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# Effect of Pomegranate Peel on Physiological Status and some Growth Parameters of the Nile tilapia, *Oreochromis niloticus*

**Suzan O.M. El-Werwary[1], Rania Nasr[2], Mohamed W. Ali[1*]**

[1]Department of Hatching & Fish Physiology, Central Laboratory for Aquaculture Research, Agricultural research Center, Egypt

[2]Department of Fish Health, Central Laboratory for Aquaculture Research, Agricultural Research Center, Egypt

[*]**Corresponding Author: Wafeek2009@yahoo.com**

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## ABSTRACT

Pomegranate peel, a by-product of fruit processing, can be repurposed as a dietary supplement in fish feed. This study investigated the physiological status of the Nile tilapia and evaluated the effects of pomegranate peel supplementation on their physiological and growth performance. The experiment was conducted at the Central Laboratory for Aquaculture Research (CLAR) of the Agriculture Research Center in Egypt. The Nile tilapia (*Oreochromis niloticus*) fingerlings were sourced from a farm in Sharkia Governorate, Egypt. A total of 450 fish were used in the experiment—225 for immediate use and 225 fingerlings stored as reserves (average weight: 0.75 ± 0.1g). The fish were randomly distributed into 15 glass aquaria (100L each), divided into four treatment groups and one control group. Pomegranate fruits (*Punica granatum*) were collected from the Agriculture Research Center. The fruits were peeled, washed, and dried in a forced-air oven at 40 °C for 36 hours. The dried pomegranate peel was incorporated into the diet at different concentrations: T1 (0.5g/ kg), T2 (0.1g/ kg), T3 (1.0g/ kg), and T4 (2.0g/ kg). All groups, including the control, were fed six days per week. Water quality was maintained under optimal conditions for tilapia rearing. Key parameters such as crude protein, crude fat, moisture content, and ash levels in the fish were analyzed. Results indicated that erythrocyte count, hematocrit, and hemoglobin levels were optimal at a supplementation rate of 1%. Leucocyte count increased proportionally with pomegranate peel concentration in the diet, up to 1%. The optimal level of supplementation for enhancing serum IgM was also 1%. Additionally, weight gain (WG) and feed conversion ratio (FCR) were improved with a 1% pomegranate peel dietary additive.

## INTRODUCTION

Pomegranate fruit is a highly valuable species used in juice production and medical treatments; however, most of its by-products are discarded as waste **(Iqbal *et al.*, 2008)**. The peel of pomegranate contains bioactive and polyphenolic compounds that act as antioxidants and immune stimulants **(Al-Zoreky, 2009)**. In the Nile tilapia

(*Oreochromis niloticus*), the inclusion of pomegranate peel in the diet has been shown to enhance oxidative resistance, growth rate, immune status, and feed efficiency **(El-Sayed *et al.*, 2014; Badrey *et al.*, 2019; Toutou *et al.*, 2019)**.

In fact, pomegranate leaf extract increased disease resistance to viral infections in the olive flounder (*Paralichthys olivaceus*) **(Harikrishnan *et al.*, 2010)**. Similarly, **Acar *et al.* (2018)** found that supplementing the rainbow trout diets with pomegranate seed oil up to 10 g/kg improved resistance against *Yersinia ruckeri*, enhanced growth parameters, and boosted the innate immune response. Moreover, it improved meat quality, pigmentation characteristics, and coloration in fish **(Berizi *et al.*, 2016)**.

Fish health status is often evaluated through parameters such as innate immune responses, hematological indices, and serum biochemical variables **(Yılmaz & Ergün, 2018)**. Plant extracts are being used as alternative feed additives, immune stimulants, and growth enhancers **(Badri *et al.*, 2021)**. Enzymatic antioxidant systems in aquatic species, such as glutathione peroxidase, superoxide dismutase (SOD), and catalase, play a vital role in defending against oxidative stress.

The findings of the present research support the inclusion of 1% pomegranate peel meal in the diet of the Nile tilapia (*Oreochromis niloticus*) as an optimal level to improve blood indices, immunity, and growth rate.

## MATERIALS AND METHODS

### Design of the experiment

This experiment was conducted at the Central Laboratory for Aquaculture Research, Agriculture Research Center (A.R.C.), Egypt. The Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from a farm in Sharkia Governorate, Egypt. Fish were acclimated for 15 days prior to the experiment. A total of 450 fish were used—225 active fingerlings and 225 stored as reserves—each with an average weight of 0.75 ± 0.1g.

The fish were randomly distributed into 15 glass aquaria (100L each), divided into four treatment groups and one control group. Pomegranate fruits (*Punica granatum*) were collected from the Agriculture Research Center. The fruits were peeled, washed, and dried at 40°C in a forced-air oven for 36 hours. The dried peels were ground into a powder and incorporated into the fish diet at the following concentrations: T1 (0.5g/ kg diet), T2 (1.0g/ kg diet), T3 (1.5g/ kg diet), and T4 (2.0g/ kg diet).

All fish groups were fed a commercial pellet diet containing 30% crude protein at a daily rate of 3% of body weight. Feeding was conducted six days per week for three months. Each treatment group was replicated three times, with 45 fingerlings per

replicate. Every 15 days, the fish were weighed and counted to determine survival rate, growth, and feed adjustments.

Fish proximate composition (crude protein, crude fat, moisture, and ash) was analyzed according to **AOAC (2012)**. Pomegranate peel was found to contain several phenolic compounds with antioxidant activity, including pyrogallol, ellagic acid, benzoic acid, catechin, and protocatechuic acid.

**Blood sampling, hematology, and biochemical parameters**

Five fish from each aquarium were anesthetized using MS-222. Blood was drawn from the caudal vein and samples were divided into two portions. One portion was transferred into heparinized tubes for hematological analysis, while the other was placed into clean, dry tubes for biochemical and immunological assays. The serum was separated and stored at –20°C until use **(Adel *et al.*, 2015)**.

Red blood cells (RBCs, $10^6/mm^3$) and white blood cells (WBCs, $10^3/mm^3$) were counted using an improved Neubauer hemocytometer with Hayem's and Turck's diluting fluids **(Blaxhall & Daisley, 1973)**. Hematocrit (Ht %) was measured using the standard microhematocrit method. Hemoglobin concentration (Hb, g/dL) was determined via the cyanmethemoglobin method. Leukocyte differentiation was performed on Giemsa-stained blood smears. Serum immunoglobulin M (IgM) was measured according to **Siwicki *et al.* (1994)**, with IgM content calculated as the difference in total protein content.

**Growth performance and body composition**

Prior to sampling, fish were fasted for one day. After three months, all fish were individually weighed. The following growth parameters were calculated according to **NRC (2011)**:

- **Weight Gain (g)** = Final weight – Initial weight
- **Weight Gain (%)** = [(Final weight – Initial weight) ÷ Initial weight] × 100
- **Survival Rate (%)** = (Number of surviving fish ÷ Initial number of fish) × 100
- **Feed Conversion Ratio (FCR)** = Dry feed intake (g) ÷ Weight gain (g)

Body composition was analyzed based on methods from **AOAC (2012)**. Moisture content was measured by oven drying at 105°C for 24 hours. Protein was determined using the Kjeldahl method. Lipid content was assessed by Soxhlet extraction, and ash content was measured by incineration in a muffle furnace at 600°C.

### Statistical analysis

Data were analyzed using one-way ANOVA. Means were compared at a significance level of $P \leq 0.05$, and standard error of the mean (SEM) was also calculated. All statistical analyses were performed using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA).

## RESULTS

Erythrocyte (RBC) counts were significantly higher in fish fed with 1% pomegranate peel (T3), recording $1.392 \pm 0.013 \times 10^6/mm^3$, compared to those fed 0.5% (T1: 1.163 ± 0.017), 0.1% (T2: 1.292 ± 0.015), 2% (T4: 1.076 ± 0.012), and the control group (1.051 ± 0.016) (Table 2). Hematocrit percentage was also significantly improved in the T3 group (28.56 ± 0.6%) compared to T1 (25.72 ± 0.7%), T2 (26.34 ± 0.8%), T4 (25.33 ± 0.7%), and the control (25.42 ± 0.8%).

Hemoglobin concentration showed the highest value in T3 (7.89 ± 0.07 g/dL), followed by T4 (7.35 ± 0.03 g/dL), T2 (7.15 ± 0.04 g/dL), T1 (6.98 ± 0.05 g/dL), and the control (7.05 ± 0.09 g/dL).

Leukocyte (WBC) counts were significantly elevated in T3 ($31.8 \pm 1.5 \times 10^3/mm^3$) compared to T1 (20.7 ± 1.5), T2 (21.75 ± 0.8), T4 (16.5 ± 2.1), and the control (15.8 ± 0.9).

Furthermore, serum immunoglobulin M (IgM) levels were enhanced in the T3 group compared to T1, T2, T4, and the control, as shown in Table (1).

**Table 1.** Hematological parameters including erythrocyte count, hemoglobin concentration, hematocrit percentage, and leukocyte count in the Nile tilapia (*Oreochromis niloticus*) at the end of the experiment

| Index | Control | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| RBC ($10^6$/mm3) | $1.051 \pm 0.016^d$ | $1.163 \pm 0.017^c$ | $1.292 \pm 0.015^b$ | $1.392 \pm 0.013^a$ | $1.076 \pm 0.012^d$ |
| WBC ($10^3$/mm3) | $15.8 \pm 0.9^c$ | $20.7 \pm 1.5^b$ | $21.75 \pm 0.8^b$ | $31.8 \pm 1.5^a$ | $16.5 \pm 2.1^c$ |
| HCT (%) | $25.42 \pm 0.8^b$ | $25.72 \pm 0.7^b$ | $26.34 \pm 0.8^b$ | $28.56 \pm 0.6^a$ | $25.33 \pm 0.7^b$ |
| Hb (g/dL) | $7.05 \pm 0.09^d$ | $7.15 \pm 0.04^c$ | $7.35 \pm 0.03^b$ | $7.89 \pm 0.07^a$ | $6.98 \pm 0.05^d$ |

Dietary supplementation with pomegranate peel significantly enhanced the antioxidant status in the liver of the Nile tilapia (*Oreochromis niloticus*) after 90 days of feeding. Superoxide dismutase (SOD) activity was at its highest in fish from the T3 group (33.45 ± 2.15), compared to lower levels in T1 (22.53 ± 1.54), T2 (24.75 ± 2.15), T4 (17.16 ± 1.58), and the control (14.85 ± 1.47) (Table 2). Similarly, catalase (CAT) activity increased significantly in T3 (26.13 ± 0.58), while values were lower in T1 (18.27 ± 0.45), T2 (19.00 ± 1.12), T4 (13.82 ± 0.74), and control (12.13 ± 0.55). Glutathione peroxidase (GSH) levels were also elevated in T3 (80.12 ± 4.21), in contrast to T1 (67.54 ± 2.91), T2 (70.65 ± 3.22), T4 (55.38 ± 4.15), and control (52.76 ± 3.12).

Interestingly, total antioxidant capacity (TAC) values were lower in T3 (22.42 ± 2.32) compared to higher values recorded in T1 (39.16 ± 1.29), T2 (38.12 ± 2.23), T4 (37.32 ± 2.22), and control (44.32 ± 2.28). Malondialdehyde (MDA), a marker of lipid peroxidation, was reduced in T3 (3.14 ± 0.12), while higher levels were observed in T1 (3.59 ± 0.34), T2 (3.53 ± 0.17), T4 (3.76 ± 0.30), and control (4.53 ± 0.30) (Table 2).

Serum immunoglobulin M (IgM) concentrations were significantly higher in T3 (153.4 ± 5.21µg/ mL), compared to T1 (122.4 ± 6.57µg/ mL), T2 (125.2 ± 4.16µg/ mL), T4 (124.7 ± 3.46µg/ mL), and the control (86.3 ± 4.22µg/ mL).

**Growth performance**

The initial weights of the fish ranged from 0.81 ± 0.01 g to 0.83 ± 0.01g. Final weights were highest in T3 (62.95 ± 1.35g), followed by T1 (54.87 ± 1.55g), T2 (55.78 ± 1.88g), T4 (52.95 ± 0.35g), and control (53.98 ± 1.70g). Weight gain (WG) was significantly higher in T3 (62.12 ± 0.34g) compared to T1 (53.65 ± 0.15g), T2 (54.05 ± 0.55g), T4 (54.97 ± 0.87g), and control (52.14 ± 0.54g) (Table 3).

Survival rates ranged from 86% to 96%, with the highest observed in T1 (96.00 ± 1.50%) and the lowest in the control (86.00 ± 1.71%). Other groups showed survival rates of T2 (91.33 ± 1.15%), T3 (90.00 ± 2.00%), and T4 (89.34 ± 1.60%).

Feed conversion ratio (FCR) was lowest in T3 (1.32 ± 0.04), indicating superior feed efficiency. Higher FCR values were recorded in T4 (1.49 ± 0.03), T1 (1.43 ± 0.03), T2 (1.41 ± 0.06), and control (1.46 ± 0.02) (Table 3).

After three months of feeding the Nile tilapia with diets supplemented with pomegranate peel, the average moisture content ranged from 73.19 ± 3.21% to 72.14 ± 3.45%. Crude protein content varied between 66.72 ± 0.31% in T4 and 68.27 ± 0.23% in T3. The lipid content ranged from 17.18 ± 0.24% in T3 to 18.52 ± 0.16% in T4. Ash content ranged from 14.39 ± 0.03% in T1 to 14.76 ± 0.02% in T4 (Table 4).

**Table 2.** Measurements of antioxidant parameters in the liver tissue of the Nile tilapia (*Oreochromis niloticus*) after feeding with pomegranate peel meal

| Items | Control | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Superoxide Dismutase (SOD) | 14.85 ±1.47$^{c}$ | 22.53±1.54$^{b}$ | 24.75±2.15$^{b}$ | 33.45±2.15$^{a}$ | 17.16 ±1.58$^{c}$ |
| Total antioxidant capacity (TAC) | 44.32±2.28$^{a}$ | 39.16±1.29$^{b}$ | 38.12±2.23$^{b}$ | 22.42±2.32$^{c}$ | 37.32±2.22$^{b}$ |
| Catalase (Cat) | 12.13±0.55$^{c}$ | 18.27±0.45$^{b}$ | 19.00±1.12$^{b}$ | 26.13±0.58$^{a}$ | 13.82±0.74$^{c}$ |
| Glutathione peroxidase (GSH) | 52.76±3.12$^{c}$ | 67.54±2.91$^{b}$ | 70.65±3.22$^{b}$ | 80.12±4.21$^{a}$ | 55.38±4.15$^{c}$ |
| Malondialdehyde (MDA) | 4.53±0.30$^{a}$ | 3.59±0.34$^{b}$ | 3.53±0.17$^{b}$ | 3.14±0.12$^{c}$ | 3.76±0.30$^{b}$ |
| Immunoglobulin M IgM (µg/ml) | 86.3±4.22 $^{c}$ | 122.4±6.57 $^{b}$ | 125.2±4.16 $^{b}$ | 143.4±5.21 $^{a}$ | 124.7±3.46$^{b}$ |

**Table 3.** Effect of dietary pomegranate peel meal on growth performance parameters of the Nile tilapia (*Oreochromis niloticus*) after three months of feeding

| **Items** | Control | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Initial weight (g) | 0.83± 0.01$^{a}$ | 0.82± 0.02$^{a}$ | 0.81± 0.01$^{a}$ | 0.83 ± 0.02$^{a}$ | 0.81±0.02$^{a}$ |
| Final weight (g) | 53.98±1.70$^{b}$ | 54.87±1.55$^{b}$ | 55.78±1.88$^{b}$ | 62.95±1.35$^{a}$ | 52.95±0.35$^{b}$ |
| Gain (g) | 53.65±0.15$^{b}$ | 54.05±0.55$^{b}$ | 54.97±0.87$^{b}$ | 62.12±0.34$^{a}$ | 52.14±0.54$^{b}$ |
| FCR (%) | 1.46 ± 0.02$^{a}$ | 1.43± 0.03$^{a}$ | 1.41± 0.06$^{a}$ | 1.32± 0.04$^{b}$ | 1.49 ± 0.03$^{a}$ |
| Survival rate (%) | 86.00±1.71$^{b}$ | 90.00±2.00 $^{b}$ | 91.33±1.15$^{b}$ | 96.00±1.5$^{a}$ | 89.34±1.6$^{b}$ |

**Table 4.** Average values of moisture, crude protein, lipids, and ash content in the Nile tilapia after three months of feeding with pomegranate peel meal

| | Control | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Moisture | 73.08±2.31$^{a}$ | 73.19±3.21$^{a}$ | 72.91±2.54$^{a}$ | 72.14±3.45$^{a}$ | 73.00±2.76$^{a}$ |
| Protein | 67.45±0.24$^{b}$ | 67.69±0.32$^{b}$ | 67.75±0.53$^{b}$ | 68.27±0.23$^{a}$ | 66.72±0.31$^{c}$ |
| Lipid | 18.13±0.17$^{a}$ | 17.92±0.25$^{a}$ | 17.78±0.19$^{a}$ | 17.18±0.24$^{b}$ | 18.52±0.16$^{c}$ |
| Ash | 14.42±0.04$^{b}$ | 14.39±0.03$^{b}$ | 14.47±0.04$^{b}$ | 14.55±0.03$^{a}$ | 14.76±0.02$^{c}$ |

## DISCUSSION

Our investigation in the Nile tilapia (*Oreochromis niloticus*) demonstrated that dietary inclusion of pomegranate peel meal positively influences physiological status and growth performance. The aquaculture industry could benefit by converting pomegranate peel by-products into valuable dietary additives **(Iqbal *et al.*, 2008; Dawood & Koshio, 2020; Dawood *et al.*, 2020a)**.

Hematological parameters such as RBC count, hemoglobin, and hematocrit levels were negatively affected at higher inclusion levels of pomegranate peel (2.0g/ kg diet), likely due to reduced feed intake leading to anemia **(Fazio, 2019; Dawood *et al.*, 2020b)**. Similarly, reductions in RBCs, hemoglobin, and hematocrit were observed in the Nile tilapia fed high concentrations of pomegranate meal **(Badrey *et al.*, 2019)**.

On the other hand, our data showed that pomegranate peel had immunostimulatory effects, evident through increased leukocyte counts across different treatment groups. This aligns with findings by **Badrey *et al.* (2019)**, who reported enhanced hematological parameters and immune responses in the Nile tilapia fed with pomegranate peel meal. Likewise, **Harikrishnan *et al.* (2010)** observed improved disease resistance in the olive flounder, attributed to increased white blood cell counts following dietary pomegranate inclusion. In rainbow trout (*Oncorhynchus mykiss*), RBCs and hemoglobin levels were also elevated when diets were supplemented with pomegranate seed oil.

In fact, increased serum total protein and immunoglobulin M (IgM) levels were observed in our study, especially in fish from treatment 1, indicating enhanced immune status. These findings are consistent with **Acar *et al.* (2018)** and **Badrey *et al.* (2019)**,

who reported elevated serum IgM and protein levels in tilapia fed pomegranate peel. Pomegranate's active compounds are known to strengthen the immune system **(Dügenci *et al.*, 2003)**.

Our findings further revealed that pomegranate peel supplementation enhanced liver antioxidant activity (Table 3). In contrast, elevated levels of alkaline phosphatase (ALP) and malondialdehyde (MDA), an indicator of lipid peroxidation and cellular damage, were reduced in fish fed with 1% pomegranate peel.

The optimal growth response was observed at the 1% supplementation level, which produced significantly better results than higher concentrations, which led to performance similar to the control group. This is in line with the hypothesis that growth can be stimulated by certain medicinal herbs due to enhanced feed palatability and intake **(Sarhadi *et al.*, 2020; Srichaiyo *et al.*, 2020)**. The high fiber and polyphenol content of pomegranate peel may contribute to improved growth performance, but excessive amounts could impair feed intake and reduce digestive enzyme activity **(Madrigal-Carballo *et al.*, 2009; Zheng *et al.*, 2017)**.

A direct relationship was observed between pomegranate peel supplementation and feed conversion ratio (FCR). In the rainbow trout, diets containing high levels of pomegranate peel failed to yield beneficial FCR values, consistent with our findings in Nile tilapia, where higher inclusion rates were associated with reduced growth and feed intake **(Badrey *et al.*, 2019)**.

Survival rate was highest in fish fed the 1% pomegranate peel diet compared to those in the 0, 0.1, 0.5, and 2% groups. High survival rates are indicative of better health conditions, and were similarly reported in fish fed pomegranate-supplemented diets by **Dawood *et al.* (2014)** and **Magouz *et al.* (2020)**.

## CONCLUSION

Our observations indicate that incorporating 1% pomegranate peel meal into the diet of the Nile tilapia (*Oreochromis niloticus*) is an optimal level for improving blood indices, enhancing immune response, and promoting growth performance. Further studies are recommended to explore the broader medical applications of pomegranate peel, particularly its potential in aquaculture health management and therapeutic use in other fields.

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