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# Dietary Effects of Dandelion (*Taraxacum officinale*) as a Food Additive on the Growth Performance, Body Composition and Health Status of Cultured *Oreochromis niloticus*

**Asmaa Maghawri[1], Samar S. Marzouk[2], Mai Nashaat[2]***

[1]Department of Aquaculture Diseases Control, Fish Farming and Technology Institute (FFTI), Suez Canal University, 41522 Ismailia, Egypt

[2]National Institute of Oceanography and Fisheries, (NIOF), Cairo, Egypt

***Corresponding author: mainashat85@gmail.com**

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## ABSTRACT

This study demonstrates that supplementing the Nile tilapia (*Oreochromis niloticus*) diets with dandelion leaves (DL) can significantly improve growth, body composition, immune responses, and overall health. Fish fed the highest DL concentration (2.00g/ kg) exhibited the best growth parameters, including weight, total length, weight gain, specific growth rate, and survival rate, compared to the control group. Additionally, body protein and lipid content increased, while moisture and ash content decreased in these fish. Hematological and bio-somatic indices, including total protein, glucose, antioxidant enzymes, and reduced oxidative stress markers, were significantly better in fish fed DL. Immune system responses, such as increased levels of glutathione, lysozyme, and nitric oxide, were enhanced, indicating an improved immune health. Furthermore, histological analysis showed improvements in the intestinal structure of the treated fish. These findings suggest that dandelion leaf supplementation, particularly at 1.00 and 2.00g/ kg, can greatly enhance the growth, immune function, and overall well-being of the Nile tilapia, making it a promising natural feed additive for sustainable fish farming.

## INTRODUCTION

In recent years, as farming water quality deteriorates and aquaculture density increases, fish have become more vulnerable to intestinal diseases, leading to antibiotic overuse and significant food safety concerns **(Tan & Sun, 2020)**. To address these issues, immunostimulants that are pollution-free and non-residual have been proposed as alternatives in sustainable aquaculture **(Ringø & Song, 2016)**. Plant extracts, recognized as functional feed additives, are increasingly used to improve immunity, gut health, and growth in fish due to their wide availability, cost-effectiveness, environmental friendliness, and broad-spectrum bactericidal properties. Additionally, these plant-based additives are metabolized by fish with minimal residual effects, making them a promising option in aquaculture **(Nayak, 2010; Awad & Awaad, 2017; Tan *et al.*, 2017)**.

Dandelion, a perennial herb belonging to the Asteraceae family, contains active compounds such as polysaccharides, phenolic acids, and flavonoids, which are known for their therapeutic effects in alleviating intestinal inflammation, liver diseases, and dyspepsia symptoms **(Schütz *et al.*, 2006; You *et al.*, 2010)**. Dandelion extract has been reported to exhibit bactericidal, antioxidant, immune-boosting, anti-inflammatory, and antioxidant properties **(Qian *et al.*, 2014)**. Previous studies have demonstrated the positive effects of dietary dandelion extracts on fish species such as golden pompano, enhancing intestinal morphology, growth performance, bactericidal capacity, physical barrier function, and immune responses **(Tan *et al.*, 2018)** of the hybrid grouper **(Mirghaed *et al.*, 2019)** and rainbow trout **(Sun *et al.*, 2019; Shekarabi *et al.*, 2021)**.

The objective of the current study was to investigate the effects of dietary supplementation with dandelion leaves (*Taraxacum officinale*) on the growth, body composition, somatic and blood indices, and immune responses of *Oreochromis niloticus* (the Nile tilapia).

## MATERIALS AND METHODS

### 1. Diet preparation

The powdered dandelion leaves (*Taraxacum officinale*) were purchased from the local herbal markets in Ismailia, Egypt. Concentrations of DL (0, 0.5, 1.0 and 2.0g/ kg) were well combined with 1.2mm commercial pellets that contained 38% protein from the Skretting Company in Egypt. As indicated in Table (1), diets for treated groups were made in accordance with the methods of **Sirakov *et al.* (2018)**.

### 2. Feeding experiments and experimental conditions

A total of 120 healthy Nile tilapia (*Oreochromis niloticus*) fingerlings, with an average weight of 4.59 ± 0.02g, were collected from the Fish Farming and Technology Institute (FFTI) at Suez Canal University, Egypt. The fish were then transported alive to the research unit. The 120 fingerlings were divided into four equal groups, with three replicates (10 fish per replicate) for each treatment. Prior to the experiment, the fish were acclimated for two weeks in twelve fully equipped aquariums (32×44×73cm) with a capacity of 60 liters of dechlorinated water. They were fed a regulated ration twice daily at 10:00 a.m. and 3:00 p.m. during the acclimation period. The aquarium water was regularly changed, with 25% of the water being replaced. Throughout the experiment, the water parameters were maintained within the following ranges: dissolved oxygen (5.51–7.00mg/ L), pH (7.6–8.3), temperature (22–24°C), and total ammonia nitrogen (TAN) concentrations (0.11–0.13mg/ L).

For a period of 45 days, the Nile tilapia were fed a basic diet supplemented with dandelion leaves (DL) at the following concentrations:

DL0: Control group (no dandelion leaves supplement)

DL1: 0.50 g/kg of dandelion leaves

DL2: 1.00 g/kg of dandelion leaves

DL3: 2.00 g/kg of dandelion leaves

Each group was fed their respective diet daily, and the growth, body composition, immune responses, and other relevant parameters were evaluated throughout the study.

**Table 1.** Feed and chemical content of the components in the diet with *T. officinale* doses

| **Component** | **Feed Composition (%)** | | | |
|---|---|---|---|---|
| Fish meal | 16.22 | 16.26 | 16.56 | 17.04 |
| Soybean meal | 48.5 | 48.47 | 48.44 | 48.27 |
| Maize | 18.53 | 18.52 | 18.5 | 18.44 |
| Wheat | 12 | 12 | 12 | 12 |
| Fish oil | 3 | 3 | 3 | 3 |
| *Vitamin (mineral premix) | 1.75 | 1.75 | 1.50 | 1.25 |
| *Taraxacum officinale* (g/kg diet) | 0.0 | 0.5 | 1 | 2 |
| **Components** | **Chemical composition (%)** | | | |
| Crude protein | 38 | 38 | 38 | 38 |
| Crude fat | 8.5 | 8.45 | 8.3 | 8.25 |
| Fiber | 5.2 | 5.17 | 5.00 | 5.1 |
| Ash | 7.5 | 7.46 | 7.3 | 7.2 |
| Nitrogen-free extract | 40.8 | 40.58 | 39.9 | 39.5 |
| *Taraxacum officinale* (g/kg diet) | 0.00 | 0.5 | 1 | 2 |

*Vitamins (mineral premix): contains the next mixture ($g/kg^{-1}$): folic acid 1.5; cholecalciferol 0.1; mentioned 21; retinyl acetate 0.67; thiamine 6.5; pyridoxine 5.5; riboflavin 11; p-aminobenzoic acid 40; ascorbic acid 130; Inositol 49; choline chloride 350; biotin 0.1; butylated hydroxy toluene 1.5:$MgSo_4$ 33.5; $CaHPO_4$, $2H_2O$ 30.5; $MgCl_2$ 54.7; Ca $(HPo_4)_2$, $H_2O$ 219; $NaHco_3$ 95.6; $FeSo_4$ $H_2o$ 10;NaCl 172.4; $CuSo_4$ 5; KCi 100; $Na_2$ Seo 35; $ZnSo_4$ 10 and $H_2O$ 0.4.

### 3. Growth performance parameters and fish body composition

On the 45th day of the study, 16 hours after the last feeding, the fish were weighed to determine their final weight. This final weight was then compared to the weight recorded on the first day to calculate the weight gain (WG). The survival rate was also estimated based on the number of fish remaining in each tank. Additionally, the specific growth rate (SGR) was calculated using the following formula:

**WG =FW–IW.**

**SGR(%/day) = 100 (ln FW–ln IW)/ T**

**Condition factor (K) = W/L3 × 100**

**ADG = FW–IW/T**

Where, W= fish weight; L= fish length; IW=initial weight (g); FW=final weight (g); T= period (days); and ln **=** the natural log**.**

Three fish from each group had their total body composition (protein, moisture, ash, and lipids) assessed utilizing the techniques explained by the Association of Official Analytical Chemists **(AOAC, 1997)**.

### 4. Hepatosomatic, spleenosomatic and gonadosomatic indices

The internal organs liver, spleen, and gonads were removed when the fish was dissected. A digital sensitive balance was used to weigh the organs. To calculate the hepatosomatic index (HSI), spleenosomatic index (SSI), and gonadosomatic index (GSI), the following formulas are used, as per **Pandit and Gupta (2019)**:

**HSI= Liver weight (g)/ fish weight (g) ×100**

**SSI= Spleen weight (g)/ fish weight (g) ×100**

**GSI = Gonads weight (g)/ fish weight (g) ×100**

### 5. Blood sampling

Following a day of fasting, three fish individuals from each group were given a small amount of anesthesia using a 50µl solution of clove oil. Using the **Noga (2010)** approach, two tubes were prepared to collect samples of blood from the caudal vein.

### 6. Hematological parameters

Using the techniques described by **Tran-Duy *et al.* (2008)**, the following parameters were measured: hematocrit test (Hct), total red blood cells (RBCs), total leukocyte count (TLC), hemoglobin content (Hb), lymphocytes, monocytes, granulocytes, platelets, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

**7. Biochemical parameters**

The method of **Lowry *et al.* (1951)** was used to determine the serum total protein (TP) concentration. Following the approach of **Samadaii and Bahrekazemi (2020)**, the serum levels of calcium (Ca), globulin (GL), albumin (ALB), creatinine (CR), uric acid (UA), and urea (U) were each measured. Serum liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were identified using commercial kits, as outlined by **Reitmann (1957)**.

**8. Evaluation of antioxidant enzymes activity and oxidative stress marker**

The activity of antioxidant enzymes in liver tissue from each group was assessed. Reagents were purchased from a bio-diagnostic company (Diagnostic and Research Agency). The traditional procedure outlined by **Del Rio *et al*. (2003)** was used to determine malondialdehyde (MDA; CAT no. 25.29). The technique of **Paoletti *et al*. (1986)** was employed to measure the activity of superoxide dismutase (SOD; SD 25.21). The reduced glutathione (GSH) assay kit (CAT no. GR 25.11) was used to measure GSH levels. The methods of **Beers and Sizer (1952)** were used to determine catalase activity (CAT; CAT no. CA 25.17).

**9. Respiratory burst (RB), lysozyme activity and nitric oxide**

Serum lysozyme activity, nitric oxide (NO; CAT NO. NO 25 33), and respiratory burst activity (RBA) were determined for all groups using the procedures outlined by **Stolen *et al*. (1990)**.

**10. Histopathological examinations**

Liver, spleen, and intestinal samples were kept for 24 hours in 10% neutral formalin prior to being moved to 70% ethanol to ensure full preservation. The stain Hematoxylin and eosin (H & E) was employed for preparing and staining the specimens in accordance with **Bancroft and Gamble (2008)**.

**11. Statistical analysis**

After presenting the results as means ± standard error (SE), IBM SPSS Statistics 20.0 was used to evaluate them using a one-way analysis of variance (ANOVA). According to **Dytham (2011)**, Duncan's multiple-range test was employed to identify group differences, with significance set at $P \leq 0.05$.

## RESULTS

**1. Growth performance and somatic indices of *O. niloticus* after feeding with *T. officinale***

Table (2) shows a highly significant difference ($P \leq 0.05$) within groups for most growth parameters, including weight (Wt), total length (TL), weight gain (WG), average

daily gain (ADG), and specific growth rate (SGR). However, the condition factor (K) showed no significant difference ($P > 0.05$) between groups. The DL3 group exhibited the highest growth rates across all factors, along with the highest survival rate of 96.7%.

Hepatosomatic index (HSI), spleenosomatic index (SSI), and gonadosomatic index (GSI) also showed highly significant differences between groups ($P \leq 0.05$). The highest values for HSI were recorded in the DL1 and DL3 groups, while the highest SSI was observed in the DL2 group. The highest GSI was detected in the DL1 group.

**Table 2.** The influence of dietary *T. officinale* concentrations on *O. niloticus* growth performance and somatic indices

| Groups / Factor | *Taraxacum officinale (g/kg diet)* | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P value*** |
| **Wt (g)** | 12.11±0.99 $^{c}$ | 12.3±1 $^{c}$ | 15.45±2.19 $^{b}$ | 20.39±1.06$^{a}$ | 0.00 |
| **TL (cm)** | 9.01±0.34 $^{b}$ | 9.3±0.35 $^{b}$ | 9.84±0.46 $^{b}$ | 10.98±0.22 $^{a}$ | 0.00 |
| **WG (g)** | 7.11±0.9 $^{b}$ | 7.3±1.01 $^{b}$ | 9.46±2.14$^{b}$ | 15.4±1.07$^{a}$ | 0.00 |
| **ADG (g)** | 0.15±0.02 $^{b}$ | 0.16±0.02 $^{b}$ | 0.21±0.05 $^{b}$ | 0.34±0.02 $^{a}$ | 0.00 |
| **SGR%** | 1.89±0.19 $^{b}$ | 1.93±0.18 $^{b}$ | 2.29±0.34 $^{b}$ | 2.99±0.15 $^{a}$ | 0.01 |
| **Condition Factor (K)** | 1.64±0.06 $^{a}$ | 1.52±0.07 $^{a}$ | 1.53±0.05 $^{a}$ | 1.66±0.08 $^{a}$ | 0.30 |
| **SR%** | 83.3 | 80 | 86.7 | 96.7 | - |
| **HSI** | 1.21±011 $^{b}$ | 2.87±0.27 $^{a}$ | 1.09±0.28 $^{b}$ | 3.37±0.21 $^{a}$ | 0.00 |
| **SSI** | 0.22±0.02 $^{b}$ | 0.24±0.03 $^{b}$ | 2.18±0.48 $^{a}$ | 0.34±0.04 $^{b}$ | 0.00 |
| **GSI** | 0.41±0.03 $^{b}$ | 1.24±0.3 $^{a}$ | 0.65±0.23 $^{b}$ | 0.23±0.02 $^{b}$ | 0.00 |

Note: a-c means that there were statistically significant differences between groups values at ($P \leq 0.05$) within the same row, n = 10/group. Weight (Wt), total length (TL), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), condition factor (K) and survival rates (SR). Hepatosomatic index (HSI), spleenosomatic index (SSI) and gonadosomatic index (GSI). A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

## 2. Effect of *T. officinale* concentrations on fish body composition of *O. niloticus*

Table (3) shows that there is a highly significant difference between groups in all fish body composition parameters ($P \leq 0.05$). Protein and lipids recorded a highly significant value in DL3 group, and the minimal values were detected in control one DL0. Moisture and ash values were significantly higher in the DL0 group, while the lowest values were observed in the DL3 group.

**Table 3.** Effect of *T. officinale* concentrations on fish body composition (wet weight %) of *O. niloticus*

| Groups / Factor | ***Taraxacum officinale* (g/kg diet)** | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P value*** |
| **Protein** | 12.46±0.02 $^{d}$ | 12.73±0.04 $^{c}$ | 13.23±0.03 $^{b}$ | 13.79±0.02 $^{a}$ | 0.00 |
| **Moisture** | 77.24±0.03 $^{a}$ | 76.36±0.05 $^{c}$ | 76.49±0.02 $^{b}$ | 75.19±0.03 $^{d}$ | 0.00 |
| **Ash** | 1.72±0.01 $^{a}$ | 1.42±0.1 $^{c}$ | 1.53±0.01 $^{b}$ | 1.39±0.01 $^{d}$ | 0.00 |
| **Lipid** | 4.85±0.03 $^{c}$ | 5.12±0.03 $^{b}$ | 5.19±0.03 $^{b}$ | 5.29±0.02 $^{a}$ | 0.00 |

Note: a-d means that there were statistically significant differences between group values at ($P \leq 0.05$) within the same row, n = 10/group. A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

## 3. Hematological parameters

Table (4) shows that all hematological parameters detected a highly significant difference between groups ($P \leq 0.05$). The higher group values in all total and differential blood count were recorded in DL3 group than other groups. DL0 group recorded the lowest values in Hb, RBCs, HCT, MCV, MCH, MCHC, lymphocyte and Eosinophil counts, while lowest count in PLT and total leukocyte count were recorded in DL1 and DL2 groups, respectively.

## 4. Biochemical parameters

Table (5) shows that all biochemical parameters detected a highly significant difference between groups ($P \leq 0.05$). DL3 group recorded the highest values of ALT, AST, TP, ALB and GL with lowest values of CR, U and UA. Other groups ranged in parameters between high and low, as DL0 group was the lowest in most of them except in CR and U values were high.

**Table 4.** Influence of dietary *T. officinale* concentrations on hematological parameters of *O. niloticus*

| Groups / Factor | *Taraxacum officinale* (g/kg diet) | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P* Value** |
| **Hb (g/dl)** | 5.21±0.02 $^{d}$ | 5.51±0.02 $^{c}$ | 6.41±0.02 $^{b}$ | 7.61±0.01 $^{a}$ | 0.00 |
| **RBCs ($mm^3$)** | 1.22±0.01 $^{d}$ | 1.24±0.01 $^{c}$ | 1.43±0.01 $^{b}$ | 1.56±0.01 $^{a}$ | 0.00 |
| **HCT (%)** | 15.62±0.03 $^{d}$ | 16.52±0.03 $^{c}$ | 19.22±0.03 $^{b}$ | 22.82±0.03 $^{a}$ | 0.00 |
| **MCV ($\mu m^3$)** | 127.19±0.31 $^{d}$ | 133.19±0.31 $^{c}$ | 134.18±0.3 $^{b}$ | 146.18±0.28 $^{a}$ | 0.00 |
| **MCH (g/dl)** | 42.09±0.15 $^{d}$ | 44.19±0.31 $^{c}$ | 45.18±0.3 $^{b}$ | 48.19±0.31 $^{a}$ | 0.00 |
| **MCHC (g/dl)** | 33.19±0.31 $^{d}$ | 34.18±0.3 $^{c}$ | 36.18±0.31 $^{b}$ | 38.2±0.29 $^{a}$ | 0.00 |
| **PL (mcL)** | 76.19±0.31 $^{c}$ | 58.19±0.31 $^{d}$ | 169.18±0.32 $^{b}$ | 172.41±0.32 $^{a}$ | 0.00 |
| **TLC (µl)** | 90.29±0.04 $^{c}$ | 96.28±0.31 $^{b}$ | 89.23±0.31 $^{d}$ | 103.19±0.3 $^{a}$ | 0.00 |
| **Neutrophil (%)** | 50.28±0.31 $^{c}$ | 51.28±0.31 $^{b}$ | 39.21±0.3 $^{d}$ | 55.75±0.27 $^{a}$ | 0.00 |
| **Lymphocyte (%)** | 43.23±0.31 $^{d}$ | 46.78±0.28 $^{c}$ | 52.26±0.31 $^{b}$ | 58.54±0.27 $^{a}$ | 0.00 |
| **Monocyte (%)** | 6.55±0.16 $^{c}$ | 4.83±0.19 $^{d}$ | 8.49±0.27 $^{b}$ | 10.26±0.31 $^{a}$ | 0.00 |
| **Eosinophil (%)** | 0.05±0.02 $^{b}$ | 0.06±0.02 $^{b}$ | 0.07±0.03 $^{b}$ | 0.15±0.04 $^{a}$ | 0.05 |

Note: a-d means that there were a statistically significant differences between group values at ($P \leq 0.05$) within the same row, n=10/group. Hemoglobin (Hb), red blood cells (RBCs), hematocrit (HCT), (MCV), mean corpuscular volume (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PL), and (TLC) total leukocyte counts. A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

**Table 5.** Influence of dietary *T. officinale* concentrations on biochemical parameters of *O. niloticus*

| Groups / Factor | *Taraxacum officinale* (g/kg diet) | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P Value*** |
| **ALT (U/L)** | 25.19±0.31 $^{c}$ | 43.19±0.31 $^{b}$ | 22.09±0.3 $^{d}$ | 88.19±0.3 $^{a}$ | 0.00 |
| **AST (U/L)** | 33.19±0.31 $^{c}$ | 27.18±0.3 $^{d}$ | 48.15±0.2 $^{b}$ | 55.3±0.1 $^{a}$ | 0.00 |
| **TP (g/dl)** | 2.09±0.15 $^{d}$ | 2.99±0.06 $^{c}$ | 4.12±0.03 $^{b}$ | 5.09±0.15 $^{a}$ | 0.00 |
| **ALB (g/dl)** | 1.42±0.03 $^{c}$ | 1.62±0.01 $^{b}$ | 1.42±0.03 $^{c}$ | 1.82±0.02 $^{a}$ | 0.00 |
| **GL (g/dl)** | 0.61±0.02 $^{d}$ | 1.62±0.03 $^{c}$ | 2.72±0.03 $^{b}$ | 3.42±0.09 $^{a}$ | 0.00 |
| **CR (g/dl)** | 0.11±0.02 $^{b}$ | 0.24±0.03 $^{a}$ | 0.14±0.02 $^{b}$ | 0.14±0.03 $^{b}$ | 0.01 |
| **U (g/dl)** | 41.09±0.15 $^{a}$ | 38.09±0.2 $^{b}$ | 37.08±0.14 $^{c}$ | 34.05±0.16 $^{d}$ | 0.00 |
| **UA (g/dl)** | 1.62±0.03 $^{b}$ | 3.18±0.02 $^{a}$ | 1.01±0.01 $^{c}$ | 0.71±0.02 $^{d}$ | 0.00 |

Note: a-d means that there were a statistically significant differences between group values at ($P \leq 0.05$) within the same row, n=10/group. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), Globulin (GL), creatinine (CR), urea (U) and uric acid (UA). A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

**5. Oxidative biomarkers and antioxidant enzymes:**

Table (6) shows that all oxidative enzymes detected a highly significant difference within groups ($P \leq 0.05$), MDA recorded a higher value in DL1, followed by DL0 groups, while the lowest value was in DL3 group. SOD, CAT and GPx recorded the higher values in DL3 group while the lowest value was recorded in DL0 and DL1. GSH has a higher value recorded in DL2 group followed by DL3, while the lowest value was recorded in DL0.

**Table 6.** Influence of dietary *T. officinale* concentrations on oxidative biomarkers and antioxidant enzymes in *O. niloticus*

| Groups / Factor | ***Taraxacum officinale* (g/kg diet)** | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P* value** |
| **MDA** nmol/g.tissue | 5441.95±19.66 $^{b}$ | 5495.89±10.11 $^{a}$ | 5258.82±7.77 $^{c}$ | 43665.59±6.14 $^{d}$ | 0.00 |
| **SOD** U/g. tissue | 1479.64±3.89 $^{c}$ | 1119.6±3.24 $^{d}$ | 1823.11±6.03 $^{b}$ | 3356.99±7.68 $^{a}$ | 0.00 |
| **GSH** mg. /g. tissue | 12.79±0.04 $^{d}$ | 15.57±0.08 $^{c}$ | 17.57±0.08 $^{a}$ | 16.09±0.15 $^{b}$ | 0.00 |
| **CAT** (U/g. tissue) | 4.09±0.15 $^{d}$ | 5.09±0.15 $^{c}$ | 5.72±0.03 $^{b}$ | 6.05±0.05 $^{a}$ | 0.00 |
| **GPx** U/g. tissue | 19.19±0.31 $^{d}$ | 20.09±0.15 $^{c}$ | 25.19±0.31 $^{b}$ | 30.19±0.31 $^{a}$ | 0.00 |

Note: a-d means that there were a statistically significant differences between group values at ($P \leq 0.05$) within the same row, n=10/group. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and glutathione peroxidase (GPx). A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

**6. The respiratory burst (RB), lysozyme activity and nitric oxide (NO)**

Table (7) shows that the activities of respiratory burst, lysozyme, and nitric oxide exhibited highly significant differences ($P \leq 0.05$) between groups. The highest values were recorded in the DL3 group, followed by the DL2 group, while the lowest values were observed in the DL0 and DL1 groups.

**Table 7.** Influence of dietary *T. officinale* levels on the activity of RB, lysozyme activity and NO in *O. niloticus*

| Groups / Factor | *Taraxacum officinale* (g/kg diet) | | | | |
|---|---|---|---|---|---|
| | **0 (DL0)** | **0.5 (DL1)** | **1 (DL2)** | **2 (DL3)** | ***P value*** |
| **RB (mg/mL)** | 0.24±0.01 [c] | 0.23±0.02 [d] | 0.31±0.01 [b] | 0.35±0.01 [a] | 0.00 |
| **Lysozyme (µg /mL)** | 155.68±0.77 [d] | 165.09±1.14 [c] | 177.83±0.38 [b] | 183.85±1.03 [a] | 0.00 |
| **NO (µmol /L)** | 96.23±0.31 [c] | 87.87±0.38 [d] | 160.88±0.78 [b] | 255.69±0.77 [a] | 0.00 |

Note: a-d means that there were a statistically significant differences between group values at ($P \leq 0.05$) within the same row, n=10/group. Respiratory burst (RB) and nitrix oxide (NO). A one-way ANOVA test was applying using SPSS statistical program. The same row values with the same superscript are not statistically significant ($P > 0.05$).

## 7. Histological and histopathological results

As shown in Plate 1, the liver and spleen of the control group (DL0) exhibit uniform cells with no evidence of deformities in the hepatocytes or splenic cells (Plate 1a and e). The other treated groups showed mild effects on the cells, likely due to adaptation stress or blood and tissue sampling during the experimental period, which may result in a temporary, non-significant effect on the tissue of immune organs such as the liver and spleen.

The liver of the DL1 group shows mild edema and moderate congestion in hepatic cells and the hepatopancreas, while the splenic tissue shows moderate depletion of white pulp and melanomacrophage cells (Plate 1b and f). The liver of the DL2 group exhibits mild necrosis in hepatic cells and the hepatopancreas, with mild depletion in the spleen's white pulp, although the red pulp appears normal (Plate 1c and g). The liver of the DL3 group shows moderate edema in hepatocytes and the hepatopancreas, while the splenic tissue shows moderate depletion of white pulp, with normal red pulp aggregations (Plate 1d and h).

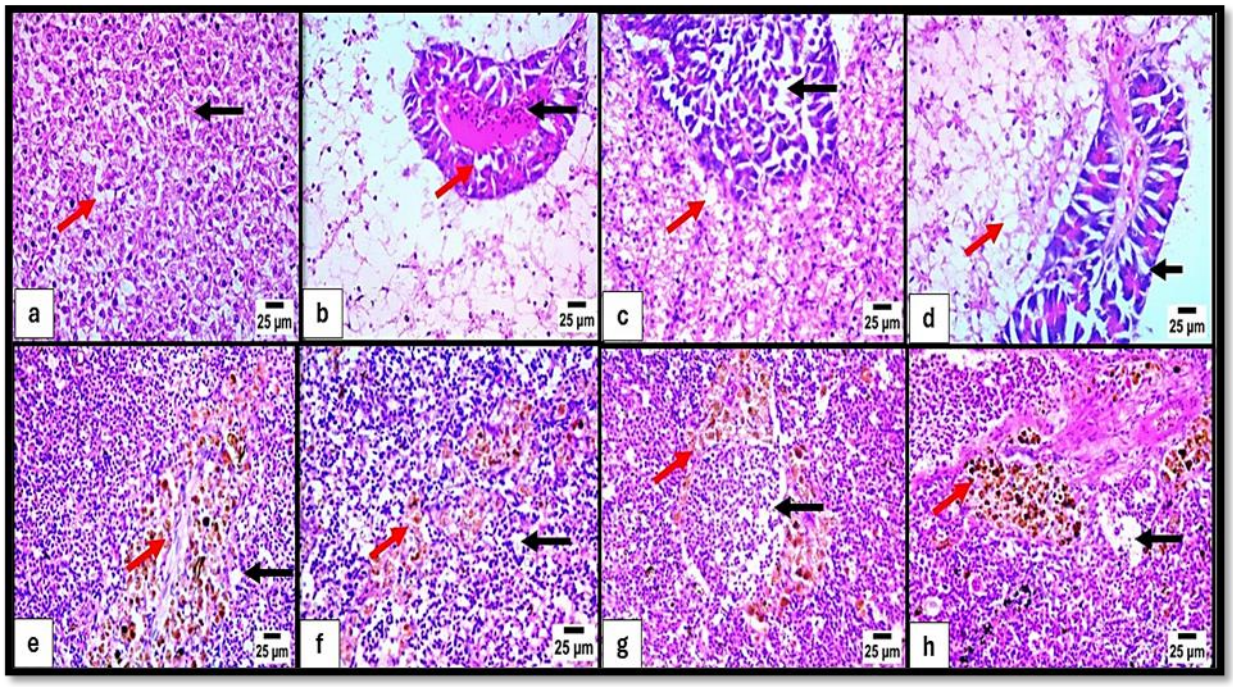


**Plate 1.** Photomicrographs of histopathological sections of livers and spleens of *O. niloticus* fed different doses of *Taraxacum officinale*. Liver of DL0 (control group), showing no marked pathological changes in hepatocytes (H&E, 25µm) **(a)**. Liver of DL1 group mild edema in hepatocytes (red arrow) with moderate congestion of blood vessels of hepatopancreas (black arrow) (H&E, 25µm) **(b).** Liver of DL2 group showing mild necrobiotic changes in hepatocytes (red arrow) with moderate edema in hepatopancreas (black arrow) (H&E, 25µm) **(c)**. Liver of DL3 group showing moderate degeneration in hepatocytes (red arrow) with mild edema in hepatopancreas (black arrow) (H&E, 25µm) **(d).** Spleen of DL0 (control group), showing no marked pathological changes in white pulps (black arrow) and melanomacrophage center (red arrow) (H&E, 25µm) **(e).** Spleen of DL1 group revealed moderate depletion of the white pulp (black arrow) and melanomacrophage centers (red arrow) (H&E, 25µm) **(f)**. Spleen of DL2 group showing mild depletion of white pulps (black arrow) with normal red pulps (red arrow) (H&E, 25µm) **(g)**. Spleen of DL3 group showing moderate depletion of white pulps (black arrow) and normal aggregates of red pulps (red arrow) (H&E, 25µm)

## Intestine histopathological change and intestinal villi length

As shown in Plate 2, the intestinal tissue of the control group (DL0) displays normal epithelial cells with a regular arrangement of intestinal villi (a). In contrast, the other treated groups showed changes in some parts of the intestinal tissue, including mild depletion, particularly at the tips of the intestinal villi. This suggests intracellular interactions between the diets and the fish intestinal tissue (b, c, and d).

As shown in Fig. (1), the length of the intestinal villi recorded a minimal average in the DL1 group, while the maximal average was observed in the DL3 group. This indicates the high efficiency of the food additive, especially at higher doses, which increases the area available for food absorption, promoting growth rates and enhancing fish health.

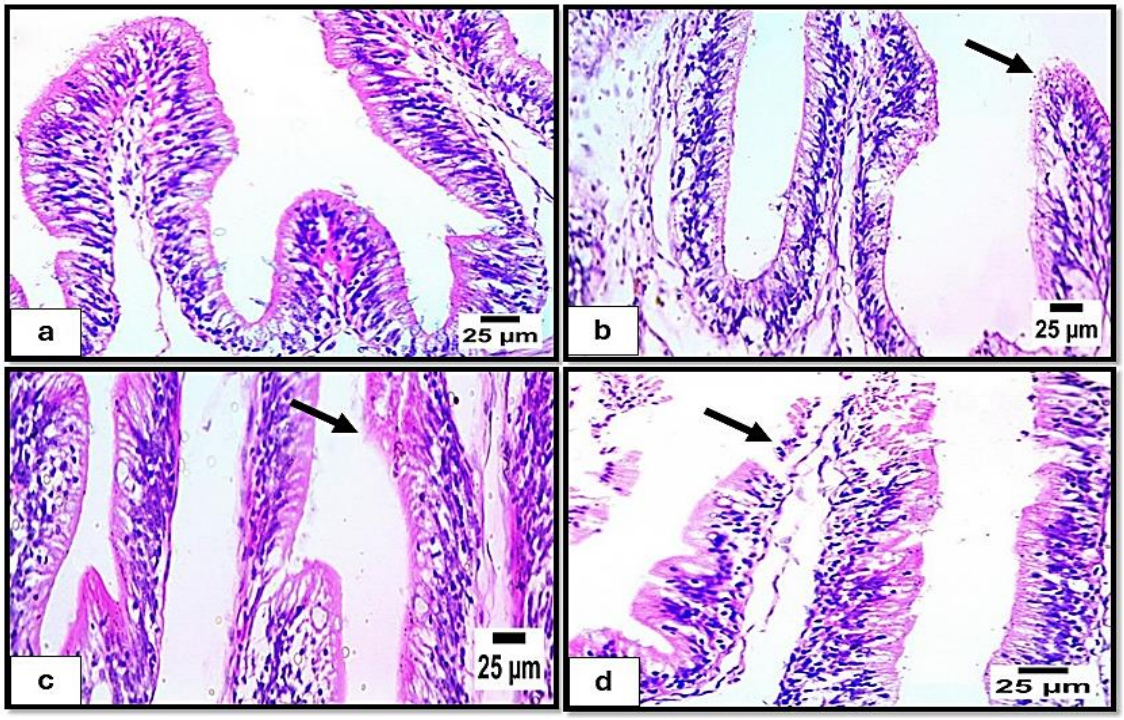


**Plate 2.** Photomicrographs of histopathological sections of intestine of *O. niloticus* fed various doses of *Taraxacum officinale*. DL0 group appearing normal histological structure of intestinal villi (H&E, 25µm) **(a)**. DL1 group showing regular arrangement of intestinal cells with mild depletion of the tips of epithelial tissue (black arrow) (H&E, 25µm) **(b).** DL2 group showing normal epithelial cells with mild depletion of the intestinal villi (black arrow) (H&E, 25µm) **(c).** DL3 group showing regular epithelial cells arrangement with observed tissue depletions in the tips (black arrow) (H&E, 25µm)

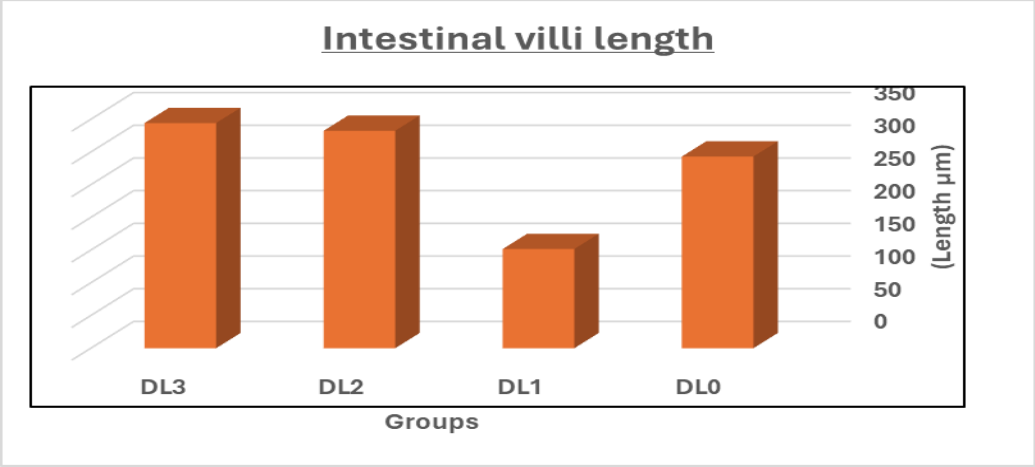


**Fig. 3.** The intestinal villi in control and treated groups of *O. niloticus* fed different doses of *Taraxacum officinale*. DL1 group recorded minimal average length (151.69µm), followed by control group DL0 (293.05µm). The maximum average length recorded in DL3 group (344.31µm) followed by DL2 group (332.45µm)

## DISCUSSION

Plant extracts and byproducts contain several effective compounds that have been identified as efficient substitutes for conventional antibiotics, vaccines, and chemotherapy. Therefore, active extracts from certain plants could be used as immune enhancers in aquaculture. Additionally, these extracts are often low-cost, locally available, functionally effective against a variety of opportunistic pathogens, rapidly biodegrade, and are environmentally friendly **(Harikrishnan *et al.*, 2011)**. Currently, researchers believe that dietary additives that regulate the microbiota of fish intestines can enhance fish health through their activities, establishing a connection between fish gut microbiota, growth performance, and immunity **(Nayak, 2010; Newaj-Fyzul & Austin, 2015)**.

The present trial found highly significant differences ($P \leq 0.05$) within groups for most growth parameters, with a significant increase in growth performance in the DL3 group (2g/ kg) compared to the other groups, which also exhibited a higher survival rate of 96.7%. However, the condition factor (K) showed no significant difference ($P > 0.05$) within groups, which concurs with the findings of **Tan and Sun (2020)**, who demonstrated that dietary dandelion root extract (DE) could enhance the development and gut microbiota of golden pompano.

In comparison with the control group, the 0.50 and 1.00g/ kg DE additive groups showed increases in weight gain and feed intake. Furthermore, **Tan *et al.* (2018)** showed that dietary supplementation with dandelion extracts at a concentration of 1g/ kg significantly enhanced intestinal immune function, promoted intestinal growth, and strengthened the intestinal physical barrier in the golden pompano. These factors may have contributed to the fish's increased appetite and improved utilization of dietary nutrients after being fed DE-supplemented feed.

At the same time, **Khalil *et al.* (2021)** demonstrated a significant increase in the condition factor (K) in G3, followed by G4 and G2, in contrast to G1, in *Oreochromis niloticus* fed dietary extracts of *Rosmarinus officinalis*. Additionally, their results showed a significant decrease in FCR in G3 and G4, along with improved FBW, WG, WG%, and SGR.

However, **Tan *et al.* (2017)** reported that feeding dandelion plant extracts to the golden pompano for eight weeks resulted in significant increases in feed intake (FI), protein digestibility rate (PDR), weight gain (WG), and specific growth rate (SGR), particularly when the dietary dandelion extract levels were increased from 0 to 1.00g/ kg. The higher dose of 2.00g/ kg used in *Oreochromis niloticus* may have had a greater effect than the 1.00g/ kg dose used for the golden pompano (*Trachinotus auratus*), which could

explain the discrepancies between the results obtained by **Tan *et al.* (2017)** and the current investigation. They also observed that an excessive consumption of dandelion extracts could raise metabolic energy consumption, potentially having harmful consequences, stressing the fish, and impeding their growth and feed utilization. Future research is needed to determine the ideal dosage of dandelion supplementation for feeding *O. niloticus*.

**Sirakov *et al.* (2019)** discovered that upon comparing the survival rate of carps fed a diet supplemented with 0.8% dandelion extract to that of the control group, the survival rate remained unaffected. Additionally, fish fed dandelion-supplemented feed had significantly higher values for growth parameters (final weight, average weight gain, and specific growth rate) than the control group. On the other hand, **Yan *et al.* (2012)** reported that feeding the rockfish fingerlings (*Sebastes schlegelii*) dandelion supplements had no impact on their feed efficiency (FE) or growth.

A good assessment of a fish's health, quality, and physiological condition is its body composition, which is determined by measuring its water, fat, protein, and ash content **(Love, 1997)**. These measurements are considered accurate indicators of a fish's health and physiological activities **(Saliu *et al.*, 2007)**. In this study, protein and lipid levels were significantly higher in the DL3 group compared to the control group. Moisture and ash values were significantly higher in the control group, while the DL3 group showed lower values. **Ji *et al.* (2007)** reported that adding herbal adjuvants to fish diets can enhance lipid metabolism, which aligns with the findings of this study. However, this result contradicts that of **Dada (2012)**, who found no significant differences in protein or ash content in the bodies of *O. niloticus* fingerlings fed herbal powder supplements ($P > 0.05$). Furthermore, **Hassan *et al.* (2018)** found that carcass composition in the Nile tilapia was not significantly affected by a 1% rosemary treatment.

In terms of HSI, SSI, and GSI, significant differences ($P \leq 0.05$) were observed within groups, with increases in HSI in the DL1 and DL3 groups, SSI in the DL2 group, and GSI in the DL1 group. These findings are similar to those of **Khalil *et al.* (2021)**, who reported significant increases in HSI and SSI in G3, followed by G4 and G2, compared to the control group in *Oreochromis niloticus* fed dietary *Rosmarinus officinalis* extract. These results differ from those of **Xue *et al.* (2022)**, who demonstrated that feeding Dandelion extract (DE) supplementation did not significantly impact the viscero-somatic and hepatosomatic indices of the common carp ($P > 0.05$), consistent with research on the hybrid groupers **(Sun *et al.*, 2019)**.

Blood parameters are considered indicators of overall fish health and are valuable for assessing the body's response to dietary supplements **(Yue *et al.*, 2015)**. In this study, all hematological parameters were significantly improved in the DL3 (2g/ kg) group. The

lowest platelet (PLT) and white blood cell (WBC) counts were observed in the DL1 (0.5g/ kg) and DL2 (1g/ kg) groups, respectively. The DL0 (0g/ kg) group had the lowest values for Hb, RBCs, HCT, MCV, MCH, MCHC, lymphocyte, and eosinophil counts. The results suggest that the *T. officinale* meal improved the health of *O. niloticus* fish. However, **Sirakov *et al.* (2019)** found that dandelion supplementation did not affect RBCs or hemoglobin in carp, while the hematocrit in the control group (CF) was higher than in the treated groups. This discrepancy could be attributed to lower oxygen levels in the tanks of the control group during the experiment. Carp fed dandelion extract supplementation showed impacts on MCV and MCHC in their blood.

Albumin and globulin are the primary proteins in serum, while alkaline phosphatase (ALP) is a crucial enzyme involved in several vital processes in all living organisms. Proteins are significant substances in serum and are used as immune indicators to assess a fish's health. It is believed that higher plasma total protein (TP) levels, along with ALP levels, correlate with an enhanced innate immune response in fish **(Zhou *et al.*, 2015)**. In the current study, the DL3 (2g/ kg) group recorded higher values of TP, albumin (ALB), and globulin (GL) compared to the control group (DL0). These results align with those of **Tan *et al.* (2017)**, who demonstrated an increase in TP levels and ALP in the golden pompano fed with dietary dandelion extracts. Additionally, **Xue *et al*. (2022)** found that the common carp supplemented with dandelion extract (DE) had higher levels of globulin, TP, and albumin, while showing lower levels of triglycerides, urea nitrogen, and cholesterol. However, **Sirakov *et al.* (2019)** reported that carp fed dandelion extract supplementation had a 21.6% higher plasma TP level than fish fed diets without the supplement. These findings suggest that the *T. officinale* meal improved the immune response of *O. niloticus* fish.

The vital amino transferases, AST and ALT, are widely recognized for their critical roles in amino acid metabolism and as significant indicators of liver health. Elevated levels of AST and ALT are often used to measure fish stress responses and are typically indicative of impaired liver function. Previous research has shown that the dandelion extract may offer substantial protection against ethanol-induced hepatocellular damage **(Al-Malki *et al.*, 2013)**. According to the current study, the DL3 (2g/ kg) group exhibited lower levels of creatinine (CR), urea (U), and uric acid (UA), along with higher levels of ALT and AST liver enzymes. In contrast, the DL0 (0g/ kg) group showed higher CR and U values, suggesting that the dandelion meal positively impacted waste products resulting from high body metabolism. These results differ from those of **Tan *et al.* (2017)**, who found that the golden pompano administered dandelion extracts, as a dietary supplement, had significantly lower plasma ALT and AST levels compared to the control group, indicating a protective effect on liver function. Dandelion extracts may prevent lipid peroxidation in cell membranes and may suppress the release of AST and ALT enzymes

into the plasma due to their antioxidant and anti-radical properties. As a result, dandelion extracts, particularly those derived from their high polysaccharide content, have hepatoprotective effects and are beneficial to liver function. In this context, **Zou *et al.* (2023)** found that adding 6% DE to the daily diet significantly reduced liver damage following ammonia exposure in the common carp.

Superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) are essential enzymes in reducing oxidative stress **(Adeshina *et al.*, 2021)**. In this study, the DL1 (0.5g/ kg) group had the highest levels of malondialdehyde (MDA), followed by the DL0 (0g/ kg) group. The DL3 (2g/ kg) group showed the lowest values for SOD, GPx, and CAT, with the highest recorded in the DL0 (0g/ kg) and DL1 (0.5g/ kg) groups. The GSH value was highest in the DL2 (1g/ kg) group, followed by DL3 (2g/ kg), and lowest in the DL0 (0g/ kg) group. These findings suggest that dietary dandelion extract (DE) exhibits antioxidant activity, with the antioxidant properties increasing as the concentration of DE in the diet increases. This is consistent with the results of **Tan *et al.* (2017)**, who found that the dandelion extract-treated groups had significantly lower MDA content and significantly higher activity of liver antioxidant enzymes (SOD, T-AOC, CAT, GSH-Px, and GSR), as well as plasma antioxidant enzymes (GSR, T-AOC) compared to the control group. These findings indicate that dandelion extract improved the antioxidative status of golden pompano, prevented the production of free radicals, and reduced injury caused by lipid peroxidation. Additionally, **Tan *et al.* (2018)** found that dietary DE significantly increased intestinal antioxidant activity in the golden pompano by upregulating intestinal CAT and GPx mRNA levels and enhancing SOD, CAT, and T-AOC activities, while downregulating intestinal keap1 mRNA levels and lowering MDA content. Furthermore, the current findings align with those of **Yilmaz (2019)**, who reported a positive correlation between the innate immune response and antioxidant enzyme activity in fish or shellfish fed diets with various feed additives.

Lysozyme and respiratory burst (RB) activities play significant roles in the non-specific immunological defense system. It has been shown that applying plant immunostimulants to several fish species increases their complement, lysozyme activity, and IgM levels **(Adel *et al.*, 2015; Zhou *et al.*, 2015)**. Nitric oxide is an essential molecule involved in immunological, circulatory, and neurological systems, and it plays a role in various physiological processes **(Förstermann & Sessa, 2012)**. In the current trial, respiratory burst, lysozyme activity, and nitric oxide levels increased significantly in the DL3 (2g/ kg) group, followed by the DL2 (1g/ kg) group. The DL0 (0g/ kg) and DL1 (0.5g/ kg) groups had the lowest values. The results indicate that dietary dandelion extract functions as an effective immunostimulant in *Oreochromis niloticus*. These findings are consistent with those of **Tan *et al.* (2017)**, who found that IgM and lysozyme levels were significantly higher in golden pompano fed dandelion extracts compared to the control

group. However, **Khalil *et al.* (2021)** observed that serum levels of nitric oxide (NO) decreased significantly with increasing the levels of *Rosmarinus officinalis* extract (RE) in the diet. Meanwhile, the serum activity of lysozyme increased significantly in the treated groups but decreased as the RE levels increased in *O. niloticus*.

Regarding histopathological changes, no significant differences were observed between the control and treated groups due to the mild to moderate effect of dietary dandelion leaves (*Taraxacum officinale*) on the internal organs (intestine, liver, and spleen). Intestinal villi length showed a minimal average in the DL1 group and a maximal average in the DL3 group. This suggests that the food additive is highly effective, particularly at higher dosages, which increases the area of the body where food is absorbed, thereby boosting growth rates and improving fish health. These results align with those of **Tan *et al.* (2018)**, who discovered that dietary *Taraxacum officinale* extracts could improve intestinal morphology, including increases in villus number, villus width, villus length, and muscle thickness in both the foregut and hindgut of golden pompano (*Trachinotus ovatus*), with statistically significant results ($P \leq 0.05$). Additionally, **Raja *et al.* (2022)** observed that increasing Rosmarinus officinalis extract (RE) levels enhanced intestinal surface absorption and gastrointestinal function. In this respect, **Awad and Awaad (2017)** noted that toxins were linked to changes in intestinal shape, such as deeper crypts and shorter villi. Increased tunnel sizes, villus numbers, and villus height or width are thought to indicate a greater surface area available for nutrient absorption in aquatic animals.

## CONCLUSION

Our investigation concluded that feeding *Oreochromis niloticus* a diet supplemented with dandelion for 45 days significantly enhanced its immune system, body composition, and growth performance, particularly at supplementation levels of 1.00 and 2.00g/ kg. Based on our findings, dietary supplementation with dandelion leaves (DL) provides considerable benefits to *Oreochromis niloticus* and may serve as an effective feed additive in aquaculture.

## ETHICAL STATEMENT

The Faculty of Science's Ethics of Animal Use in Research Committee (EAURC) at Suez Canal University, Egypt, provided the ethical guidelines for all experimental protocols involving animals (Approval number: REC347/2024).

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