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**RESEARCH ARTICLE**

# Effect of Olive Leaves and Propolis Extracts on Growth Performance, Immunological Parameters and Economic Efficiency using Nile Tilapia (*Oreochromis niloticus*)

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**Abstract**

This study aimed to evaluate the effects of olive leaves ether extract (EEOL) and ethanol extract of propolis (EEP) on growth performance, body composition, nutrient digestibility, immunological parameters and economic efficiency in Nile tilapia, *Oreochromis niloticus*. Three Isonitrogenous (crude protein 35%), isocaloric (Digestable energy, 2900kcal) diets were fed to Nile tilapia averaging 19.7 ±0.94 g. A basal control diet had no additives, second and third diets containing 0.1% EEOL (1g/kg) and 0.4% EEP (4 g/kg) for successive 12 weeks. Results revealed that the dietary addition of EEOL and EEP had significant effects on growth performance parameters and protein utilization (P≤0.029). In addition, dietary supplement of EEP was more prominent than with EEOL and propolis extracts on fish final body gain and protein utilization. The carcass content from dry matter, protein and fat contents recorded higher values in EEOL or EEP supplemented groups. Furthermore, nutrient digestibility was improved by adding EEOL and EEP. The total leucocytes count, lymphocytes %, monocytes %, phagocytic activity, plasma total protein and globulin showed higher levels in fish groups with dietary addition of EEOL and EEP extracts than in fish fed the control diet.

In terms of economic analysis, EEOL and EEP extract diet revealed the lowest cost per kg live weight of fish, along with the highest economic return and net profit. Addition of EEOL or EEP extracts to diet resulted in a more economically efficient production than in fish fed the control diet. Our results suggested that the dietary inclusion of EEOL (1g/kg) or EEP extracts (4g/kg) markedly enhanced growth performance, body composition, feed digestibility, immune status and economic efficiency using Nile tilapia.

**Keywords:** Growth Performance, Body Composition, Nutrient Digestibility, Immunity, Economic Efficiency, Costs of Fish.

## Introduction

Fish is an economic source of animal protein compared to other animal protein sources. In most developing countries, fish comprising about 30% of the total animal protein consumption per capita [1] as it accounts for over 50% of the total intake from animal protein [2], especially that it is a virtually unique and rich source of omega-3 fatty acids [3]. Nile tilapia, *Oreochromis niloticus*, is a highly popular fresh water fish due to its rapid growth rate and capacity to develop in unusual and undesirable conditions. In Egypt, aquaculture are emphasized and there are many restrictions that threaten rapid growing and sustainable development of aquaculture industry, such as costs of production, feed availability, and technologies lack for feed manufacture [4]. On the other hands, the vast use of antimicrobials, for not only treatment and control of diseases but also as growth promoters, in domesticated animals and fish increased the probability of bacterial

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resistance, the development of drug-resistant microorganisms and antibiotic deposits in fish and environment [5]. In addition, antibiotics lead to changes in the normal fish-gut flora, which is beneficial to the fish [6]. Enhancement of the immune system is a promising method for controlling fish diseases [7], avoiding the undesirable effects of antibiotics. In fact, the use of immunostimulants is, by far, a safer alternative for enhancement of fish non-specific immune response, antibody production and/or up-regulation of inflammatory response [8, 9]. Natural immunostimulants are biocompatible, eco-friendly and safe for human health as well as environment [10]. Examples for these natural immunostimulants are olive leaves extract (of plant origin) and propolis (of animal origin). Extracts of olive leaves contain important compounds such as oleuropein and poly-phenolic compounds. Oleuropein has various pharmacological and health promoting properties including anti-arrhythmic, spasmolytic, immune-stimulant, cardio-protective (by inhibiting low-density lipoprotein oxidation), hypotensive, anti-inflammatory (responsible for inhibition of 5-lipoxygenase enzyme), hypoglycemic, antiviral, cytostatic and an enzyme-modulator effect due to its antioxidative properties, its antioxidant capacity estimated to be 400% higher than vitamin C and almost double that of green tea or grape seed extract [11- 13]. Poly-phenolic compounds, on the other hand, affect the growth of probiotic bacteria and other microorganisms. For example, they can prevent the growth of food pathogens and food-spoiling microorganisms [14].

Propolis, a green–brown viscous substance produced by bees and mixed with secreted beeswax, is a multifunctional composite utilized by bees in building, maintenance and security of their hives [15]. Propolis extract has been identified to have more than 300 compounds. The key active of propolis extract components are flavonoids and phenolic acids, of about 25–30%, with many pharmacological and biological actions as antimicrobial, anti-inflammatory, anti-allergic and, immunopotentiation, vasodilator and antitumor effects, as well as, propolis has been used as a natural immune and growth promotor [16, 17].

Studies aiming at enhancing both health and productive efficiency of fish are of great importance. Given the fact that Nile tilapia is considered to be the most intensively cultivated freshwater fish in Egypt and, as far as we know, there is no available information on the use of olive leaves extracts and insufficient information on the use of propolis in Nile tilapia. Therefore, our objectives aimed at testing the effect of extracts of olive leaves and propolis on growth promotion, body composition, nutrient digestibility, immunological parameters and their economic efficiency using Nile tilapia.

## Materials and Methods

The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

### *Experimental Fish and Culture*

A total of 180 Nile tilapia *Oreochromis niloticus*, were purchased from central laboratory for aquaculture research, Abbassa, Abu-Hammad, Sharkia, Egypt). There were divided into equal three triplicate groups (each replicate contained 20 fish). The fish were stocked in clean concrete pond (3x1x1 m), and the replicate of each fish group was stocked in its corresponding cage for two weeks to be acclimatized before starting the experiment. The cement ponds were supplied with dechlorinated water and air supplied by a large air pump. Water temperature was 29 ±0.5ºC, and dissolved oxygen (Oxygen meter, YSI Company model 56, Yellow Springs, OH, USA) was 5.7 ±0.02 mg/L and were measured once a day, while pH was 7.5 (Orion pH meter; Abilene, TX, USA). The fish groups were kept under photoperiod of 14 L: 10 D. The mean water temperature, dissolved oxygen found to be 26.3±1 ºC, 5.4±0.05 mg/l, pH, ammonium (NH4), nitrite and nitrate were measured and found to be 6.9 ±0.04, 0.3 mg/l, 0.035 mg/ l and 5.6 mg/ l, respectively.

### *Preparation of Ethanol Extract of Propolis (EEP)*

EEP was prepared according to [18]. Briefly, crude propolis was obtained from a bee farm. EEP was made by addition of absolute ethanol (30 ml) to 3 g propolis in air-sealed containers, which were constantly stirred in darkness at room temperature for 24 hrs. The extract was

then purified two times, dried and stored in air-sealed bottles at 4ºC until use.

### *Preparation of Ethanol Extract of Olive Leaves (EEOL)*

Fresh herbal olive leaves were harvested, cleaned and dried at 37°C for 3 days and then grounded well. Dried herbal powder was then soaked in 70% ethanol (1:1 ratio) for 48 hrs [19]. Later, the solvent-extract was filtered with filter paper and centrifuged for 5 min at 5000 rpm. In order to obtain dried extract, first the solvent was removed by rotator evaporator (RE-301, China) at 40°C, then solvent free extract was dried by using freeze drier system (Operon: fdb-5503, Korea), according to the previously described method [20]. Finally, the freeze dried herbal extract was stored at 4°C in air-sealed bottles until use.

### *Experimental diets and feeding trial*

Three isonitrogenous (CP, 35%), isocaloric (DE, 2900 kcal) experimental diets were formulated; Control, EEOL and EEP with no, 1g/kg EEOL and 4g/kg diet EEP, respectively (Table 1). Separate dietary ingredients were grounded, then weighted according to the diet formula and the previous fed additives added by such concentration, thoroughly mixed and finally manufactured into pellets of suitable size (< 1mm in diameter, that should be easily consumed by fish) using a pelleting machine. The diets were air dried and stored in air-sealed plastic bags and kept at 5˚C until used. Fish groups were manually fed their respective diets 3 times/day (at 09:00 h AM, 12:00 h PM and 15:00 h PM) at a level of 3% of body weight till satiation. Fish from each replicate in each group were weighted every three weeks and feed allowance was adjusted accordingly. To minimize stress after weighting, feeding was discontinued for 24 hours [21].

**Table1: Dietary formulation and proximate composition of the basal diet for Nile tilapia, (*Oreochromis niloticus*)**

| Ingredients | % | Proximate analysis | % |
|---|---|---|---|
| Fish meal | 18 | Dry matter | 91.60 |
| Poultry-byproducts | 14 | Crude protein | 35.10 |
| Soy bean meal | 18 | Ether extract | 10.07 |
| Yellow corn | 31.7 | Crude fiber | 3.12 |
| Corn gluten | 5.2 | Ash | 7.40 |
| Wheat bran | 6 | Nitrogen free extract | 34.28 |
| Vegetable oil | 5 | Calcium | 1.55 |
| L-Lysin HCL | 0.2 | Phosphorus | 1.16 |
| D L-Methonine | 0.2 | Lysin | 2.13 |
| Calcium dibasic phosphate | 0.5 | Methionine | 0.93 |
| Vitamin & mineral premix* | 1.2 | DE (Kcal/ kg)** | 2927 |

*Vitamin and Mineral mixture (alfakema): Each 1 kg contains, Vit. A 580000 IU, vit. D3 8600 IU, Vit. E 720 mg, Vit. K3 142 mg, Vit. C 0.1 mg, Vit. B1 58 mg, Vit. B2 34 mg, Vit. B6 34 mg, Vit. B12 58 mg, Folic acid 86 mg, Pantothenic acid 8 mg, Manganese sulfate 65 mg, Zinc methionine 3000 mg, Iron sulfate 2000 mg, Copper sulfate 3400 mg, Cobalt sulfate 572 mg, Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (Carrier substance) till 1000 g. ** Digestible energy calculation based on values of protein 3.5 kcal/g, fat 8.1 kcal/g, NFE 2.5 kcal/g. According to Santiago *et al.*, [52]

### *Digestibility trial*

At the end of the 12 weeks, apparent digestibility for the 3 groups was determined by addition of chromic oxide at a rate of 5 g/kg diet (0.5%) as a digestibility marker, as previously described [22]. Fish, randomly selected, of each group were reared in separate glass aquaria (15 fish/ aquarium, 150x40x30 cm) which contained aerated dechlorinated water (26-29 °C). Fish were manually fed, until apparent satiation, the diets containing chromic oxide two times/day (at 10:00 h AM and 14:00 h PM) for one week as an adaptation period, then for two weeks as a collection period. The feces were carefully collected at 18:00-19:00 h using a fine net. The collected feces from each group were dried at 70 °C for 72 h then at 105 °C for 3 hrs. After that, dried feces were packed in air-sealed plastic bags and kept in a dry cool place until digestibility analyses.

***Chemical analysis***

Proximate chemical analyses were made for diet ingredients and for a sample of fish at the beginning and at end of the experiment, according to the standard methods for digestibility analysis [23], Chromic oxide content of each diet and of the collected feces were analyzed using the acid digestion method described previously [24]. Protein, fat and fiber content were also determined in the feces to assess protein, fat and fiber digestibility.

***Calculation of growth indices and digestibility***

Fish was weighted individually in each treated group to obtain: average weight gain, weight gain %, Feed intake, feed conversion ratio, specific growth rate, protein efficiency ratio, and protein retention efficiency, as well as condition factor and survival rate were calculated according to Zehra *et al.*, [25]

Apparent Digestibility Coefficient (DC) of dry matter and nutrients of each diet were calculated from the following equations, according to NRC [26].

*Digestibility DM (%) = [(% indicator in feces - % indicator in feed) / % indicator in feces] **

*Digestibility nutrient (%) = [100 – {(% indicator in feed / % indicator in feces) * (% nutrient in feces / % nutrient in feed)}] * 100*

***Blood parameters' determination***

At the end of each experiment, blood samples obtained from the caudal vessels, were collected from a 5 random sample of fish/group, into a clean sterilized tube containing EDTA as an anticoagulant. A portion of blood sample was used for determination of total leukocyte count (TLC) and differential leukocyte counts. Plasma was obtained from the remaining portion by centrifugation (3000 rpm for 10 min) directly after collection and then stored at -20ºC till further analyses.

Plasma total protein concentration (g/dl) was determined by the biuret method as described by Henry [27], and albumin concentration (g/dl) was measured according to the method mentioned by Doumas *et al.*, [28] using a commercially available diagnostic kit (Spinreact Co., Santa Coloma, Spain). The difference between total protein and albumin was considered to be globulin concentration (g/dl). The blood analysis was done in biochemistry lab at faculty of veterinary medicine, Zagazig University

***Determination of phagocytic % and phagocytic index***

A portion of blood sample was carefully layered above an equal quantity Ficoll-Paque™ PREMIUM (density 1.077 g/ml; GE Healthcare), and the isolation of mononuclear blood cells followed manufacturers' instructions. The phagocytic activity of the mononuclear blood cells was assessed in 24-well gelatin-plasma coated plates using Tetramethyl-Rhodamine-Isothiocyanate (TRITC) labeled *Candida albicans*. *Candida albicans* WDCM 00054 Vitroids was purchased from sigma. The number of phagocytic cells was determined according to method described by Goddeeris *et al.,* [29] with slight modifications, in brief 300 µl RPMI-1640 (supplied with antibiotics and 10% pooled serum of the same fish group) and TRITC labeled *C. albicans* (at ratio 10 yeast/cell) were added to each well. The plate was incubated for 30 min at 37ºC in humidified $CO_2$ incubator. After incubation, the cells were washed 3 times with cold PBS, and then fixed with 300 µl of cold freshly prepared 2% paraformaldehyde in PBS. The cells were observed under inverted Microscope. The total number of phagocytic cells was counted and the number of the *C. albicans* per mononuclear cell was counted. The test was done by the help of immunologist Dr/ hossam Abdullah in department of bacteriology , faculty of veterinary medicine, zagazig university**.**

- *Phagocytic % = Number of mononuclear cells p hogocytized C. albicans / Total number of mononuclear cells*
- *Phagocytic index = Total number of C. albicans in 100 mononuclear cell / 100*

***Calculation of economic efficiency***

The economic efficiency was calculated from the input-output analysis as per the prevailing market price of the experimental diets and fish at the time of the experiment, according to El- Rafa, [30] as follows:

EE= NP/AVC x 100 where, NP is the net profit and AVC is the average variable costs.

The following indices were also calculated: feed cost (FC), total costs (TC), total return (NR), net profit (NP) as the following equation:

AVC= average variable costs which equal feed costs

AFC= average fixed cost (fixed in all groups, which consists of labor, medical treatment, selling price of fish)

TC (total costs) = AVC+AFC

TR (total returns) = selling return from fish

NP= TR-TC

***Statistical analyses***

Effect of different diets on all over growth performance parameters was evaluated using was evaluated using one-way analysis of variance (ANOVA), and Post hoc comparisons whenever appropriate, using Duncan's test. The analysis of variance revealed that difference between replicate are non-significant. All statistical procedures were performed using PASW statistics 18 (SPSS Inc., USA). Statistical significance was considered at $P \leq 0.05$.

**Results**

***Growth indices and digestibility***

There were significant higher fish final weight, weight gain, protein efficiency ratio and protein retention efficiency and lower feed intake was found in fish groups fed diets containing ethanol extract of olive leaves (EEOL) and ethanol extract propolis (EEP) (Table 2). The dietary supplementation of EEOL and EEP significantly increased weight gain %, condition factor (K), specific growth rate and improved feed conversion ratio than in the control group, however no significant differences were observed between EEP and EEOL groups. Fish survival in this study ranged from 92.50% to 94.58% and was not significantly different among the dietary treatments.

Diets had significant effects on digestibility of DM, CP, fat and crude fiber ($P \leq 0.002$; Table 2). The highest digestibility of DM, CP and fat was obtained in group supplemented with EEP followed by the group supplemented with EEOL and finally the control one ($P < 0.05$). Also, the digestibility of CF was significantly higher ($P < 0.05$) (in groups supplemented with EEP and EEOL 87.73- 88.77) respectively, than in the control group (84.82), with no significant difference between EEP and EEOL groups.

**Table2: Effect of dietary supplement with olive leaves extract or propolis extract on all over growth performance and nutrient digestibility of Nile tilapia (mean ±SE).**

| Parameters | Control group | Olive group | Propolis group | P-value |
|---|---|---|---|---|
| Initial BW (g) | 19.73±0.15 | 19.47±0.95 | 19.83±0.46 | 0.08 |
| Final BW (g) | 38.01±0.9$^{c}$ | 44.49 ±0.67$^{b}$ | 48.27±1.07$^{a}$ | 0.05 |
| Body gain (g) | 18.28±0.85$^{c}$ | 25.03 ±0.90$^{b}$ | 28.44±0.75$^{a}$ | 0.001 |
| Body gain (%) | 92.82±4.1$^{b}$ | 129.48 ±1.06$^{a}$ | 143.44±3.5$^{a}$ | 0.005 |
| Specific growth rate (%) | 0.78 ±0.02$^{b}$ | 0.99 ±0.05$^{a}$ | 1.06±0.06 $^{a}$ | 0.008 |
| Feed intake (g) | 48.68±0.44$^{a}$ | 44.50 ±0.84$^{b}$ | 41.38±1.23$^{c}$ | 0.006 |
| Feed conversion ratio | 2.66±0.16$^{a}$ | 1.77±0.11$^{b}$ | 1.45±0.09$^{b}$ | 0.001 |
| Condition factor | 1.67±0.98$^{b}$ | 2.21±0.21$^{a}$ | 2.29±0.10$^{a}$ | 0.003 |
| Protein efficiency ratio | 1.07±0.05$^{c}$ | 1.60±0.06$^{b}$ | 1.96±0.10$^{a}$ | 0.005 |
| Protein retention efficiency (%) | 16.02±0.20$^{c}$ | 27.46±0.85$^{b}$ | 36.85±2.00$^{a}$ | 0.005 |
| Survival (%) | 92.50±1.10 | 94.58±1.10 | 94.17±1.10 | 0.07 |
| Nutrient digestibility % | | | | |
| Dry matter | 73.57±0.13$^{c}$ | 78.79±0.20$^{b}$ | 80.18±0.07$^{a}$ | 0.001 |
| Crude protein | 86.42±0.23$^{c}$ | 90.02±0.15$^{b}$ | 92.29±0.09$^{a}$ | 0.001 |
| Fat | 93.58±0.05$^{c}$ | 95.54±0.21$^{b}$ | 96.49±0.12$^{a}$ | 0.001 |
| Crude fiber | 84.82±0.55$^{b}$ | 87.73±0.41$^{a}$ | 88.77±0.38$^{a}$ | 0.001 |
| $Cr_2O_3$ in diet | 0.51 ±0.01 | 0.51 ±0.00 | 0.50 ±0.00 | 0.09 |
| $Cr_2O_3$ in feces | 1.92 ±0.02 $^{c}$ | 2.42 ±0.018 $^{b}$ | 2.54 ±0.0 $^{a}$ | 0.001 |

Within-the same row different superscript letters denote significant difference ($P<0.05$).
*****Initial body composition at the beginning of the experiment

### *Whole body composition*

Dietary supplementation of EEOL and EEP significantly increased final whole-body dry matter, protein, lipid and ash contents (P<0.001; Table 3) in comparison with the control diet. It is to be noted that, final whole-body dry matter and protein contents were significant higher with dietary supplementation of EEP than EEOL (P< 0.05); however, no significant differences were found in final lipid and ash contents when either EEOL or EEP was supplemented in the diet.

**Table3: Effect of dietary supplement with olive leaves extract or propolis extract on body composition of Nile tilapia (mean ±SE)**

| Nutrient (%) | Initial* | Control group | Olive group | Propolis group | P-value |
|---|---|---|---|---|---|
| **Dry matter** | 19.9±0.09$^{d}$ | 22.23±0.29$^{c}$ | 23.89±0.09$^{b}$ | 25.05±0.13$^{a}$ | 0.001 |
| **Protein** | 56.43±0.20$^{d}$ | 58.71±0.48$^{c}$ | 60.94±0.29$^{b}$ | 62.58±0.12$^{a}$ | 0.001 |
| **Fat** | 15.26±0.54$^{d}$ | 16.20±0.17$^{c}$ | 18.38±0.10$^{a}$ | 18.68±0.15$^{a}$ | 0.006 |
| **Ash** | 16.42±0.06$^{d}$ | 16.63±0.17$^{c}$ | 17.32±0.11$^{b}$ | 17.43±0.07$^{a}$ | 0.001 |

### *Effect on immunological parameters and plasma proteins*

Regarding to the immune status of the study fish, total leukocyte counts (TLC), neutrophils, lymphocytes %, monocytes %, phagocytic % and phagocytic index were significantly higher (P< 0.05) after dietary inclusion of EEOL and EEP when compared with the control one (Table 4). The TLC, lymphocytes %, monocytes % and phagocytic % were significantly higher in EEOL group than in EEP group; however there was no significant difference in phagocytic index in between. Furthermore, neutrophils % was significantly higher in EEOL and EEP groups than in control group. However, eosinophils % and basophils % were significantly lower in EEOL or EEP groups in comparison with the control group (P≤0.01). Plasma total protein and globulin levels significantly increased (P< 0.05) in EEOL and EEP groups (Table 4) compared to the Control group, with no difference in between.

**Table 4: Effect of dietary supplement with Olive Leaves extract or Propolis extract immunological parameters and plasma proteins of Nile tilapia (mean ±SE)**

| Items | Control group | Olive group | Propolis group | P-value |
|---|---|---|---|---|
| Total leukocytic Count (x10$^{3}$ /µl) | 35.67±0.33$^{c}$ | 41.00±0.58$^{a}$ | 38.33 ±0.67$^{b}$ | 0.001 |
| Neutrophils % | 22.67±0.33$_{b}$ | 24.33±0.33$^{a}$ | 24.67 ±0.67$^{a}$ | 0.001 |
| Eosinophils % | 5.33±0.33$^{a}$ | 0.33±0.33$^{c}$ | 2.00 ±0.58$^{b}$ | 0.001 |
| Basophils % | 6.00±0.58$^{a}$ | 0.67±0. 33$^{c}$ | 2.33±0.33$^{b}$ | 0.001 |
| Lymphocytes % | 61.33±0.33$^{c}$ | 66.33±0.33$^{a}$ | 64.33±0.33$^{b}$ | 0.001 |
| Monocytes % | 4.67±0.33$^{c}$ | 8.33±0.33$^{a}$ | 6.67±0.58$^{b}$ | 0.001 |
| Phagocytic % | 82.33±0.33$^{c}$ | 97.67±0.88 $^{a}$ | 95.67±0.33$^{b}$ | 0.001 |
| Phagocytic index | 1.19±0.12$^{b}$ | 2.06±0.05$^{a}$ | 1.85±0.03$^{a}$ | 0.001 |
| Total protein (g/dl) | 6.80±0.21$^{b}$ | 7.40±0.06$^{a}$ | 7.27± 0.03$^{a}$ | 0.001 |
| Globulin (g/dl) | 3.83±0.41$^{b}$ | 5.17±0.03$^{a}$ | 4.90 ± 0.10$^{a}$ | 0.001 |
| Total albumin (g/dl) | 2.97±0.34 | 2.23±0.09 | 2.37± 0.09 | 0.09 |

Within-the same row different superscript letters denote significant difference (P<0.05).

***Economic efficiency evaluation***

The effect of experimental diets on economic efficiency in different fish groups is presented in (Table 5). The feeding costs (AVC) were 0.58, 0.57and 0.57 Egyptian Pound (E.P.) for control, EEOL and EEP, respectively. The significantly highest (P< 0.05) total return of obtained gain was in fish fed on diet containing EEP (1.93 E.P.) followed by the fish fed on diet containing EEOL (1.77 E.P.), while the lowest was found in control group (1.55 E.P.). The highest net return was in group fed on diet containing EEP (1.00 E.P.) followed by EEOL group (0.85 E.P.), while the lowest was recorded in control group (0.58 E.P.). The significantly highest (P< 0.05) economic efficiency was in fish fed on diet containing EEP (93.08 %) followed by the fish fed on diet containing EEOL (77.96 %.), while the lowest was reported in control group (52.04%).

**Table 5: Effect of dietary supplement with olive leaves extract or propolis extract on Economic efficiency of Nile tilapia.**

| Groups | AVC[1] | AFC[2] | TC[3] | TR[4] | NP[5] | EE[6] % |
|---|---|---|---|---|---|---|
| **Control group** | 0.58±0.08 | 0.35±0.02 | 0.93±0.01 | $1.52^{c}$±0.44 | $0.58^{c}$ ±0.17 | $100.35^{c}$ ±2.1 |
| **Olive group** | 0.57±0.03 | 0.35±0.03 | 0.92±0.02 | $1.77^{b}$±0.41 | $0.85^{b}$ ± 0.25 | $147.12^{b}$±2.23 |
| **Propolis group** | 0.57±0.06 | 0.35±0.09 | 0.92±0.01 | $1.93±0.33^{a}$ | $1.00^{a}$±$0.33^{a}$ | 172.87 ±$2.30^{a}$ |
| **P-value** | 0.10 | 0.09 | <0.001 | <0.001 | <0.001 | <0.03 |

Within-row different superscript letters denote significant difference (P<0.05).
[1] Average variable costs (feed costs)
[2] Average fixed costs
[3]Total costs= AVC+AFC
[4]Total Return
[5] net profit = TR-TC
[6] Economic efficiency = NP/AVC*100, selling cost of fish= 40 EP, price of kg diet (12-13-14 EP ), respectively.

## Discussion

In the present study, dietary inclusion of 1 g/kg olive leaves extract (EEOL) and 4 g/kg propolis extract (EEP) markedly improved growth performance, protein utilization and digestibility (of DM, CP,EE and CF) in Nile tilapia, *Oreochromis niloticus*. Previous studies in rainbow trout (*Oncorhynchus mykiss*) also showed that the incorporation of 2-4 g/kg EEP increased growth performance and feed utilization [31]. Using in Nile tilapia fingerlings (8 gm), using a rather higher dose than used in this study, 10 g/kg EEP or crude propolis increased the growth performance and decreased the feed conversion ratio in Nile tilapia [32]. Olive leaves extract at each levels of one and five g/kg resulted in reduction of food conversion and improvement of some blood and immune parameters in common carp fingerlings [33].

In birds, the antimicrobial and/or antioxidant activities of the components of EEP contributed to better intestinal health and improved digestion and absorption, and thereby improving the growth performance [34]. Olive leaves extract (EEOL) proved to have non-carbohydrate prebiotics which in turn are able to stimulate the growth of probiotic bacteria [35], useful for fish growth and feed utilization. In addition, oleuropein, one of the components of EEOL, is able to activate pepsin [36] and improve feed conversion, utilization of nutrients and health status by protecting the epithelial cells in the gastrointestinal tract [37].

Similarly, inclusion of *Eucommia ulmoides* olive leaf at a level of 0.1, 0.15, 2 and 4% in grass carp significantly increased growth rate and FCR [38]. All of these properties together improve digestion and nutrient absorption with a subsequent increase in fish weight, average weight gain and weight gain %, as observed in this study.

On the other hand fish with better growth performance, feed utilization and digestibility, fed on EEOL or EEP, also had the highest whole-body dry matter, protein, lipid and ash contents suggesting that supplementation of 1 gm/kg EEOL or 4 gm/kg EEP improved body composition of the Nile tilapia. In accordance with our results, dietary supplementation of propolis extract improved whole body composition in juvenile eel [39]. The improvement in BW and BWG of

broiler consumed 450 mg propolis is likely due to the presence of micronutrients and high content of flavonoids and phenolic resin acids in propolis, which improve a helpful microbic in the gut and mirrored on positive effects on health and metabolism [40, 41].

Moreover, our results suggest that supplementation of 1 g/kg olive leaves extract and 4 g/kg propolis extract not only did improve growth performance, nutrient digestibility and body composition but also enhanced the cellular immunity (non-specific immunity) in Nile tilapia evidenced by an increase in the blood cells count (TLC, lymphocytes and monocytes percentages) and plasma levels of globulins. Increased percentage of circulating blood mononuclear cells (lymphocytes and monocytes) led to increased *C. albicans* phagocytosis, and eventually to higher phagocytic % and phagocytic index. These results were in agreement with [37] who reported that Olive leaves extract improved the cellular immune response in grass carp. Also, Ahmed *et al*., [42] found that addition of oleuropein to diet of layer at any levels resulted in significant ($p<0.01$) increased in plasma protein, globulin, white blood cells (WBC), and lymphocytes count while heterophils count and H/L ratio significantly ($p<0.01$) decreased. Karimi *et al.,* [33] found that using of olive leaves extract at both 1 and 5 gm/ kg in common carp fingerlings resulted in significant increase in the number of WBCSs and lymphocytes. In addition, oleuropeins from EEOL are converted inside the body into elenoic acid, which prevents the replication of viruses and bacteria, and can enhance nitric oxide production by macrophages. Also, the results of Zemheri-Navruz et *al.,* [43] show that feeding common carp with a diet containing olive leave extract (1 g/kg) over a period of 60 days might be adequate to improve fish immune parameters, and survival rate against *E. tarda*.

The mechanism of antimicrobial activity of propolis is complex and could be attributed to the synergistic activity between phenolic and other compounds [44]. Cinnamic acid, one of components of EEP, stimulates lymphocyte proliferation and induces IL-1 and IL-2 production, [45]. Some mechanisms of the activity of propolis on bacterial growth have been reported, such as inhibition of cell division, bacterial cytoplasm, cell membrane and cell wall collapse, bacteriolysis and protein synthesis inhibition [46]. In addition, EEP caused inhibition of microbial oxygen consumption decreasing micro-organisms vitality [47]. Increased globulins level, thought to be immunoglobulins, was reflected in plasma total protein concentration, which was also higher in EEOL and EEP groups compared to the Control group. The gainful impact of propolis on protein divisions might be because of the stimulating impact on liver showing an anabolic activity favoring protein synthesis and its safeguarding impact to the body protein from degeneration.

The cost of feeds constituted the largest share of the total cost of production. Feeding costs (AVC) is critical in aquaculture as it represents between 40% and 50% of the total productive cost [48].Fixed costs are cost depreciation, labor, electricity, equipment and building. The fixed costs represented a minor proportion of the total costs while the total variable costs represented the major portion of the total costs. The revenues included sales of fish. Net profit and economic efficiency were significantly higher in group fed on fed on diet containing EEP followed by the fish group fed on diet containing EEOL These findings are strongly confirmed the findings of Shreif and El-Saadany [41] who found that ,Treated groups with propolis gave more net revenue and feed economic efficiency than the control group. . On other hand, supplementation of diet with EEP and EEOL increased the economic efficiency of Nile tilapia production by increasing the gain and enhancement of fish immune system. Similarly, addition of feed additives to fish diets improved immunity, productivity and economic efficiency of fish via improving fish body weight [ 49], weight gain [50]., feed conversion ratio and efficiency [51].

## Conclusion

In conclusion, dietary supplementation of 1 g/kg EEOL and 4 g/kg EEP significantly improved the growth performance, nutrient digestibility, body composition and significantly enhanced the immune status of Nile tilapia, *Oreochromis niloticus*. Ethanol Extract of propolis (EEP) was more prominent as a growth promoter in Nile tilapia. Meanwhile, better immunity was achieved when using Ethanol Extracts of olive Leaves (EEOL) in the diet. Also, adding of EEOL and EEP showed a great economic efficiency and net profit in production of Nile tilapia.

## Conflict of interest

The authors have no conflict of interests to declare.

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**الملخص العربي**

**تأثير مستخلصات أوراق الزيتون والبروبوليس على أداء النمو والمعايير المناعية والكفاءة الاقتصادية باستخدام أسماك البلطي النيلي**

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هدفت هذه الدراسة إلى توضيح آثار مستخلص أوراق الزيتون ومستخلص صمغ النحل في علائق أصبعيات البلطي النيلي على معدلات النمو ومعدلات التحويل الغذائي، ومعاملات الهضم ومكونات الجسم والكفاءة الاقتصادية، والحالة المناعية وبعض متغيرات مصل الدم. غذيت الأسماك على ثلاثة علائق متساوية في نسبة البروتين الخام (٣٥ %) والطاقة المهضومة (٢٩٠٠ كيلوكالوري/كجم) مع أضافة تركيز ٤ جم/كجم عليقة من مستخلص صمغ النحل و١ جم/ كجم من مستخلص أوراق الزيتون على العليقة الثانية والثالثة لمدة ١٢ اسبوع كما يلي- :المجموعة الأولى غذيت على العليقة الأساسية بدون اضافات (المجموعة الضابطة)، المجموعة الثانية غذيت على العليقة الأساسية مع اضافة ٤ جم /كجم مستخلص صمغ النحل، المجموعة الثالثة غذيت على العليقة الأساسية مع اضافة ١ جم /كجم من مستخلص صمغ النحل. أظهرت النتائج أن الإضافة الغذائية لمستخلص أوراق الزيتون ومستخلص صمغ النحل كان لها تأثير كبير على معدلات النمو ومعدلات التحويل الغذائي مقارنة مع المجموعة الضابطة. بالإضافة إلى ذلك، فإن إضافة مستخلص أوراق الزيتون كان أكثر بروزًا من مستخلص صمغ النحل على الوزن النهائي للأسماك ومدي الاستفادة من البروتين. وقد لوحظ زيادة نسبة المادة الجافة، البروتين، الدهون، والرماد في جسم الاسماك في نهاية التجربة في مجموعتي الاسماك التي غذيت على عليقة تحتوي على مستخلص صمغ النحل ومستخلص أوراق الزيتون بينما اقل قيمة لوحظت في المجموعة الضابطة. علاوة على ذلك، تم تحسين هضم المواد الغذائية عن طريق إضافة مستخلص أوراق الزيتون ومستخلص صمغ النحل. أظهر عدد بعض متغيرات مصل الدم من (الجلوكوز، البروتين الكلي، الألبيومين، الجلوبيولين، الكولسترول، الجلسيريدات الثلاثية و مستويات أعلى في مجموعات الأسماك التي تحتوي على مستخلص صمغ النحل و مستخلص أوراق الزيتون.من حيث التحليل الاقتصادي، أعلي كفاءة اقتصادية كانت في مجموعة الاسماك التي غذيت علي عليقة تحتوي علي مستخلص صمغ النحل يليه مستخلص أوراق الزيتون مقارنة مع المجموعة الضابطة. اقترحت نتائجنا أن أضافة مستخلص أوراق الزيتون (1 جم / كجم) أو مستخلص صمغ النحل4 (جم / كجم) يعزز أداء النمو بشكل ملحوظ، تكوين الجسم، هضم الأعلاف، الحالة المناعية والكفاءة الاقتصادية في البلطي النيلي.