100	100	100
Proximate analysis (%) on DM basis:							
Dry matter	87.98	87.93	87.45	86.98	86.87	86.89	86..87
Crude Protein	30.22	29.52	28.82	29.58	28.95	29.6	28.99
Ether Extract	6.58	6.68	6.31	6.71	6.38	6.72	6.41
Ash	6.14	6.3	6.19	6.31	6.21	6.32	6.22
Crude Fiber	5.77	5.07	4.37	5.14	4.51	5.16	4.55
Nitrogen free extract	51.29	52.43	54.31	52.26	53.95	52.2	53.83
GE.[4]	443.87	445.56	445.87	445.48	445.78	445.44	445.79
P:E Ratio[3]	66.25	65.03	66.45	64.94	66.40	64.64	66.25

[1]Diet No.1 a control diet without dried olive cake, diets No.2 and diet No.3 containing 25 and 50 % dried olive cake (DOC) , respectively. Diets No.4 and diet No.5 containing 25 and 50 % dried olive cake treated with low concentration of ZAD® (LCOC) of the diet, Diets No.6 and diet No.7 containing 25 and 50 % dried olive cake treated with high concentration of ZAD® (HCOC),respectively.

[2]Vitamin mixture/kg premix containing the following: 3300IU vitamin A, vitamin D410 I vitamin E,2660 mg vitamin B1,133mg vitamin B2, 580 mg vitamin B6 ,410 mg vitamin B12- 50 mg biotin , 9330 mg Colin chloride, 4000 mg vitamin C,2660 mg Inositol, 330 mg Para -amino benzoic acid, 9330 mg niacin, 26.60mg pantothenic acid.

[3]Mineral mixture/kg premix containing the following 325 mg Manganese, 200mg Iron,25 mg Copper, 5 mg Iodine, 5mg Cobalt.

[4]GE=Gross Energy: - Gross energy was calculated as 5.65, 9.45 and 4.12 Kcal per gram of protein, lipid and carbohydrate, respectively after (NRC, 2011).

[5]P/E ratio = Protein to energy ratio mg crude protein/Kcal GE.

Measurements of Water Quality Parameters

The averages of water quality parameters which include; water temperature (ºC),pH, Dissolved oxygen concentration (DO mg/l), total ammonia nitrogen (TA-N mg/l), nitrite concentration (NO_2-N mg/l) and nitrate concentration (NO_3-N mg/l) are presented in Table (4).The water quality parameters showed no significant differences (P≤0.05) in water quality parameters in all treatments. These results are in accordance with finding of Labib (2010) and Labib *et al. (*2016) and Mabrouk *et al*.(2011).

Table (4).Water quality parameters of the different experimental treatments for rearing monosex Nile tilapia (*O. niloticus*) fingerlings.

Experimental Treatments*1	Temperature (^{0}C)	pH	DO*2 (mg/L)	TAN*3 (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)
D1	26.91^a±0.05	7.90^a±0.10	5.47^a±0.58	2.20^a±0.05	0.18^a±0.01	2.40^a±0.04
D2	26.90^a±0.10	7.94^a±0.08	5.45^a±0.04	2.27^a±0.06	0.20^a±0.01	2.42^a±0.06
D3	27.10^a±0.09	7.93^a±0.09	5.55^a±0.09	2.30^a±0.10	0.17^a±0.05	2.36^a±0.05
D4	27.10^a±0.13	8.03^a±0.14	5.44^a±0.07	2.18^a±0.10	0.16^a±0.01	2.39^a±0.10
D5	26.90^a±0.04	7.80^a±0.01	5.44^a±0.09	2.32^a±0.09	0.17^a±0.04	2.35^a±0.04
D6	27.10^a±0.12	8.00^a±0.05	5.55^a±0.03	2.22^a±0.01	0.17^a±0.12	2.40^a±0.05
D7	26.90^a±0.07	8.04^a±0.06	5.53^a±0.05	2.31^a±0.06	0.18^a±0.03	2.36^a±0.10
Mean*4	26.99	7.92	5.50	2.25	0.19	2.39
C.V. %	0.11	26.96	7.95	9.16	16.66	5.37
LSD(P≤0.05)	0.048	0.33	0.53	0.489	0.069	0.303

*1Diet No.1 a control diet without dried olive cake, diets No.2 and diet No.3 containing 25 and 50 % dried olive cake (DOC) , respectively. Diets No.4 and diet No.5 containing 25 and 50 % dried olive cake treated with low concentration of ZADO® (LCOC) of the diet, Diets No.6 and diet No.7 containing 25 and 50 % dried olive cake treated with high concentration of ZADO® (HCOC), respectively.

*2DO: dissolved oxygen; *3TAN: total ammonia nitrogen; *4All the mean in the same column did not significantly different at (P< 0.001).

Growth performance , feed and nutrient utilization

The effect of DOC, LCOC and HCOC at different levels (25 and 50 %) instead of YCM on growth performance parameters and feed and nutrients utilization of Nile tilapia fingerlings are summarized in Table (5) and Table (6). Significant (P≥0.05) differences have been observed among values of total weight gain and specific growth rate (SGR%/day) of diets from 1 to 7.The results showed that the replacement of DOC (D2 and D3) ,LCOC (D4 and D5) and HCOC (D6 and D7) at different levels (25 and 50 %) with yellow corn meal gave a growth performance preferred by the control diet(D1).Also, On the other hand, DOC, LCOC and HCOC at different levels 25 % instead of YCM gave a better growth performance than 50%. The results showed that treatment of dried olive cake with low concentration of probiotic exogenous fibrolytic enzymes ZAD® (LCOC) instead of YCM gave a better growth performance(total weight gain and specific growth rate) for Nile tilapia than high concentration at 25% and 50%. Harmantepe *et al* . (2016) found that olive cake can be incorporated to diets of juvenile hybrid tilapia up to 120 g kg^{-1} without any adverse effect on fish growth and feed utilization. Also, Ighwela (2015) indicate that olive mill waste improved its growth performance value in practical feeds for Nile tilapia fry. These results agree with the findings of El-Sayed *et al*. (2006) who found that growth performance of Nile tilapia fed the control diet were significantly (P≥0.05) higher than diet with 25% pits. Also, similar results have been reported for probiotics use in diets for tilapia by El-Dakar *et al*. (2007); Salem (2008) and Carnival *et al*.(2006).

The same trend was observed for feed and nutrients utilization (protein efficiency rate, PER ;protein productive value ,PPV % and energy utilization ,EU %) with increasing replacement of DOC,LCOC and HCOC instead of yellow corn meal from 25 to 50 % ,the PER ,PPV and EU values had significantly ($P < 0.05$) decreased. On the other hand, DOC, LCOC and HCOC at the same levels 25% gave a better PER, PPV and EU values than 50%.Also, the results showed that treatment of dried olive cake with low concentration of probiotic exogenous fibrolytic enzymes ZAD® (LCOC) instead of YCM gave a better PER ,PPV and EU values for Nile tilapia than high concentration at 25% and 50%. These results are agree with the finding of El-Sayed *et al*. (2006) who found that feed and nutrient utilization of Nile tilapia fed the control diet were significantly (P≥0.05) higher than diet with 25% pits. Also, similar results have been reported for probiotics use in diets for tilapia by El-Dakar *et al*., (2007); Salem (2008) and Carnevali*et al*.(2006).

The feed conversion ratio (FCR) tended to rise with the increase of dried olive cake levels. FCR of fish fed with Diet 2 and Diet 4 was significantly lower ($P < 0.05$) compared to fish fed on D1 ,D3,D5,D6 and D7.On the other hand, feed conversion ratio has improved at DOC (D2) and LCOC(D4). On the other hand, it is possible to say that the feed conversion rate has improved for Nile tilapia, which has been fed on D2 and D4 compared with other diets. Similar results were reported by Ighwela (2015) that disagree with the finding of Harmantepe*et al* . (2016) who show any negative effect of dietary olive cake in juvenile hybrid tilapia (O. niloticus × O. aereus) and indicated that they could be used up to 120 g kg^{-1} level. Labib *et al* . (2016) found that the best feed conversion ratio, protein efficiency ratio, protein productive value and energy utilization were obtained with fish fed on diets containing 30% wet date without 0.05% Thyme leave extract replaced the corn meal followed by fish fed with 0.05% Thyme leave extract replaced the corn meal . El-Kholy (2012) found that tilapia hybrid (*O.niloticus*× *O.aureus*) fingerlings achieve increasing by low level (150 mg/ kg) dietary addition of sage herbs. The low achievement of fish fed DS (diet No. 10, 11 and 12) are documented by the work Mabrouk *et al*. (2011) with agreement of the present finding. The higher performance of tilapia fed WD may have been due to their low NFE compared with DDD or DS. In support, Yousif *et al*. (1996) noted that the poor performances of tilapia fed date may have been due to their high contents of simple sugars ,whereas tilapia are known to utilize complex sugars more efficiently than simple sugars. These results are similar to results obtained by Belal *et al*. (2015), found that there were no significant difference in growth performance and feed and nutrient utilization when feeding Nile tilapia (*O. niloticus*) fish on diets containing date fiber.

The results from the present study indicate that replacement of DOC, LCOC and HCOC instead of YCM in diets for Nile tilapia (*O. niloticus*) up to a level of 25 % without having a significant negative effect on growth (final weight, SGR) and feed utilization (FCR, PER) parameters. It was detected in this experiment that growth performance was slowed down in the fish fed with the increase of DOC level in diet. This negative effect on growth may be from high fibre, NDF, ADF, ADL, total phenolics, tannins and condensed tannin involved in DOC. It is well-known that high dietary fibre decreases growth performance. Dietary fibre influences absorption and movement of nutrients through the gastrointestinal tract. Fibre can bind nutrients such as fat, protein and minerals and reduce their bioavailability (Richter *et al.,* 2003). Quantities of NDF, ADF and ADL which are among the components of cell wall were high in diets which low growth performance Richter *et al.,* (2003) reported that they obtained low growth performance in diets with high NDF and ADF quantity. Higgs *et al.* (1982) reported that high fibre, tannin, phytic acid and glucosinolates in juvenile Chinook salmon diets cause poorer growth performance. In the studies conducted on Nile tilapia fish, Richter *et al.* (2003) reported that the increase in values of NDF, ADF, total phenolics non haemolyticsaponin and phytic acid in the diet might depress the growth, and Dongmeza *et al*. (2006) reported that the diets with high total phenolics and saponins might depress the growth. Rojas and Verreth (2003) associated the reason of decrease in the parameters of growth and feed utilization of Tilapia fed with diets including coffee pulp with high dietary fiber levels and antinutritionals in diet (K level, polyphenols, tannins and caffeine). McCurdy and March (1992) reported that sinapine and tannins reduced the digestion of proteins. Likewise, Yigit and Olmez (2011) stated that increasing fibre in tilapia diets caused the decrease in protein digestibility.

Table (5). Effect of the experimental treatments of rearing Nile tilapia *(O.niloticus)* fry on carcass composition (%).

Experimental treatments*	Dry matter (%)	On dry matter basis (%)			Energy content Kcal/100g
		Crude protein	Ether extract	Ash	
D1	28.16^{d}±0.02	57.66^{d}±0.02	22.33^{g}±0.001	20.01a±0.005	535.99g±0.19
D2	28.70^{a}±0.001	58.56^{a}±0.005	23.10^{a}±0.001	18.35^{g}±0.005	548.31a±0.03
D3	28.52^{b}±0.02	58.29^{c}±0.005	22.82^{c}±0.001	18.90e±0.005	544.15d±0.028
D4	28.64^{a}±0.01	58.51^{b}±0.001	22.97^{b}±0.001	18.52f±0.01	546.83c±0.056
D5	28.31^{c}±0.03	58.00^{d}±0.01	22.47^{e}±0.001	19.50d±0.01	539.52e±0.028
D6	28.25^{c}±0.10	57.80^{f}±0.001	22.41^{f}±0.001	19.79b±0.01	537.54f±0.001
D7	28.27^{c}±0.05	57.89^{e}±0.001	22.47^{e}±0.001	19.64c±0.10	5547.52b±0.001
Mean**	28.41	58.10	22.66	19.24	542.84
C.V. %	0.42	0.011	0.31	0.03	0.006
LSD(P≤0.05)	0.066	2.36	0.001	0.016	0.087

*Diet No.1 a control diet without dried olive cake, diets No.2 and diet No.3 containing 25 and 50 % dried olive cake (DOC) , respectively. Diets No.4 and diet No.5 containing 25 and 50 % dried olive cake treated with low concentration of ZAD® (LCOC) of the diet, Diets No.6 and diet No.7 containing 25 and 50 % dried olive cake treated with high concentration of ZAD® (HCOC), respectively.

Table (6). Effect of the experimental treatments on growth performance and feed utilization for growing of monosex Nile tilapia *(o.niloticus)* fry

Treatments*[1]	Initial Weight (g/fish)	Growth performance		Feed conversion ratio	Protein utilization		Energy utilization (%)
		Weight gain (g/fish)	SGR*[2] (%/day)		PER*[3]	PPV*[4] (%)	
D[1]	$0.94^{a}\pm0.005$	$10.26^{f}\pm0.05$	$2.76^{f}\pm0.001$	$1.96^{a}\pm0.001$	$1.69^{g}\pm0.001$	$17.64^{g}\pm0.01$	$17.64^{g}\pm0.12$
D2	$0.94^{a}\pm0.01$	$16.47^{a}\pm0.03$	$3.24^{a}\pm0.009$	$1.75^{g}\pm0.001$	$1.94^{a}\pm0.001$	$20.14^{a}\pm0.02$	$20.14^{a}\pm0.017$
D3	$0.96^{a}\pm0.005$	$13.22^{c}\pm0.05$	$2.99^{c}\pm0.001$	$1.89^{e}\pm0.001$	$1.84^{c}\pm0.001$	$18.97^{c}\pm0.001$	$18.97^{c}\pm0.001$
D4	$0.94^{a}\pm0.01$	$14.31^{b}\pm0.03$	$3.10^{a}\pm0.003$	$1.81^{f}\pm0.001$	$1.87^{b}\pm0.03$	$19.07^{b}\pm0.001$	$19.07^{b}\pm0.002$
D5	$0.95^{a}\pm0.01$	$12.21^{d}\pm0.02$	$2.92^{d}\pm0.01$	$1.93^{d}\pm0.001$	$1.79^{d}\pm0.001$	$18.02^{e}\pm0.0.01$	$18.02^{e}\pm0.017$
D6	$0.94^{a}\pm0.005$	$12.25^{c}\pm0.05$	$2.94^{d}\pm0.001$	$1.94^{c}\pm0.001$	$1.74^{f}\pm0.001$	$18.42^{d}\pm0.01$	$18.42^{d}\pm0.01$
D7	$0.94^{a}\pm0.001$	$11.28^{e}\pm0.01$	$2.85^{e}\pm0.01$	$1.95^{b}\pm0.001$	$1.77^{e}\pm0.001$	$17.80^{f}\pm0.003$	$17.79^{f}\pm0.003$
Mean*	0.94	12.86	2.97	1.89	1.80	18.58	18.58
C.V. %	1.14	0.39	0.38	1.56	0.11	0.39	0.11
LSD(P≤0.05)	0.025	0.12	0.027	0.006	0.006	0.048	0.99

*[1] Diet No.1 a control diet without dried olive cake, diets No.2 and diet No.3 containing 25 and 50 % dried olive cake (DOC) ,
respectively. Diets No.4 and diet No.5 containing 25 and 50 % dried olive cake treated with low concentration of ZAD® (LCOC) of the diet, Diets No.6 and diet No.7 containing 25 and 50 % dried olive cake treated with high concentration of ZAD® (HCOC), respectively.

*[2] Specific growth rate (%) = 100 (final weight–initial weight) /time (days).

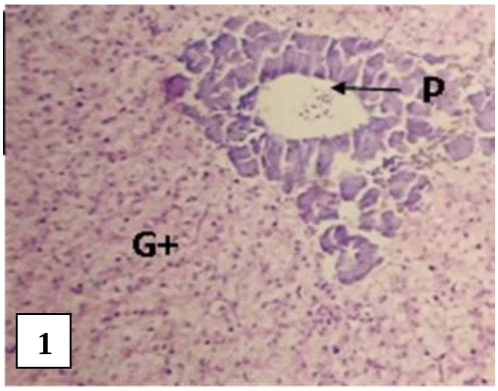
*[3] PER=Protein efficiency ratio

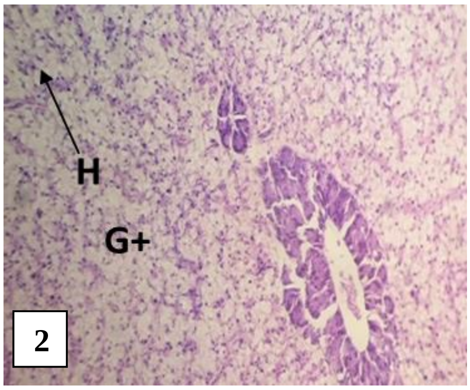
*[4] PPV=Protein productive value

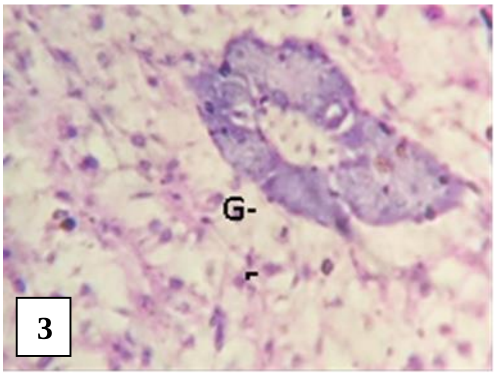
Histological Studies:

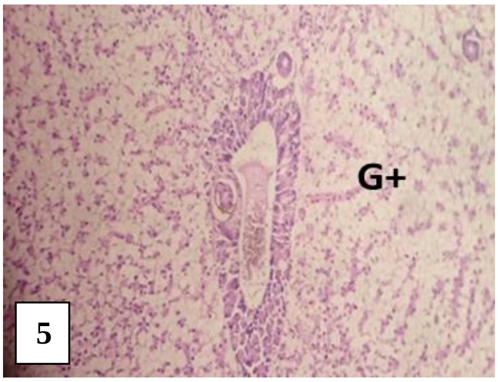
The severity of histological changes of hepatopancrease of fish fed diets contained DOC are presented in Fig (6) (1-7) and Table (10). The severity of histological changes were increased with increasing DOC inclusion level as the decrease of glycogen content and increase of hepatic changes with 50% DOC. The supplementation with ZAD® reduced the severity of changes especially with 25% DOC inclusion level.

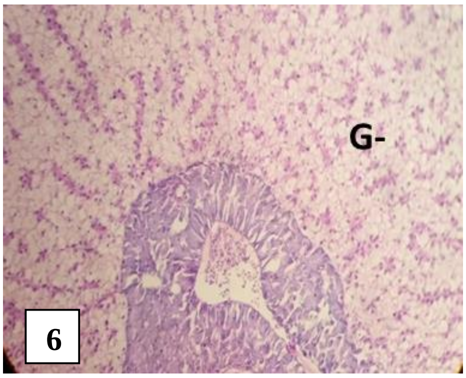
The histological investigation of intestine and histopathological degrees of Nile tilapia fed diets contained DOC with or without ZAD® supplementation Fig (7) (1-7) and Table (1**1**). The results showed that there are a marked increase in villi length, width and area of absorption in fish fed 25% DOC. Whoever, increasing DOC substitution to 50% negatively affected the histology of intestinal wall and decreasing the morphometric parameters of villi and area of absorption. In accordance, the oil cake as soya bean cake induced intestinal histological changes as width and cellular infiltration of the submucosa and lamina propria, and reduction in the supranuclear vacuolization of the enterocytes which increased with increasing the inclusion level in Atlantic salmon, Salmo salar, (Krogdahl et al., 2003). Also, Nordrum et al. (2000) showed that inclusion of soyabean meal reduced intestinal mucosal wet weight. Moreover, Van den Ingh et al. (1991) reported that there are a shortened in microvilli of enterocytes of fish fed diet contained high inclusion level of soya bean cake. Sayehban et al. (2017) reported that the inclusion of 100 g/kg olive pulp in the diets increased jejunum relative weight and jejunum length.











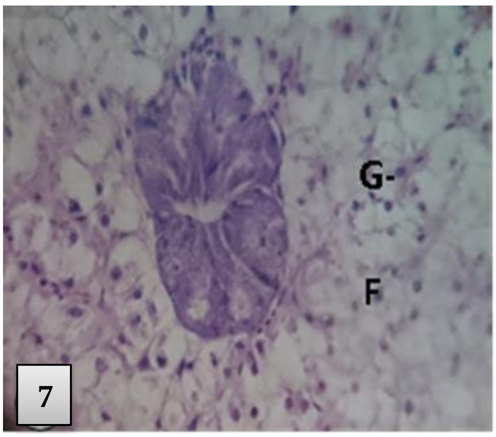


Fig.(7):Light microscopy of the hepatopancreas of Nile tilapia, Oreochromis niloticus, after feeding on different experimental diets (hematoxylin and eosin stain (X 160).

Where:(P): Pancreatic acini, (H): Hepatic cells, (G-): Low glycogen content, (G+): High Glycogen Content, (F): Hepatic fatty change.

Table (10): Effect of Yellow corn Replacer by different levels of Dried olive cake with or without ZAD® on hepatopancreas histological alteration of Nile Tilapia (Oreochromis niloticus).

Diets	Histopathological degrees			
	Hepatic and pancreatic acini	Hepatocytic Glycogen content	Hepatocytes nuclear position	Hepatic fatty change
Diet 1	_	++	In middle	_
Diet2	_	+++	In middle	_
Diet 3	_	+	In distal	++
Diet 4	_	+++	In middle	_
Diet 5	_	++	In distal	_
Diet 6	_	+	In distal	_
Diet 7	_	+	In distal	++

Where: score: (_) description : normal level
score: (+) description : low-level
score: (++) description : focal moderate level
score: (+++) description : focal extensive level.

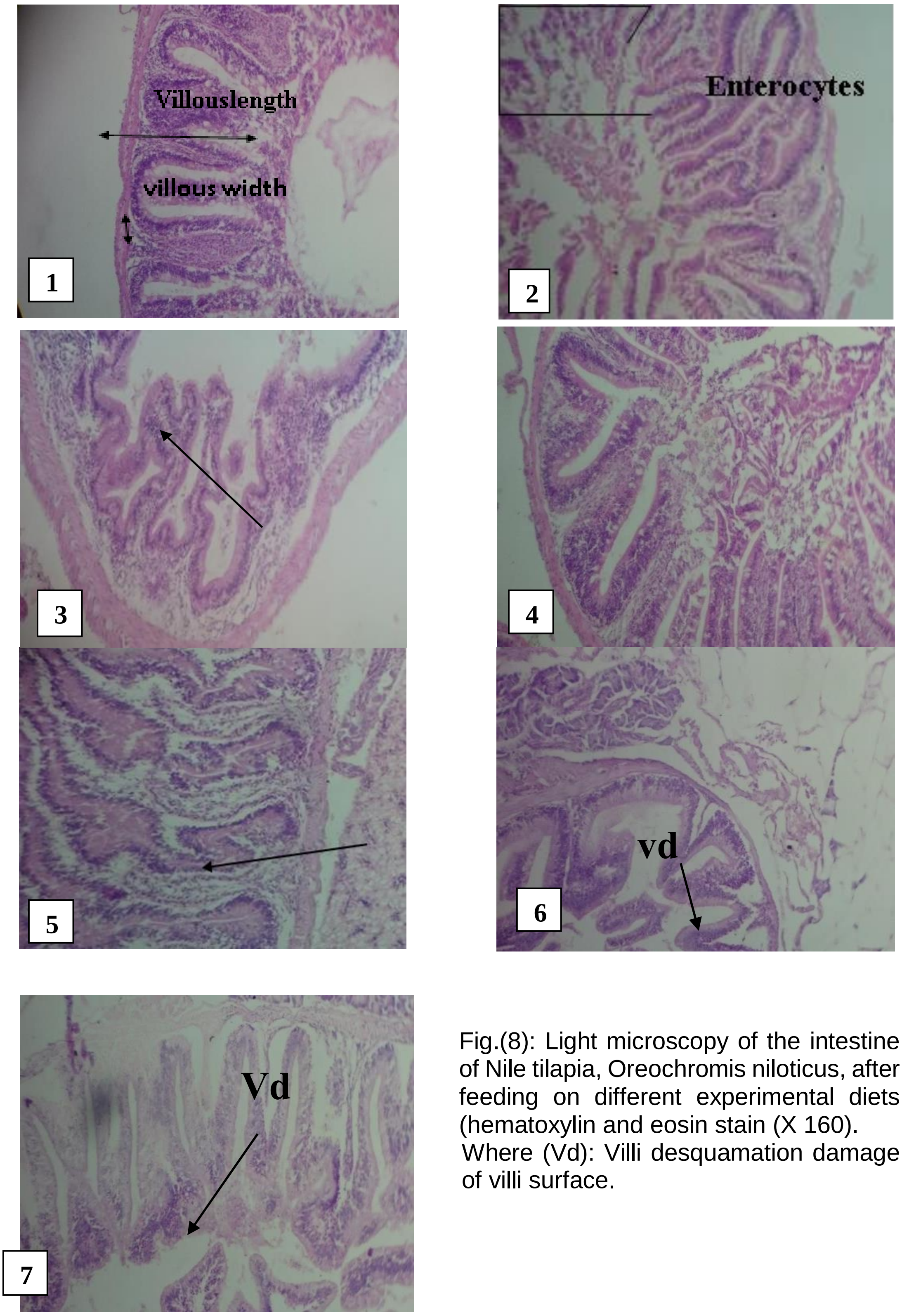


Fig.(8): Light microscopy of the intestine of Nile tilapia, Oreochromis niloticus, after feeding on different experimental diets (hematoxylin and eosin stain (X 160).
Where (Vd): Villi desquamation damage of villi surface.

Table (11): Effect of Yellow corn Replacer by different levels of Dried olive cake with or without ZAD® on Intestine histological alteration of Nile Tilapia (Oreochromis niloticus).

Diets	Histopathological Degrees			
	Villi length (µm)	Villi width (µm)	1Area of absorption (mm)	2Villi desquamation
Diet 1	910	250	227.50	_
Diet2	1230	262	322.26	_
Diet 3	530	190	100.70	+
Diet 4	1120	225	252.00	_
Diet 5	550	180	99.00	++
Diet 6	600	205	123.00	++
Diet 7	840	175	147.00	+

1 Area of absorption= (villi length x villi width) (mm)
2 Villi desquamation:damudge of villi surface.
Where :score : (-) description : normal level
score : (+) description : low level
score : (++) description : focal moderate level .

Economical evaluation of diet:

Calculations of economic efficiency of the tested diets based on the cost of feed and cost of one kg gain in weight of Nile tilapia and its ratio with the control group are shown in Table (12) the highest Cost per kg gain (L.E) were recorded for the diet No.1 (16.50 L.E) where control diet. The economic evaluation revealed the superiority of diet No. 2 which included contained 25 % dried olive cake (DOC since it provides the least cost (13.30 L.E) for producing 1 kg gain followed by diet No.4 which contained 25 % dried olive cake treated with low concentration of ZAD® (13.63 L.E). These results may indicate that the costs of one kg of diet declined by the incorporation of non-conventional energy sources due to its low price and this is in agreement with Belal et al. (2015) and Labib et al. (2016).

Table (12): Feed cost (LE) for producing one kg weight gain.

Item	Experimental diets1						
	D1	D2	D3	D4	D5	D6	D7
Cost/kg diet (LE)2	8.42	7.6	7.35	7.53	7.31	7.54	7.33
FI/fish	20.1	28.82	24.99	24.89	23.57	23.76	21.99
final weight /fish	11.19	17.41	14.18	15.24	13.16	13.18	12.22
FCR	1.96	1.75	1.89	1.81	1.93	1.94	1.95
Consumed feed to produce I kg fish (kg)3	1.80	1.66	1.76	1.63	1.79	1.80	1.80
Feed cost per kg fresh fish (LE)4	15.12	12.58	12.95	12.30	13.09	13.59	13.19
Relative % of feed cost /kg fish 5	100	83.18	85.64	81.31	86.57	89.87	87.21
Feed cost /1 kg gain (LE)6	16.50	13.30	13.89	13.63	14.11	14.63	14.29
Relative % of feed cost of kg gain 7	100	80.59	84.17	82.59	85.49	88.63	86.61
Decrease in feed cost %	0.00	9.48	22.02	-2.42	1.93	15.70	6.87

1Diet No.1 a control diet without dried olive cake, diets No.2 and diet No.3 containing 25 and 50 % dried olive cake (DOC) ,
respectively. Diets No.4 and diet No.5 containing 25 and 50 % dried olive cake treated with low concentration of ZAD® (LZDOC) of the diet, Diets No.6 and diet No.7 containing 25 and 50 % dried olive cake treated with high concentration of ZAD® (HZDOC), respectively.
2Cost of 1 kg ingredients used were 15 L.E for fish meal, 9 L.E for soybean meal, 4 L.E for wheat bran, 4 L.E for yellow corn , 0.25 L.E for dried olive cake, 0.35 L.E for dried olive cake treated with low concentration of ZAD®, 0.35 L.E for dried olive cake treated with high concentration of ZAD®, 5 L.E for sunflower oil , 2 L.E for Vit. ,and Min.4 L.E for L-Methionine and L-Lysine and 0.5 L.E for CaCo3. Egypt Feed Ingredients Price at end of 2016.
3Consumed feed to produce I kg fish) kg)=Feed intake per fish per period/ final weight per fish Kg/Kg.
4 Feed cost per kg fresh fish (LE) = Cost /kg diet (LE) X consumed feed to produce 1kg fish (kg) .
5Respective figures for step 3/ highest figure in this step.
6Feed cost /1Kg gain (LE) = Feed intake per Kg gain X Cost /kg diet (LE).
7Respective figures for step 5/ highest figure in this step.

CONCLUSIONS

From the previous results, it could be concluded that the used diets containing dried olive cake or dried olive cake treated with low concentration of ZAD® as a dietary energy source in formulation instead of 25% Yellow corn have positive effects . Finally, it could be concluded that the growth performance , feed and nutrient utilization parameters obtained at the results of the study did not show any negative effect of dried olive cake in Nile tilapia (*O. niloticus*) and indicated that they could be used up to 25 %, whether dried olive cake or dried olive cake treated with low concentration of ZAD®.

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الملخص العربي

تحسين الاستفادة من تفل الزيتون المعالج بتركيزات مختلفة من إنزيمات التحلل الخارجي (الزاد) في علائق البلطي النيلي

عبد الجليل خيرت أبو عيانة[1]، طارق محمد أحمد سرور[2] ، محمد أحمد عبد الله زكى [3]، عبدالله تاج الدين منصور[2]

[1]مركزبحوث الصحراء

[2]كلية الزراعة سابا باشا جامعة الإسكندرية

[3]كلية الزراعة الشاطبى جامعة الإسكندرية

الدراسه الحاليه تهتم بتحسين القيمة الغذائية لتفل الزيتون (مصدر من مصادر الطاقة) بالمعالجة بتركيزات مختلفة من أنزيم الزاد ليحل إحلال جزئيا محل الأذرة الصفراء (25،50% على التوالي) في تغذية زريعه أسماك البلطي النيلى وتأثيرها على أداء النمو وكفاءة الاستفادة الغذائية وتركيب الجسم والمعايير الفسيولوجية لزريعه أسماك البلطي النيلي .تم عمل تجربة تغذية في 21 حوض زجاجي بمقاس (30×40×100 سم) لمدة 84 يوم على زريعه أسماك البلطي النيلى ذات وزن متقارب بمتوسط (0.02 ± 0.9 جم / زريعه) وتم تكوين سبعة علائق تجريبية متزنة في محتواها من البروتين (30 %) في سبعة معاملات (كل معاملة ثلاثة أحواض وكل حوض يحتوى على خمسه عشر زريعه) لدراسة تأثير أحلال تفل الزيتون محل الأذرة الصفراء بمعدل (25 ، 50 % على التوالى) الجاف غير المعامل أو المعامل بتركيزين مختلفين من أنزيمات التحلل الخارجي (الزاد) وذلك في تغذية صغار أسماك البلطي النيلى وتأثيرها على أداء النمو وكفاءة الاستفادة الغذائية وتركيب الجسم .والعلائق السبع هي كالتالي : العليقة الأولى (عليقة قياسية خالية من تفل الزيتون) ، أما العلائق الثانية والثالثة فهي خاصة بتفل الزيتون الجاف الغير معالج (25 ، 50 % تفل زيتون جاف على التوالي) أما العلائق الرابعة والخامسة فهي خاصة بتفل الزيتون المعالج بالتركيز المنخفض من الزاد (25 ، 50 % تفل زيتون معالج بتركيز منخفض من الزاد(10^4خليه/مل) على التوالي) أما العلائق السادسة والسابعة فهي خاصة بتفل الزيتون المعالج بالتركيز العالي من الزاد (25 ، 50 % تفل زيتون معالج بتركيز عالي من الزاد (10^{12}خليه/مل) على التوالي).وتم تغذية الزريعه بمعدل 3 % بناء على وزن الزريعه الحية وتم تعديل مستوى التغذية كل أسبوعيين وتوزع الوجبة اليومية على مرتان يوميا . وفى نهاية التجربة تم أخذ أوزان الأسماك وتجفيفها وتقدير المادة الجافة والبروتين والدهن والرماد والطاقة مقارنة بالمعاملات المختلفة وكذلك الأسماك في بداية التجربة وتم تحديد أفضل المعاملات بناء على معايير النمو والاستفادة من الغذاء والطاقة وتحسين مواصفات لحوم الأسماك الكيميائية .

أظهرت النتائج المتحصل عليها أنه ينصح بإحلال مكونات عليقه أسماك البلطي النيلي بنسبة 25 % من تفل الزيتون الجاف أو المعالج بالتركيز المخفف من الزاد بدلا من الأذرة الصفراء وليس لها تأثير على الحالة الصحية للأسماك.