J. Agric. Sci. Mansoura Univ., 29(5): 2243 - 2252, 2004

# UTILIZATION OF OLIVE PULP BY-PRODUCTS AS AN UNCONVENTIONAL FEEDSTUFF IN DIETS OF NILE TILAPIA, *Oreochromis niloticus*,FINGERLINGS

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## ABSTRACT

A feeding experiment was conducted to evaluate the effect of olive pulp at different levels on growth performance, feed and nutrients utilization, body composition, plasma glucose, total plasma lipids (TPL) and total plasma proteins (TPP), liver enzymes and cost-benefit analysis of Nile tilapia fingerlings. Ten glass aquaria with the dimensions of 70x30x40 cm were used to stock 10 fish/aquarium averaging 9 g/fish. five experimental diets were formulated to contain 0, 5, 10,15 and 20% of Olive pulp instead of wheat milling by product. All the diets were isonitrogenous and isoenergitics. Fish fed 3-5% of body weight daily. The feed amount was given three times daily, six days a week for 77 days. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new fish weight.

*Results indicated that no significant differences* (P>0.05) were observed in growth performance among all fish groups fed varied levels of olive pulp. Yet, there was a tendency of Nile tilapia fed 5% olive pulp to increase their body weight, gain and SGR. Lower amounts of feed intake were obtained when fish fed diets had 15 & 20% olive pulp. No significant differences (P>0.05) were observed in FCR, PER, PPV% and ER% among all fish groups fed diets containing varied levels of olive pulp. However, carcass fat content and plasma glucose and plasma total lipids were increased by increasing levels of olive pulp in contrast and total plasma protein values were decreased. Economic analysis showed lower incidence cost and higher profit index by increasing olive pulp levels in Nile tilapia diets.

**Keywords:** Nile tilapia, Olive pulp, Growth, Cost-benefit analysis

## INTRODUCTION

The importance of developing cheap nutritionally balanced artificial diets for fish has been increased dramatically (Osman *et al.*, 2003). Incorporation of cheap untraditional feedstuffs, particularly energy sources in animal and fish diets may participate in solving the problem of feed shortage and alleviate the pollution problems. Olive pulp is one of the most important agro-industrial by-products that may be serve as unconventional energy sources. Olive pulp as energy source (Khamis *et al.*, 1989; Mohamed,et al .,1997 and El-Aiatt, 1999; Mousa, 2000 and 2001and Mousa and Abdel Samee, 2002). More than 95% of the estimated world's olive crops are grown in the Mediterranean area. Annual world's olive production amounts to 804 million tones, which gave rise to three million tones crude olive cake after industrial processing (Abdel-Naby, 1998). Khamis *et al.* (1989) reported that the annual available olive pulp and date seeds in Egypt were estimated to be about 7600 tones giving about 5935 tons of dry matter and about 3433 tons of total digestible nutrients.

Publications on olive pulp used in fish feeding are very little, except El-Aiatt (1999) who found that weight gain of hybrid tilapia fed 30% olive

waste diet was lower than those fed the control diet. The present study, therefore, was carried out to investigate the effect of different levels of olive pulp on growth performance, feed conversion, protein and energy efficiencies, body composition, blood analysis and cost–benefit analysis of Nile tilapia, *O. niloticus*, fingerlings.

## MATERIALS AND METHODS

**Experimental facilities:-**

This work was carried out at the Wet Lab., Animal Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Ten glass aquaria with dimensions of 70x 30x 40 cm, 70-L total net volume, were used in this study. All aquaria were filled with dechlorinated tap water stored 24 hours before use. Fingerlings of Nile tilapia, *O. niloticus* were obtained from the Maryot Fish Farming Co, Alexandria Governorate, Egypt. Fish were transported in fiberglass tanks (300 L). After arrival, all fish were kept for one week to alleviate stresses and to be adapted to the new conditions. Ten fish in the same initial weight (9 g/fish) were selected and randomly allotted into each experimental aquarium. Fish were fed the control diet for two weeks, during this period healthy fish of the same weight replaced dead one.

**Experimental diets:-**

Five experimental diets were formulated to contain 0, 5, 10, 15 and 20% of olive pulp instead of wheat milling by-product. Each diet was fed to two randomly assigned (duplicate) aquariums. Ingredients composition of the experimental diets is presented in Table (1). Fishmeal was home made by collecting small fish and non-saled fish named locally "Wazafa" from fish market to dry in oven at 60 ºC to dryness. Then dry fish were grounded and sieved prior to keep at –20 ºC until use as an animal protein source. Soybean meal 48% was used as a plant protein source. While yellow corn, wheat bran and wheat milling by-product were served as energy sources. The experimental diets were isocaloric by using sunflower oil in different levels depended on the energy content in the rest ingredients.

The experimental diets were prepared by mixing dry ingredients with water and were pelleted using a meat mincer with a 1-mm holes diameter. The pellets were air dried and stored at -20° C until use. Fish fed 5, 4 and 3 % of the body weight daily for the 1-4, 5-8 and 9-11$^{th}$ week, respectively. The feed amount was given at three times a day (9 00, 1200 and 1500 hrs) in equal proportions. Feeding was performed for six consecutive days a week, for 77days. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new weight of fish.

**Environmental conditions:-**

Fish were reared at 28±2 ˚C and 8.6±0.1 pH. Third water volume of all aquaria was exchanged daily, except the weighing day where about two thirds of water volume were changed.

**Analytical methods:-**

Diets and fish samples were analyzed according to AOAC (1990). The gross energy contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.2 kcal/g of protein, lipid and carbohydrate, respectively (NRC, 1993). Digestible energy content was calculated from standard physiological fuel values as 4 and 9 kcal/g of protein and carbohydrate and lipid, respectively (Garling and Wilson, 1976).

**Table 1: Ingredients composition of the experimental diets**

| Ingredients | Diets No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Fish meal | 15 | 15 | 15 | 15 | 15 |
| Soybean meal | 34 | 33 | 32 | 31 | 30 |
| Yellow corn | 5 | 5 | 5 | 5 | 5 |
| Olive pulp meal | 0 | 5 | 10 | 15 | 20 |
| Wheat milling by-product | 34.5 | 30.5 | 26.5 | 22.5 | 18.5 |
| Wheat bran | 5 | 5 | 5 | 5 | 5 |
| Sunflower oil | 4.5 | 3.5 | 2.5 | 1.5 | 0.5 |
| Vitamin mix.[1] | 0.875 | 0.875 | 0.875 | 0.875 | 0.875 |
| Ascorbic acid | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 |
| Mineral mix.[2] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Total | 100 | 100 | 100 | 100 | 100 |

1. Vitamin mixture (g/100 g) was 960000 IU, 160000 IU, 0.8 g, 80 mg, 0.32 g, 0.12 g, 0.8 g,, 0.8 mg 1.6 g, 80 mg, 4 mg, 40 g. of vitamin A, D3, E, K, B1, B2, B6, pantothenic acid, B12, niacin, biotin, choline chloride, respectively.

2. Mineral mixture (g/100g) was 12.75, 72.85, 0.55, 0.25, 0.02, 5, 2.5, 0.08, 0.05, 0.01 and 6 mg of $MgSO_4.7H_2O$, $CaHPO_4.2H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.4H_2O$, $CaI_2.O_6.6H_2O$, KCl, $FeSO_4.7H_2O$, $CuSO_4.5H_2O$, $CoSO_4.7H_2O$, $CrC_3.6H_2O$ and NaCl, respectively.

**Blood analysis:-**

Blood was collected using heparinized syringes from the caudal vein of all the experimental fish at the termination of the experiment. Blood samples were centrifuged at 3000 rpm for 15 minutes to allow separation of plasma which was subjected to determine plasma total protein (Armstrong and Carr, 1969), total lipid (Frings *et al.*, 1972) and glucose (Trinder, 1969). The activities of aspartate aminotransferase (AST) and alanine aminotrnsferase (ALT) were determined colorimetrically by using commercial kits (Quimica Clinica Aplicada, S.A., Aspain) according to the method of Reitman and Frankel (1957).

**Cost-benefit analysis:-**

To evaluate cost-benefit analysis of olive pulp inclusion in the diets of Nile tilapia fingerlings, incidence cost (IC) and profit index (PI) were calculated according to New (1985).

**Statistical analysis:-**

Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1982) using a completely randomized design (CRD). Differences were subjected to Duncan's Multiple Range-Test (Duncan, 1955) at a significance level of 0.05. Program of MSTAT-C was used to compute the statistical analysis (MSTAT-C, 1988).

## RESULTS AND DISCUSSION

**Nutritive value of olive pulp:-**

Data presented in Table (2) show that olive pulp used in the present study had a moderate percentages of crude protein (11.56%) and crude fiber (16.53%) with higher percentages of ether extract (21.29%) and nitrogen free extract (38.02%) while ash was 2.6% on dry matter basis. The present results are in agreement with (Abasa *et al.* 1987 and Al-Shanti, 2003) except the ether extract that was higher than those obtained by the previous studies. This may be due to differences of oil extraction methods. The percentage of olive pulp differs according to the variety and degree of maturity of the fruit (Abdel Gabber, 1979), oil extraction procedures (Al-Shanti, 2003), the presence or absence of pis and elapsed time and treatment of pulp between pressing and processing (Belidasakis; 1984). In addition some varities of olive fruit had higher oil content ranged from 43.5 to 50.82% (Kiritskis, 1998 and Azza, 2002) such these varities wasted fatty pulp in case of bad oil extraction.

**Table 2: Chemical analysis of the experimental diets.**

| Item | Olive pulp | Diet No. | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Dry matter % | 94.68 | 94.41 | 94.1 | 93.99 | 94.12 | 94.23 |
| **% on the DM basis:-** | | | | | | |
| Crude protein , CP | 11.56 | 30.48 | 30.56 | 30.64 | 30.72 | 30.81 |
| Ether extract | 21.29 | 9.20 | 9.72 | 10.23 | 10.75 | 11.26 |
| Crude fiber | 16.53 | 2.90 | 3.69 | 4.49 | 5.29 | 6.09 |
| NFE | 48.02 | 46.6 | 45.34 | 44.07 | 42.79 | 41.52 |
| Ash | 2.60 | 10.82 | 10.69 | 10.57 | 10.45 | 10.32 |
| **Calculated energy (kcal/100 g diet)** | | | | | | |
| Gross energy , GE | - | 456 | 456 | 456 | 456 | 456 |
| Digestible energy, DE | - | 391 | 391 | 391 | 391 | 391 |
| P/E ratio mg CP/kcal DE | -- | 77.95 | 78.16 | 78.36 | 78.57 | 78.8 |

**Growth performance and surviving:-**

Survival rate of all fish groups fed varied levels of olive pulp was100%. Data presented in Table (3) show the growth performance and feed and nutrient utilization of fish fed different levels of olive pulp. Results confirm a great tendency of Nile tilapia fed 5% olive pulp to increase body weight, gain and SGR. However, statistical analysis showed that no significant differences ($P>0.05$) among all fish groups fed varied levels of olive pulp. Results in the present study agree to a satisfactory extent with those of Tortuero *et al.* (1989); Morani (1990); El-Kerdawy (1997); Mousa and Abdel-Samee (2002) and Mousa and Shetawi (2002). In poultry feeding, Al-Shanti (2003) found that chicks fed diet contained 5% of either extruded full-fat soybean or olive cake had significantly higher weight gain values and economic efficiency. While in fish feeding, El-Aiatt (1999) used higher levels of olive pulp in tilapia diets. He found that percentage of gain in weight decreased by the replacement of wheat flour in diets by 30% olive waste to about 62% than the control diet. The present work confirmed the above findings.

**Table 3: Growth, survival rate, feed and nutrient efficiencies utilization of Nile tilapia fingerlings fed diets containing different levels of olive pulp.**

| Item | | Diet No. | | | | | MSE[1] |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| Initial weight | g/fish | 9.74 | 9.77 | 9.72 | 9.74 | 9.74 | 0.01 |
| Final weight | g/fish | 39.94 | 41.05 | 38.5 | 37.9 | 37.85 | 0.51 |
| Gain[2] | g/fish | 30.2 | 31.29 | 28.78 | 28.16 | 28.12 | 0.51 |
| SGR[3] | % /day | 1.83 | 1.87 | 1.79 | 1.77 | 1.77 | 0.02 |
| Survival rate | % | 100 | 100 | 100 | 100 | 100 | 0.00 |
| Feed intake | g/fish | 58.9$^{ab}$ | 59.82$^{a}$ | 56.75$^{bc}$ | 55.65$^{c}$ | 54.25$^{c}$ | 0.77 |
| FCR[4] | | 1.85 | 1.8 | 1.86 | 1.87 | 1.82 | 0.01 |
| PER[5] | | 1.79 | 1.82 | 1.76 | 1.75 | 1.79 | 0.01 |
| PPV[6] % | | 29.38 | 30.85 | 28.54 | 28.71 | 26.72 | 0.67 |
| ER[7] % | | 18.87 | 20.35 | 17.76 | 17.96 | 17.6 | 0.47 |

*Values in the row having a common superscript letter are not significantly different (P>0.05).

1. ***Standard error of the means derived from the analysis of variance***
2. **Gain =** ***(final weight - initial weight).***
3. **Specific growth rate =** ***100(Ln final weight - Ln initial weight)/ day).***
4. **Feed conversion ratio =** ***DM intake/weight gain.***
5. **Protein efficiency ratio =** ***weight gain/protein intake.***
6. **Productive protein value =** ***100(protein gain / protein intake).***
7. **Energy retention % =** ***100 x (gross energy gain / gross energy intake).***

**Feed intake and feed utilization:-**

The results of feed intake under the present study (Table, 3) indicated that fish fed diets containing 5% of olive pulp exhibited the highest feed intake. However, lower amounts of feed intake were obtained when fish fed diets had 15 and 20% olive pulp. These results are in agreement with those obtained by many researchers (Razzaque and El-Sheikh, 1982 and Youssef *et al.*, 2001). It was clearly shown that, no significant differences (P>0.05) were observed in feed conversion ratio, protein efficiency ratio, productive protein value and energy retention among all fish groups fed diets containing varied levels of olive pulp. Razzaque and El-Sheikh (1982) found that the use of olive cake did not show any significant effects on feed intake and FCR. This means that the animal fed the ration containing olive pulp consume normally due to a good palatability of olive pulp (Khamis *et al.*, 1989). However, Omar *et al.* (1995) observed that increasing olive pulp levels (0, 5, 10, 15 and 20%) impaired FCR, especially of lambs at the high levels of 15 and 20%.

Results in the present study indicated slightly improvement of feed and protein and energy utilization when fish fed 5-10 % olive pulp. It may be due to the nature of olive pulp oil which had an adequate amount of unsaturated fatty acid (HUFA), it consists mainly of about 96% HUFA (Zaza *et al.*, 2001). The mixture of unsaturated fatty acids has been determined as essential for growth (Murray *et al.*, 1991). Furthermore, Klatt (1986) stated that dietary unsaturated fatty acids are the subjects of current interest because they have been credited with number of beneficial effects.

**Body composition:-**

Data in Table (4) show the body composition of fish fed varied levels of olive pulp. No significant differences (P>0.05) were observed in crude protein and ash content. However, ether extract content of fish fed 5% olive pulp had higher values followed by fish fed the control diet than the other treatments. Similar trend was noticed with energy content. Also, fish at the start of the experiment had lower crude protein, ether extract, ash and energy contents, but they had higher moisture than at the end of the experiment. A significant of fat content of fish fed olive pulp may be due to its higher oil content, which affected deposition of lipid in fish tissue. Increasing dietary oil in fish diets resulted in a higher fat content in many species of fish (El-Dakar *et al.*, 1988 and Shalaby, 1998).

**Table 4: Body composition (% fresh weight) of Nile tilapia fed different levels of olive pulp[ns].**

| Diets | Initial fish | Olive pulp levels (%) | | | | | SEM* |
|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 | |
| Moisture | 76.47 | 73.36 | 72.42 | 74.11 | 73.35 | 73.63 | 0.33 |
| Crude protein | 13.45 | 15.76 | 16.13 | 15.5 | 15.64 | 15.2 | 0.26 |
| Ether extract | 5.9 | 6.67 | 7.16 | 6.11 | 6.2 | 6.16 | 0.19 |
| Ash | 3.85 | 4.13 | 4.29 | 4.26 | 4.32 | 4.31 | 0.04 |
| Energy, Kcal/100 g | 99.67 | 152 | 159 | 145 | 147 | 144 | 3.64 |

**ns means not significant**

*** Standard error of the means derived from the analysis of variance.**

**Blood parameters:-**

Table (5) shows that plasma gluccse, total plasma protein and total plasma lipid were affected by different levels of olive pulp. It was clear that increasing of olive pulp levels in tilapia diets resulted in increases in plasma glucose and total lipids. On the other hand, olive pulp gave a decrease in total plasma protein. Similar results were obtained by Mousa (2000) and Al-Shanti (2003). Aspartate aminotransferase (AST) showed significantly (P>0.05) higher activity in fish fed the control diet. However, it decreased dramatically as olive pulp increased in the experimental diets. At the same time, alanine aminotransferase (ALT) did not show significance in all fish groups. These results suggested that olive oil may influence through its essential oil to improve liver function. Mousa (2000) demonstrated that no significant differences were observed in adrenal, liver and kidney functions as well as serum total protein, albumin and globulin when lambs fed different levels of olive pulp. In addition, total lipid and cholesterol increased significantly (P<0.05) by 15 and 23% respectively in growing lambs fed 25 and 30% olive pulp.

**Table 5: Blood parameters of Nile tilapia fed different levels of olive pulp.**

| Item | Experimental Diets | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Plasma glucose mg/dl | 53.12 | 52.72 | 55.48 | 63.58 | 79.47 |
| Plasma total protein g/dl | 5.31 | 3.90 | 3.89 | 3.37 | 4.52 |
| Plasma total lipid g/dl | 3.79 | 5.53 | 6.30 | 6.17 | 6.76 |
| AST[1] U/dL | 154 | 140 | 133 | 121 | 114 |
| ALT[2] U/dL | 62 | 60 | 57 | 50 | 47 |

1- Aspartate amiontransferase 2- Alanine aminotransferase

**Cost-benefit analysis:-**

Cost-benefit analysis of fish fed olive pulp is presented in Table (6). Results indicated that feed cost per kg weight gain of fish (incidence cost) decreased by 96, 97, 96 and 91% of the control diet for fish fed diets containing 5, 10, 15, and 20% olive pulp, respectively. In contrary, change in profit index ranged between 3 and10% more than those fed the control diet. Lower incidence cost and higher profit index was obtained at 20% olive pulp. Similar results were obtained by Mousa and Abdel-Samee (2002) and Abdel-Ghaffar (2002) who demonstrated that olive pulp replacement up to 20% of rabbit diets decreased feed cost per kg body weight gain by 18%, it increased considerably the economical efficiency.

Generally, it could be recommended to use safely olive pulp at 5-20% in fish feeding.

**Table 6: Cost-benefit analysis of Nile tilapia fed different levels of clive pulp.**

| Item | Diet No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Cost / kg feed | | | | | |
| Incidence cost[1] | 4.60 | 4.43 | 4.48 | 4.41 | 4.21 |
| Change % | 100 | 96 | 97 | 96 | 91 |
| Profit index[2] | 1.30 | 1.36 | 1.34 | 1.36 | 1.43 |
| Change % | 100 | 105 | 103 | 105 | 110 |

1- Incidence cost = feed cost to produce 1 kg fish
2- Profit index = value of fish /cost of feed consumed, 1 kg fresh fish equals 6 LE
Cost of 1 kg olive pulp was 0.10 LE.
Costs were as common commercial feeds in local markets (Prices in LE).

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## الاستفادة من تفل الزيتون كمصدر علفى غير تقليدي فى تغذية اصبعيات البلطـــى النيلى

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قسم الإنتاج الحيواني – كلية الزراعة-جامعة الإسكندرية

في تجربة غذائية أجريت لدراسة تأثير إضافة مستويات مختلفة من تفـل الزيتـون علـى النمـو والاستفادة من الغذاء والعناصر الغذائية، والجلوكوز والدهون الكلية والبروتينات الكلية للبلازما، والـتركيب الكيماوى لجسم الأسماك وتحليل التكلفة– الربح لأسماك البلطى النيلى. استخدمت عشرة أحواض زجاجيـة بأبعاد ٧٠ X ٣٠X ٤٠سم لتخزين عشرة أسماك لكل حوض بمتوسط وزن ٩جم/سمكة. تم تركيب اربعـة علائق تجريبية متزنة فى البروتين و الطاقة لتحتوى على صفر ، ٥ ،،٠ ، ١٥ ،٢٠ % من تفل الزيتـون. قدمت العلائق ثلاثة مرات يوميا فى ستة أيام كل أسبوع لمدة ٧٧ يوما. وكانت الأسماك توزن كل أسـبوعين ويعدل على أساسها كميات الغذاء.

أشارت النتائج أنه على الرغم من عدم وجود اختلافات معنوية فى أداء النمو للأسـماك المغـذاه على مستويات مختلفة من تفل الزيتون، إلا أن الأسماك التى تغذت على ٥% تفل زينون كانت تميـل الـى إحداث زيادة خفيفة فى وزن الجسم والزيادة الوزنية ومعدل النمو النوعى. كما أن كميات الغـذاء المـأكول الأكثر انخفاضا قد لوحظت فى حالة الأسماك التى تغذت على ١٥، ٢٠% تفل زيتـون . وأنـه لا يوجـد اختلافات معنوية فى معدل التحويل الغذائى والكفاءة النسبية للبروتين وكفاءة احتجاز الطاقة والبروتين بيـن جميع الأسماك التى تغذت على المستويات المختبرة من تفل الزيتون . فى حين سجلت زيادة كل من دهـون الجسم وجلوكوز البلازما ودهونها الكلية بزيادة مستويات تفل الزيتون فى علائق المختبرة وعلـى العكـس انخفضت قيم البروتينات الكلية فى البلازما.

كما أظهر التحليل الاقتصادي الى انخفاض التكلفة الغذائية لانتاج ١كجم سمك وزيادة دليل الربحيـة بزيادة مستويات تفل الزيتون فى علائق البلطى النيلى.