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Influence of olive leaves additive on growth and physiological parameters of *Sarotherodon galilaeus*

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ABSTRACT

A 100- day study was conducted to evaluate the effect of dried olive leaves on the growth rates, biochemical parameters, and physiological activities of the tilapia (*Sarotherodon galilaeus*), with an initial body weight 33 ± 1.0g reared in a concrete pond. Test fish fed three diets, 30 % crude protein (CP) and 3000 kcl containing 1, 2, and 3% olive additive, besides the control (0% additive). Optimum results of growth parameters, total weight gain, and specific growth rate (TWG and SGR) were recorded for fish-fed diets with 2 and 3% olive leaves. The best ratios of feed conversion (FCR) and protein efficiency (PER) were recorded with values of 1.86±0.11 & 2.0±0.42, respectively, for fish fed on the treated diet (3). Results indicated that diet containing olive leaves were palatable with optimal digestive rate. The protein content of body fish increased significantly through all the treated diets compared to that of the control. Olive additive improved plasma total protein, albumin, and globulin levels, accompanied with optimal promising findings of plasma aminotransferases (AST and ALT) activities. In addition, a significant reduction in plasma glucose, cholesterol, and triglycerides levels were detected for the three test fish-fed olive supplemented diets. In conclusion, olive leaves could improve the growth and physiological parameters in cultured *Sarotherodon galilaeus*.

INTRODUCTION

Africa production of the tilapia aquaculture represents 1.2 million tons **(FAO, 2017)**. Aquaculture in Egypt is characterized by culturing finfish and shellfish. Tilapia are the main cultured species that represented about 67% of total cultured species in 2014 **(Soliman & Yacout, 2016; Shaalan *et al.*, 2018)**. Sarotherodon genus (Family: *Cichlidae*) is restricted to West Africa, and has extended eastwards to reach the Nile and the first Rift lakes **(FAO, 2017).**

Tilapia galilaea, *Sarotherodon galilaeus* is the best fish species of the Cichlidae family; its culture system is known for its easy cultivation, disease resistance, high fertility and

high tolerance to a wide range of water quality **(El-Husseiny *et al.*, 1993; El- Waly, 1999)**.

Plant protein sources are considerable alternatives of animal protein sources and can be used in fish feed without affecting its nutritional quality **(Nithiyanantham *et al.*, 2012)**. Thus, the use of cheaper and locally available plant sources would reduce fish production cost, and thereby increase the profit **(Osman *et al.*, 1996; Munguti *et al.*, 2006)**. In this context, the use of unconventional feed sources, such as tree leaves, for the sake of increasing the animal production has gained widespread attention.

Remarkably, olive leaves contain 7.0 to 12.9% crude protein from dry matter (DM),and one olive tree produces about 25-30 kg of leaves per year (5% of the olive tree mass produced) **(Delgado-Pertinez *et al.*, 1998; Martin-Garcia *et al.*, 2006)**. Furthermore, it was reported that olive processing produced around 10% of the weight of raw material (leaves and wastes) in the form of by-products **(Kiritsakis *et al.*, 2010)**.

At the same level of olive oil, olive leaf is an excellent source of bioactive compounds such as oleuropein, verbascoside, rutin, tyrosol and hydroxytyrosol (**Sánchez-Gutiérrez *et al.*, 2021)**. Those phenolic compounds have anticholinesterase activities **(Lee and Lee, 2010)** antioxidant, and antimicrobial **(Cumaoglu *et al.*, 2011; Amoo, *et al.*, 2012)**.

Oleuropein, the predominant phenolic compound, represents 9% of the total dry weight of olive leaf **(Kiritsakis *et al.*, 2010)**, which has important biological properties **(Hashmi *et al.*, 2015)**. Oleuropein is successfully used in controlling *Salmonid rhabdovirus* and *Haemorrhagic Septicaemia* virus **(Harikrishnan *et al.*, 2010 a, b)** and as natural food additive **(Barbosa-Pereira *et al.*, 2014)**. Hence, the current study aimed to address the effects of olive leaves dried powder as a feed additive on the growth performance, the chemical composition and the physiological parameters of *Sarotherodon galilaeus*.

MATERIALS AND METHODS

Experimental design:

A 100- day experiment was organized to feed the tilapia galilae (*Sarotherodon galilaeus*) with olive additive and detect the feed effect on the fish under study. The specimens were weighed recording an initial body weight of 33 ± 1 g. Samples were stocked in a concrete pond with dimensions of 4×10×1.5m. The pond was divided into 8 equal parts (30 fish/ part), and water was changed twice a week. For acclimatization purposes, fish were held under optimal conditions for 2weeks before starting the trial.. Fish were fed twice a day at 10:30 and 14:30 h. The fish were biweekly weighed in order to adjust the daily feed amount, which was 3% of live biomass. Fish samples were collected and immediately frozen (– 20°C) and reserved for initial proximate chemical analysis.

Water quality

During the experimental period, the water quality parameters: water temperature, dissolved oxygen and pH (measured daily); total ammonia, nitrite, and nitrate were measured weekly.

Experimental diets

The leaves of olive (*Olea europaea*) of Oleaceae family were randomly collected from several trees in Vally of El Natron. Leaves were washed, sun-dried and crushed into powder. Three experimental diets (30 % CP, 3000 kcl) were prepared with olive leaves powder as an additive as follows: 1%, 2% and 3%, respectively, whereas the control diet was left without additives.The formula of experimental diets is shown in Table (1).

Table 1. The composition of experimental diet.

Feed ingredient	Experimental diet			
	Cont.	Olive leaves		
		1 %	2 %	3 %
Fish meal 62 %	21	21	21	21
Soybean meal 44%	20	20	20	20
Yellow corn	33	33	32	32
Wheat bran	18	17	17	16
Oil	6	6	6	6
Premix*	2	2	2	2
Feed additive	0	1	2	3
Total	100	100	100	100

One kg premix contained:

Vitamins: 48×10^5 I.U (A), 6×10^2 mg (B_6), 20 mg (biotin), 8×10^5 I.U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), 4×10^3 mg (pantothenic acid), 4 mg (B_{12}), 4×10^2 mg (niacin), 2×10^5 mg (choline chloride), and 400 mg (folic acid).

Minerals: 12×10^3 mg iron, 16×10^3 mg manganese, 12×10^2 mg copper, 120 mg iodine, 80 mg cobalt, 40 mg selenium, and 16×10^3 mg zinc.

Fish performance and feed utilization parameters

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

Body weight gain (**BWG**, g /fish) = [final body weight (g) - initial body weight (g)];

Daily weigh gain, (**DWG**, g /fish /day) = [BWG (g) / Experimental period (days)];

Specific growth rate (**SGR**, %g/day) = 100 [Ln final weight - Ln initial weight] / Experimental period (day);

Feed conversion ratio (**FCR**) = feed intake (g) / body weight gain (g);

Protein efficiency ratio (**PER**) = gain in weight (g) / protein intake in feed (g);

Protein productive value (**PPV**, %) =100 [protein gain in fish (g)/food protein intake (g)];

Fish Survival rate % = 100 [Initial number of fish stocked-Mortality] / Initial number of fish stocked.

Table 2. Chemical composition of experimental diets and additives (dry matter bases)

Parameter	Diet	Olive leaves
Moisture %	10.5	55.41
Dry matter %	89.5	44.6
Crude protein %	30.3	11.82
Ether extract %	10	5.97
Crude fiber %	3.6	9
Nitrogen free extract %*	46.7	62.67
Ash %	10.0	10.54
Gross energy**	457.23	
Metabolizable energy (kcal/kg)	3008.9	-
Protein/Energy ratio	98.71	-

***NFE =** Nitrogen free extract (100 – [CP + Ash + CF + EE)].

****GE** = Gross Energy calculated as 5.64, 9.44, and 4.11 Kcal/ gram of protein, lipid and carbohydrates, respectively **(NRC, 2011)**.

Physiological parameters

a. Body indices

At the end of the trial, livers, gonads and viscera of the examined fish were removed and weighed to evaluate the liver, gut and gonad indices (HSI, VSI & GSI, respectively) as follows:

HSI = (liver weight/ fish total weight)×100;

VSI = (viscera weight/ fish total weight)×100;

GSI = (gonad weight/ fish total weight)×100.

b. Blood parameters

At the end of experiment, blood samples were collected from the caudal veins of fish using heparinized syringes. Blood was centrifuged at 1008 xg for 15 min. Plasma protein (PTP) was determined following the method of **Armstrong and Car (1964)**, while plasma albumin (PA) was adjusted using the method of **Doumas *et al.* (1977)**. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method of **Rettman and Frankel (1957)**. Serum triglycerides (STG) and cholesterol (Ch)were determined according to the method described by **Stein (1986)**. Glucose concentration was measured according to the method of **Trainder (1969).** Alkaline phosphatase (ALP) activity was determined by using the method of **Williason (2003)**.

Chemical analysis

The chemical analysis of the additives, the experimental diets and the fish body were performed to determine the percentages of dry matter (DM %). Crude protein (CP %), ether extract (EE %), crude fiber (CF %) and ash (%) were adjusted according to the **AOAC method (2012)**. Nitrogen free extract (NFE %) was calculated by deducting the sum of CP%, EE%, CF% and ash% from 100.

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using general linear models' (GLM) procedure; the software used was SPSS (Version 16.0) **(SPSS, 1997)**. Duncan's multiple range tests **(Duncan, 1955)** were used to compare between means of the control and those of the treated groups.

The model of analysis used was as follows:

$\mathbf{Y_{ij} = \mu + T_i + E_{ij}}$

μ = the overall mean,

T_i = the effect of treatment,

and, E_{ij} = the random error.

RESULTS AND DISCUSSION

Water analysis

The mean values (±SD) of physico-chemical parameters were recorded as follows: water temperature 28.6±0.3°C; dissolved oxygen 5-6± 0.4 mg/L; pH 7.5±0.2; total ammonia 0.023 ± 0.01 mg/L; nitrite 0.025±0.013 mg/L and nitrate 0.8 ±0.4 mg/L. The present results showed that all water quality parameters were within the acceptable ranges for rearing the tilapia **(Makori *et al.*, 2017).**

Growth performance

The growth parameters in Table (3) shows that the best values of final body weight, body weight gain and daily weight gain were recorded for fish fed diets containing 2% and 3% olive leaves. These results may be due to the phytogenic compounds in olive feed additives which may either enhanced the feed palatability and flavor or improved nutrient absorption and digestive enzymes' activities **(Cross *et al.*, 2007; Zeng *et al.*, 2015)**. Moreover, olive leaves include oleuropein that may have a good effect on gut functions **(Pereira *et al.*, 2006)**.

The best SGR values (1.79 & 1.79 %g/day) were recorded for fish fed 2% and 3% additives. The olive leaves are rich in phenolic compounds, particularly oleuropein, which has important biological properties **(Hashmi *et al.*, 2015)**. Among the radical properties is acting as growth promoters **(Yáñez-Ruiz & Molina-Alcaide , 2008)**.

Abou-Zeid (2002) and Raky (2009a) mentioned that when (ginger and garlic) dried powders were added to the feed of the Nile tilapia, the biomass and SGR were positively affected. **Raky *et al*. (2021)** recorded that, the use of (mulberry or guava) dried leaves additive in Nile tilapia diet, were enhancement growth performance.

In the present data, best values of the survival rate (94, 97, and 96 %) were obtained for fish fed diets with olive leaves additive. Many studies recorded that feed additive in the tilapia diets revealed an enhancement of survival rate beside some other growth parameters **(Pachanawan *et al.*, 2008; Raky, 2009a, b; Shahin *et al.*, 2019).**

Table 3. Growth performance of Tilapia galilae fed on diets with dried olive leaves (Mean ± SE)**.**

Growth parameter	Experimental diet			
	Control	Olive leaves		
		1%	2%	3%
Initial weight	34^{a} ± 0.11	33^{b} ± 0.02	33^{b} ±0.08	30^{c} ± 0.1
Final weight	112^{c} ±0.5	152^{b}±1.0	173^{a} ± 0.53	171^{a}±0.54
Body weight gain	78^{c}± 0.5	119^{b}± 1.0	140^{a}± 0.51	141^{a}±0.53
Daily weigh gain	0.78^{c} ±0.01	1.19^{b} ±0.02	1.40^{a} ±0.01	1.41^{a} ±0.03
Specific growth rate	1.12^{c} ±0.020	1.53^{b} ±0.011	1.79^{a}±0.010	1.74^{a} ±0.003
Survival rate %	88^{c} ± 1.6	94^{b} ± 1.74	97^{a} ±1.82	96^{ab} ± 1.59

In all fish groups, the values of feed convertion ratio were decreased ($p<0.05$) accompanied with an increase in the PER. The best PPV% value was recorded for the fish fed diet containing 2% additive (Table 4). These parameters are used to measure the quality of fish diets and indicate whether the diet containing olive leaves was palatable for fish.

Shahin *et al.* (2019) mentioned that supplementation of 1.0 g / kg olive leaves extract or 4.0 g /kg propolis extract improved growth performance and body composition of the Nile tilapia. Additionally, **Raky *et al.* (2021)** found that dietary guava leaves improved feed intake, FCR, PER and body composition of the Nile tilapia.

Table 4 . Feed utilization of the examined fish (Mean ± SE)

Food parameters	Experimental diet			
	Control	Olive leaves		
		1%	2%	3%
Feed intake (g)	208.03^{d}±1.43	243.96^{c}±1.32	269.89^{a}±1.92	262.89^{b}±1.5
Feed conversion ratio	2.67^{a} ±0.13	2.05^{b} ±0.2	1.93^{c} ±0.09	1.86^{d} ±0.1
Protein efficiency ratio	1.4^{d} ±0.44	1.82^{c} ±0.19	1.93^{b} ±0.03	2.0^{a} ±0.04
Protein productive value %	20.82^{c} ±0.21	26.64^{b} ±0.35	27.27^{a} ±0.45	26.89^{ab}±0.51

Chemical composition of whole fish body

The whole fish body analysis of the Tilapia galilae is presented in Table (5). The body protein content was significantly increased with the increase of additive levels in test diets, while the lipid content decreased with the same levels of the additive compared to control. **(El-Ebiary and Zaki 2003**; **Abdelhamid *et al.*, 2007**; **Raky *et al.*, 2021)** stated that a negative correlation was determined between protein and fat of the *Oreochromis niloticus* and **Raky (2009b)** assessed an identical observation for the *Tilapia aurea*. On the other hand, **El-Saidy and Gaber (2002)** recorded that, a positive relationship was spotted between crude protein and crude fat contents of the fish.

Table 5. Fish body proximate analysis of *Tilapia galilae* (Mean ± SE)

Body composition	Experimental diet			
	Control	Olive leaves		
		1%	2%	3%
Moisture %	72.4^{d}±0.54	74.3^{c}±0.51	75.28^{b}±0.70	76.1^{a}±0.41
Crud protein %	54.01^{d}±0.32	57.59^{a}±0.25	57.1^{b}±0.39	56.2^{c}±0.9
Ether extract %	23.59^{a}±0.31	18.9^{c}±0.21	19.28^{c}±0.46	20.12^{b}±0.42
Ash %	19.37^{c}±0.41	21.32^{a}±0.22	20.95^{ab}±0.5	20.83^{b}±0.41
Nitrogen free extract %	3.03^{a}±0.23	2.19^{c}±0.12	2.67^{b}±0.04	2.85^{b}±0.06
Dry matter %	27.6^{a}±0.52	25.7^{b}±0.45	24.72^{c}±0.35	23.9^{d}±0.41

Physiological parameters

a. Body indices

Table (6) reveals that the higher value (1.77 ± 0.13) of hepato-somatic index was recorded for the examined fish fed diet 1%, accompanied with the lowest value (1.46 ± 0.02) of gonado-somatic index, compared to the other fish groups. These results may be due to the fact that, the olive leaves are rich in phenolic compounds, specifically oleuropein, which has important biological properties **(Hashmi *et al.*, 2015)**.

Table 6. Somatic indices of *Sarotherodon galilaeus* (Mean ± SE)

Biological parameters	Experimental diet			
	Control	Olive leaves		
		1 %	2 %	3 %
Final weight	112^{c} ± 0.57	152^{b}±1.06	173^{a} ± 0.55	171^{a} ± 0.52
Hepato-somatic index	1.95^{a}± 0.04	1.77^{b} ± 0.13	1.6^{c} ± 0.05	1.6^{c} ± 0.04
Viscera somatic index	7.86^{c} ± 0.21	8.75^{b}± 0.19	9.01^{a} ± 0.3	9.0^{a} ± 0.19
Gonado-somatic index	1.1^{c} ± 0.01	1.46^{b} ± 0.02	1.62^{a} ± 0.03	1.61^{a} ± 0.11

b. Blood parameters

The lowest plasma glucose values (89, 90, and 87 mg/dL) were recorded for fish fed the three treated diets compared the control (111.0 mg/dL). Mean values of serum triglycerides and cholesterol levels in the examined fish blood decreased in the fish fed on the olive leaves additive (Table 7).

These findings may be due to the improvement of the produced insulin from pancreace **(Raky, 2009b)**. Olive leaf comprises an excellent source of bioactive compound, oleuropein (**Sánchez-Gutiérrez*, et al.*, 2021)**, it represents 9% of total dry weight of the olive leaf **(Kiritsakis,*et al.*, 2010)** and has important biological properties **(Hashmi *et al.*, 2015)**. The present results coincide with those of **Jemai *et al.* (2008; Rahmanian *et al.* (2015)** who stated that oleuropein decreases low-density lipoprotein cholesterol and as an oxidative stability promoter of lipids**. Fazio *et al.*, (2021**) revealed that, levels of cholesterol and triglycerides were decrease with increasing the concentration of olive leaves extract in Nile tilapia diet.

Table 7. Blood parameters of examined fish (Mean ± SE)

Hematological parameter	Experimental diet			
	Control	Olive leaves		
		1 %	2 %	3 %
Glucose (mg/ dL)	$111^{a} \pm 1.06$	$89^{bc} \pm 1.1$	$90^{b} \pm 1.02$	$87^{c} \pm 1.08$
Plasma total protein (g/dL)	$3.45^{a} \pm 0.41$	$4.81^{b} \pm 0.39$	$4.47^{c} \pm 0.34$	$4.49^{c} \pm 0.28$
Plasma albumin (g/dL)	$1.44^{c} \pm 0.05$	$2.08^{a} \pm 0.04$	$1.97^{b} \pm 0.02$	$1.9^{b} \pm 0.04$
Plasma globulin (g/dL)	$2.01^{c} \pm 0.11$	$2.73^{a} \pm 0.09$	$2.50^{b} \pm 0.08$	$2.59^{ab} \pm 0.12$
Serum triglycerides (mg/dL)	$68^{a} \pm 0.9$	$55^{d} \pm 0.9$	$63^{b} \pm 1.01$	$59^{c} \pm 0.8$
Cholesterol (mg/ dL)	$134.3^{a} \pm 1.28$	$116.6^{c} \pm 1.92$	$118^{b} \pm 1.45$	$117^{bc} \pm 1.63$
Alkaline phosphatase (ALP) (U/L)	$47^{a} \pm 0.51$	$31.5^{d} \pm 0.62$	$33.6^{b} \pm 0.28$	$32.2^{c} \pm 0.47$
Alanine aminotransferase (ALT) (U/L)	$43^{a} \pm 0.60$	$34.4^{bc} \pm 0.39$	$35.5^{b} \pm 0.67$	$33.9^{c} \pm 0.58$
Aspartate amino transferase (AST) (U/L)	$121^{a} \pm 1.40$	$107^{b} \pm 1.09$	$101^{c} \pm 1.85$	$104^{c} \pm 1.37$

Present results of PTP, PA, and PG differ significantly for those fed the additive when compared to the control one. The results revealed that the mean value of PTP (4.81, 4.47, and 4.49 g/dL) improved for the three fish groups fed by olive by compared to the control (3.45 g/dL).

Fazio *et al.*, (2021) revealed that, with respect to the Nile tilapia, both the plasma total protein and the globulin albumen ratios increased when fed on 1% of olive leaves extract.

Raky *et al*. (2021) reported that plasma total protein for Nile tilapia fed on (guava or mulberry) leaves was increased.

ALP has been considered as the main track for the synthesis and dominance of amino acids that allow the interaction between carbohydrate and protein metabolism during the fluctuation of the energy requirements of the organism in different adaptive situations. Hence, the state of the liver and some other organs must be evaluated **(Schram *et al.*, 2008)**. Notably, the AST and ALT activities may be altered by a variety of biochemical and physiological conditions. The rates of Kreb's cycle activity decreased accompanied with the decrease of its intermediates. Thus, the ALT and AST were optimized by providing a-ketoglutarate **(Salah El-deen and Rogarswa, 1993)**.

The present study showed that the values of AST and ALT decreased significantly in the blood of fish fed on olive leaves that may have worked to maintain normal liver function. Those results may also be due to the action of olive powder protecting the liver cells' membrane against free radicals

The addition of olive decreased the blood ALP activity significantly, whereby the health of *Tilapia galilee* was improved resulting in the best flesh condition of fish.

The present study confirmed that, olive leaves enhanced growth parameters, feed utilization and fish health. In this respect, **Karimi Pashaki *et al.* (2018)** recorded a reducing food conversion and an improvement of some blood parameters when using five levels of olive leaves' extract for common carp fingerlings. **Raky *et al*. (2021)** recorded

that the addition of (mulberry or guava) leaves to the diets of the *O. niloticus* resulted in optimal the levels of ALP, AST and ALT enzymes.

CONCLUSION

In conclusion, the olive powder may be used as growth promoter that was proved to improve the physiological activities and the survival rates in the *Tilapia galilae* and enhance fish tolerance to environmental agents and stress.

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