



## GROWTH PERFORMANCE, FEED UTILIZATION, HEMATOLOGICAL PARAMETERS, AND HISTOLOGICAL FEATURES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FED DIETS WITH SUPPLEMENTARY HERBAL EXTRACTS UNDER PROLONGED WATER EXCHANGE

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### Abstract

Nile tilapia is known for its relative tolerance to some biotic and abiotic stressors. However, long-period water exchange may impair the health status and, thereby, the growth performance and feed utilization. In this regard, using herbal extracts may help to relieve the adverse impacts of low water quality on the productivity of this fish species. A 100-day feeding trial was designed to examine the effects of dietary supplements of *Aloe vera* leaves (AVE), and chamomile flowers, *Matricaria chamomilla* (CFE) extracts on water quality, growth performance, and well-being of Nile tilapia. Fish ( $3.95 \pm 0.05$  g,  $n=1200$ ) were distributed into five groups (15 fiberglass tanks, 2 m<sup>3</sup>) under a water exchange rate of 20% every two days for standard control (T0) without herbal supplements. Groups (T1, T2, T3, and T4) were exposed to a long water exchange period of 50% every month, wherein T1 (stressed control), T2, T3, and T4 groups were fed a diet supplemented with 0% herbal extract, 1% AVE, 1% CFE and 0.5% AVE + 0.5% CFE, respectively. Results showed that feeding on a T2 diet exhibited a clear improvement under a long period of water exchange stress, and it is almost similar to their counterparts under normal conditions (T0) in terms of specific growth rate, survival rate, feed intake, feed conversion ratio, and protein efficiency ratio. Moreover, the lowest levels of aspartate aminotransferase were obtained with T2 and T0. In addition, the highest white blood cell count and hemoglobin values were recorded with group T0, followed by T2, and T4, while there was no significant difference between T3 and T1. Fish under stressed conditions without herbal extracts (T1) showed the lowest performance and survival rate compared to T0 and T2 groups. In conclusion, feeding diet supplied with 1% *A. vera* extract to stressed fish restored their performance and well-being to the level of their counterparts under normal conditions.

**Key words:** *Aloe vera*, chamomile, well-being, feed utilization, water quality, sustainable aquaculture, stress marker

Aquaculture is an essential source of safe animal protein, and it is realized as a potential option for closing the gap between production and human consumption (El Basuni et al., 2022; Shadrack et al., 2022). Farmers and investors have been drawn to intensification methods that use contemporary technology to improve profitability because of the high rate of return on investment (Zaki et al., 2020). Although the extensive aquaculture grows, negative repercussions and stressful conditions for fish become more common (Dawood et al., 2021). As a key option to overcome water and land resource constraints, intensive farming relies on high fish densities paired with highly nitrogenous diets (25 to 55 percent) to boost production in closed or semi-closed techniques. However, water characteristics are harmed by intensification sys-

tems, particularly the accretion of inorganic nitrogen wastes (NH<sub>3</sub> and NO<sub>2</sub>) (Zaki et al., 2020). As a result, establishing alternative strategies to improve aquatic animals' health and performance is critical for aquaculture industry sectors. The traditional methods of ensuring the success of intensive culture include the water exchange to maintain its required quality (Schar et al., 2021). Today, the need to replace antibiotics and other synthetic chemicals with eco-friendly approaches is capable of strengthening aquatic organisms' health and performance (Dossou et al., 2021; Paray et al., 2021).

The combined goals of intensive yields and low pollution can be achieved through high-quality feeds, which can be better utilized, resulting in fewer waste nutrients per unit yield (Amirkolaie, 2011). Several studies

have reported that the administration of plant extracts improves the digestibility and availability of nutrients and leading to higher protein synthesis (Mohammadi et al., 2020; Reverter et al., 2017). Moreover, products originating from medicinal plants have been presented as anti-stress, growth promoters, appetite stimulators, and immune-stimulants, as well as have aphrodisiac and antimicrobial characteristics (Abdel-Latif et al., 2021) due to their content of functional bioactive ingredients (phytochemicals) such as polyphenols and alkaloids (Kuebutornye and Abarike, 2020; Reverter et al., 2017; Ahmadi-far et al., 2021).

*Aloe vera* is a well-known medicinal plant that grows effectively in various environments and exhibits antiviral, antibacterial, and immune-stimulation activities (Baruah et al., 2016; Khanal et al., 2021). It consists primarily of water, polysaccharides, glucomannan, and acemannan (Lee et al., 2001; Liu et al., 2019). The application of *A. vera* extract (0.5–2%) in Nile tilapia feeds exhibited favorable impacts on growth, nutrient utilization, and antioxidant activities (Gabriel et al., 2015 a, b). Chamomile (*Matricaria chamomilla*) is another oldest medicinal herbs cultivated for several medical purposes (Srivastava et al., 2010). Chamomile's dried flowers are high in terpenoids and flavonoids, which are essential for their beneficial effects. It was also demonstrated that a 2% dose of dried chamomile flowers enhanced all growth indices, feed conversion, and survival rate in Nile tilapia fingerlings (*Oreochromis niloticus*) (Srivastava et al., 2010). Moreover, the dietary incorporation of chamomile flower extract or meal at a level of 1% enhanced the growth and immune indices of Nile tilapia (Khalafalla, 2009) and African catfish (*Clarias gariepinus*) (Abdelha et al., 2010). The phytochemical components of *A. vera* (Azaroual et al., 2012; Dey et al., 2017; Ojha and Kumar, 2012; Sánchez-Machado et al., 2017) and chamomile (Haghi et al., 2014; Molnar et al., 2017; Šibul et al., 2020; Viapiana et al., 2016) extracts have been documented distinctly in many studies.

Nile tilapia is a widely farmed fish species due to its ability to grow in different conditions along with high marketability and preferences (Gewaily et al., 2021; Lind et al., 2019). According to Kord et al. (2022), it was demonstrated that Nile tilapia can withstand the stressful condition of zero water exchange (8 weeks) when fed probiotic-based diets. The present research aimed to investigate the potential uses and applications of herbal extracts (*A. vera* or/and chamomile) on Nile tilapia performance, health under a prolonged water change period (100 days).

## Material and methods

### Ethical approval

This study was carried out with the strict recommendations and approval of the National Institute of Oceanography and Fisheries (NIOF, Egypt) Committee for Eth-

ical Care and Use of Aquatic Animals (NIOF-IACUC, Code: NIOF-AQ4-F-22-R-023).

### Experimental diets

One kilogram of freshly collected *Aloe vera* (*Aloe barbadensis miller*) leaves or chamomile flower (*Matricaria chamomilla*) was combined with 200 mL of 70% ethanol and squeezed in a Whizzer agitated on a shaker incubator for 12 h, filtered through filter paper, and lyophilized. The separated matter was stored in tightly sealed dark containers in a freezer at  $-20^{\circ}\text{C}$  for later use (Khanal et al., 2021). A basal diet containing 30% crude protein (CP) was provided for all fish (Table 1). All ingredients were mixed well with herb extract according to the tested percentage for 15 minutes before adding oil. Water was added at a rate of 20% of the mixture mass and combined in a blender (Hobart A120) at 104 rpm using a dough hook for 5 min to produce a stiff dough. The dough was extruded using a meat grinder (2 mm). Pellets were air-dried at  $40^{\circ}\text{C}$  and stored at  $-15^{\circ}\text{C}$  until use. Fish were fed twice daily at 8 AM and 5 PM with a 5% feeding rate of total fish biomass, and the feeding rate was lowered to 3% when fish reached 50 g.

Table 1. Ingredients and proximate composition of the basal control diet

| Ingredients                               | %                            |
|-------------------------------------------|------------------------------|
| Yellow corn*                              | 19.2                         |
| Soybean meal (44% protein)                | 20.1                         |
| Corn gluten (60% protein)                 | 5                            |
| Fish meal                                 | 20.1                         |
| Rice bran                                 | 13.4                         |
| Wheat bran                                | 13.6                         |
| Wheat flour                               | 4                            |
| Soybean oil                               | 2.15                         |
| Molasses                                  | 2                            |
| Choline chloride                          | 0.07                         |
| Vitamin and mineral premix**              | 0.35                         |
| Vitamin C                                 | 0.03                         |
| Nutrient specification                    | % on dry matter basis (% DM) |
| crude protein                             | 30.1                         |
| crude lipids                              | 6.34                         |
| crude fiber                               | 5.02                         |
| ash                                       | 13.2                         |
| NFE***                                    | 40.34                        |
| calcium                                   | 1.55                         |
| total phosphorus                          | 1.33                         |
| lysine                                    | 1.66                         |
| Gross energy (kcal kg <sup>-1</sup> diet) | 4210.96                      |

\**Aloe vera* (AVE) or/and chamomile (CFE) extracts are included at the expense of yellow corn.

\*\*Premix provided each kg of feed with Biotin = 0.025 mg; Folic Acid = 1 mg; Niacin = 20 mg; Pantothenic acid = 8 mg; Vitamin A = 7000 IU; Vitamin B<sub>1</sub> = 1 mg; Vitamin B<sub>12</sub> = 0.01 mg; Vitamin B<sub>2</sub> = 4 mg; Vitamin B<sub>6</sub> = 1 mg; Vitamin D = 1400 IU; Vitamin E = 10 mg; Vitamin K<sub>3</sub> = 3 mg; Cobalt = 0.01 mg; Copper = 10 mg; Iodine = 0.05 mg; Iron = 15 mg; Manganese = 40 mg; Selenium = 0.01 mg; Zinc = 40 mg.

\*\*\*Nitrogen free extract (NFE) = 100 - (crude protein + crude lipids + crude fiber + ash).

The methods of AOAC (2000) have been used to evaluate crude lipid (EE), moisture, and crude protein (CP). Moisture was measured at 105°C for 24 hours using an oven (Labostar-LG122 Tabia Espec, Osaka, Japan). The chloroform/methanol (2:1 v/v) extraction procedure was predicted for crude lipids with a Soxhlet. Crude protein was analyzed using an automatically processed Kjeldahl (Buchi 430) using a Kjeltect method (N×6.25) using an automatic Kjeldahl system (Buchi 430/323; model 1265, Moline IL, USA), and ash content was determined by ashing at 550°C in a muffle furnace for three hours. Gross energy (GE) was estimated for formulated diets using combustion factors 5.64, 9.45, and 4.22 Kcal g<sup>-1</sup> for protein, lipids, and carbohydrates, respectively.

### Experimental design

This experiment was carried out in a fish feeding laboratory, Fish Research Station, National Institute of Oceanography, Fayoum Governorate, Egypt, according to animal handling Institutional Standards (Faculty of Agriculture, Tanta Univ.; AY<sub>2019-2020</sub>/Session 6/2020.01.13). Five different treatments were conducted in 15 fiberglass tanks (L × W × H = 3.9 × 1.0 × 0.6 m) with a water volume of 2 m<sup>3</sup> tank<sup>-1</sup> and an air pump. Fish were acclimatized to laboratory conditions for two weeks; then randomly distributed with a stocking density of 80 juveniles per tank with an average initial weight of 3.95±0.05 g (±S.E.) and coded with T0: (control positive) which were fed with basal diet without extending of the water exchange period. T1 (stressed control) fish were fed a basal diet with a long exchange water period. T2, T3, and T4 groups of fish were fed a basal diet with adding 1% extract of *A. vera* (AVE), 1% chamomile flowers (CFE), and 0.5% (AVE)+0.5% (CFE) powder, respectively. The rate of water exchange was at 20% every two days in T0, while in T1, T2, and T3 was 50% of water volume per month. The experimental period lasted 100 days under a natural light/dark routine, and feed was offered two times a day at 8 AM and 5 PM.

### Water physicochemical parameters

Water samples were collected from Nile tilapia aquaria and their properties, i.e., water temperature, pH, and dissolved oxygen concentration (DO mg L<sup>-1</sup>), were recorded every day using a multi-parameter water quality analyzer (MULP-8C), while total ammonia (TA, mg L<sup>-1</sup>), and nitrite (NO<sub>2</sub>, mg L<sup>-1</sup>) were determined weekly using standard protocols (APHA, 2005).

### Growth indicators

The following formulae were used to compute growth indicators:

Wet weight gain (WG, g) = Final body weight (FBW, g) – Initial body weight (IBW, g).

Average daily weight gain (ADWG, g day<sup>-1</sup>) = [(FBW, g – IBW, g) / time, days].

Relative growth rate (RGR, %) = [(FBW, g – IBW, g) / (IBW, g)] × 100.

Specific growth rate (SGR, % day<sup>-1</sup>) = [(Ln FBW - Ln IBW × 100) / time, days].

Fulton's condition factor (K) = (FBW, g/Final length, cm<sup>3</sup>) × 100.

Survival rate (SR, %) = (No. of fish survived/No. of fish released) × 100.

Feed intake (FI, g fish<sup>-1</sup>) = Consumed feed of each pond / No. of fish.

Feed conversion ratio (FCR) = Feed given (g)/Weight gain (g).

Protein efficiency ratio (PER) = WG, g/protein intake, g.

### Blood sampling

At the end of the trial period, five fish from each replicate tank were anesthetized using MS-222 (tricaine methane sulfonate, 0.1 g L<sup>-1</sup>, Sigma-Aldrich, USA). Blood samples were drawn by 3-mL syringes from the caudal vein and emptied into two tubes; one contained EDTA to prevent coagulation and estimate the hematological parameters, and the other tube did not contain EDTA to measure the serum parameters. After that, the hematological parameters (white blood cells, leucocytes WBC; 10<sup>9</sup> L<sup>-1</sup>, hemoglobin, HB g dL<sup>-1</sup>, and hematocrit Hct, %) were determined by following Rawling et al. (2009). Biochemical indicators such as plasma glucose (RBCs, mg dL<sup>-1</sup>, glucose-TR 1001191), serum aspartate aminotransferase (AST, U L<sup>-1</sup>, Liquizyme (4+1) E.C.2.6.1.1.), alanine aminotransferase (ALT, U L<sup>-1</sup>, Liquizyme (4+1) E.C.2.6.1.2.), and urea (mg dL<sup>-1</sup>, Perth lot. Enzymatic colorimetric Ref: 2×100 ml, Diamond) were measured electrometrically with a semi-automated analyzer (3000 Evolution, Biochemical Systems International, Arezzo, Italy). Serum lysozyme assay was measured as determined following the method of Lygren et al. (1999).

### Histopathological evaluation

At the end of the experimental period, the fish were euthanized by decapitation, then tissue specimens from the intestines, spleens, and dorsal musculatures from randomly chosen ten fish per group were collected according to standardized necropsy protocol (Meyers, 2009), immediately fixed in 10% neutral buffered formalin for 24 hours, processed for paraffin technique, sectioned at five µm thick, stained with hematoxylin and eosin dyes (Bancroft and Layton, 2019), and examined by light microscope recording any pathological changes.

### Statistical analysis

The data were statistically examined on the software SPSS (IBM® SPSS® Inc., IL, USA version 20). The Shapiro-Wilk's and Levene's tests were managed to verify variance normality and homogeneity. The differences among the treatments were determined at a significant level (P<0.05) by one-way analysis of variance (ANOVA) and Duncan's post hoc test.

## Results

### Water quality

The means of water quality indicators are displayed in Table 2. The water temperature did not differ among treatments and ranged from 23.65 to 24.25°C. The properties of water in the T0 group have the highest DO and the lowest pH levels, total ammonia, unionized ammonia, and NO<sub>2</sub> compared to the T1 group. No significant differences existed between the treated groups with herbs in DO, pH, unionized ammonia (NH<sub>3</sub>, mg L<sup>-1</sup>), and NO<sub>2</sub>. The highest concentrations of total ammonia, unionized ammonia, and nitrite were with T1 (0.6, 0.024, and 0.507, respectively), and the lowest were in T0 (0.075, 0.012, and 0.039, respectively) followed by T2 (0.4, 0.018, and 0.377 mg L<sup>-1</sup>, respectively).

### Performance variables

The growth rate, feed utilization, and survival % results of Nile tilapia juveniles are shown in Table 3. Fish in the non-stressed control group (T0) displayed better FBW, WG, ADG, RGR, SGR, SR, FCR, and PER compared to other groups. The fish group fed on the T2 diet with AVE supplement exhibited a clear improvement under a long period of water exchange stress, and it is almost similar to their counterparts under normal conditions (T0) in terms of SGR, SR, FI, FCR, and PER. The poorest results in growth variables were found in T1, T3, and T4. Moreover, the highest values of Fulton's condition factor (K) were calculated for fish in the T1, T3, and T4 groups, and the lowest value was in the T0 group. Fish under stressed conditions without herbal extracts (T1) showed the lowest survival rate compared to other groups.

Table 2. Means of water quality parameters of control groups and the experimental treatments after an experimental period of 100 days

| Items                                   | T0      | T1      | T2      | T3       | T4       | S.E.  | F-value | P-value |
|-----------------------------------------|---------|---------|---------|----------|----------|-------|---------|---------|
| Temperature (°C)                        | 24.00   | 24.25   | 23.65   | 23.87    | 24.10    | 0.58  | 0.307   | 0.86    |
| DO (mg L <sup>-1</sup> )                | 6.10 a  | 4.20 b  | 4.25 b  | 4.22 b   | 4.21 b   | 0.27  | 18.13   | 0.004   |
| pH                                      | 7.35 b  | 8.45 a  | 7.77 ab | 7.88 ab  | 7.94 ab  | 0.24  | 5.25    | 0.049   |
| Total ammonia (mg L <sup>-1</sup> )     | 0.075 c | 0.60 a  | 0.40 b  | 0.52 a   | 0.53 a   | 0.035 | 6890    | 0.001   |
| Unionized ammonia (mg L <sup>-1</sup> ) | 0.012 b | 0.085 a | 0.018 b | 0.023 b  | 0.029 b  | 0.011 | 17.16   | 0.004   |
| NO <sub>2</sub> (mg L <sup>-1</sup> )   | 0.039 c | 0.570 a | 0.357 b | 0.465 ab | 0.470 ab | 0.045 | 40.17   | 0.00    |

Means in the same row with different letters differ at P<0.05. T1 = stressed control; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE); T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE); T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE).

Table 3. Performance variables of Nile tilapia after 100 days experimental period

| Items                         | T0        | T1        | T2        | T3        | T4        | S.E.  | F-value | P-value |
|-------------------------------|-----------|-----------|-----------|-----------|-----------|-------|---------|---------|
| FBW (g)                       | 91.50 a   | 83.00 c   | 88.80 b   | 83.30 c   | 84.00 c   | 51.08 | 111.70  | 0.001   |
| WG (g)                        | 87.55 a   | 79.05 c   | 84.85 b   | 79.40 c   | 80.05 c   | 51.08 | 111.70  | 0.001   |
| ADG (g)                       | 0.87 a    | 0.79 c    | 0.85 b    | 0.79 c    | 0.80 c    | 0.005 | 111.16  | 0.001   |
| RGR (%)                       | 2216.40 a | 2221.03 c | 2148.10 b | 2008.90 c | 2026.60 c | 12.93 | 111.62  | 0.004   |
| SGR (% day <sup>-1</sup> )    | 3.14 a    | 3.05 b    | 3.11 a    | 3.05 b    | 3.05 b    | 0.006 | 93.58   | 0.006   |
| Fulton's condition factor (K) | 1.46 c    | 1.78 a    | 1.52 b    | 1.76 a    | 1.70 ab   | 0.015 | 179.45  | 0.004   |
| SR (%)                        | 98 a      | 91.5 c    | 97 a      | 93.5 b    | 93.75 ab  | 1.35  | 7.87    | 0.02    |
| FI (g fish <sup>-1</sup> )    | 100.50 b  | 98.71 c   | 101.61 b  | 102.80 b  | 107.14 a  | 1.05  | 18.11   | 0.02    |
| FCR                           | 1.15 c    | 1.25 b    | 1.17 c    | 1.30 a    | 1.34 a    | 0.013 | 359.02  | 0.006   |
| PER                           | 3.21 a    | 2.96 b    | 3.14 a    | 2.87 b    | 2.78 c    | 0.011 | 102.83  | 0.002   |

Means followed by different letters are significantly different according to Duncan's multiple range comparisons at P<0.05. Means followed by the same letter are not significantly different. T1 = stressed control; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE); T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE); T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE).

Table 4. Blood indices of Nile tilapia after 100 days trial period

| Items                                  | T0       | T1      | T2       | T3      | T4       | S.E. | F-value | P-value |
|----------------------------------------|----------|---------|----------|---------|----------|------|---------|---------|
| WBC (10 <sup>9</sup> L <sup>-1</sup> ) | 122.50 a | 90.00 d | 114.00 b | 90.50 d | 100.00 c | 2.42 | 71.17   | 0.002   |
| Hb (g dL <sup>-1</sup> )               | 8.70 a   | 5.200 d | 7.50 b   | 6.10 d  | 6.900 c  | 0.38 | 119.02  | 0.001   |
| HCT (%)                                | 19.00 b  | 14.40 c | 24.50 a  | 20.30 b | 16.50 c  | 0.83 | 42.38   | 0.001   |
| RBCs (mg dL <sup>-1</sup> )            | 39.00 b  | 59.50 a | 44.00 b  | 54.50 a | 42.00 b  | 2.14 | 33.402  | 0.003   |
| Urea (mg dL <sup>-1</sup> )            | 8.70     | 7.10    | 7.60     | 6.60    | 7.90     | 0.78 | 2.098   | 0.219   |
| AST (U L <sup>-1</sup> )               | 34.00 d  | 65.41 a | 34.10 d  | 54.70 b | 45.90 c  | 1.11 | 295.47  | 0.001   |
| ALT (U L <sup>-1</sup> )               | 7.15     | 7.70    | 7.300    | 7.60    | 7.20     | 0.46 | 0.568   | 0.698   |
| Lysozyme (ng mL <sup>-1</sup> )        | 4.65 c   | 5.00 c  | 10.85 a  | 5.85 c  | 9.00 b   | 0.72 | 29.062  | 0.001   |

Means in the same row bearing unique letters vary at P<0.05. T1 = stressed control; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE); T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE); T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE).

### Blood parameters

Nile tilapia subjected to a 100-day feeding trial revealed normal blood test results (Table 4). Insignificant differences were shown in urea and ALT between all treatments. The lowest level of AST was obtained with T2 and T0 and subsequently T4, T3, and T1, respectively. The highest values of WBC and Hb were recorded with group T0, followed by T2 and T4, while no significant difference between T3 and T1. RBCs did not significantly differ among T0, T2, and T4, and these groups were significantly lower in RBCs than T1 and T3. A significant decline in the HCT% was reported for fish in T1, and the highest HCT% was in the T2 treatment. Moreover, fish in the T2 group exhibited the highest lysozyme activity, followed by T4, while lysozyme levels did not significantly change between T3, T0, and T1.

### Histological profile

#### Intestine

Figure 1 is a representative photomicrograph of H&E-stained intestinal tissue sections showing a normal histological picture in the normal control fish; villus lined by simple or pseudostratified columnar epithelium

(red arrowhead), with goblet cells (black arrowhead) and connective tissue core (yellow arrowhead), and lamina propria containing blood (black arrow), and lymph (red arrowhead) vessels and loose connective tissue with few mononuclear (yellow arrow) (A and B). T1 fish showed desquamated epithelial lining (red arrowhead) and marked villous atrophy with necrosis of enterocytes, particularly at the tip of the villi (black arrowheads) (C), mononuclear aggregations in the lamina propria (black arrow), and increased numbers of goblet cells (arrowheads) (D). The AVE-treated fish (T2) fish showed almost normal intestinal architectures with excess mucus in the intestinal lumen (black arrowhead), tiny, desquamated cells (red arrowhead) (E), and intraepithelial lymphocytic infiltration (arrow) (F). The CFE-treated fish (T3) showed extensive mononuclear cell infiltrates (black arrowhead) with goblet cell hyperplasia (red arrowhead) (G) and intraepithelial vesicle formation (black arrowhead) goblet cell hyperplasia (red arrowhead) (H). The AVE+CFE-treated fish (T4) showed lymphocytic infiltration in the lamina propria (black arrowhead) (I) and increased numbers of goblet cells (red arrowheads) (J).

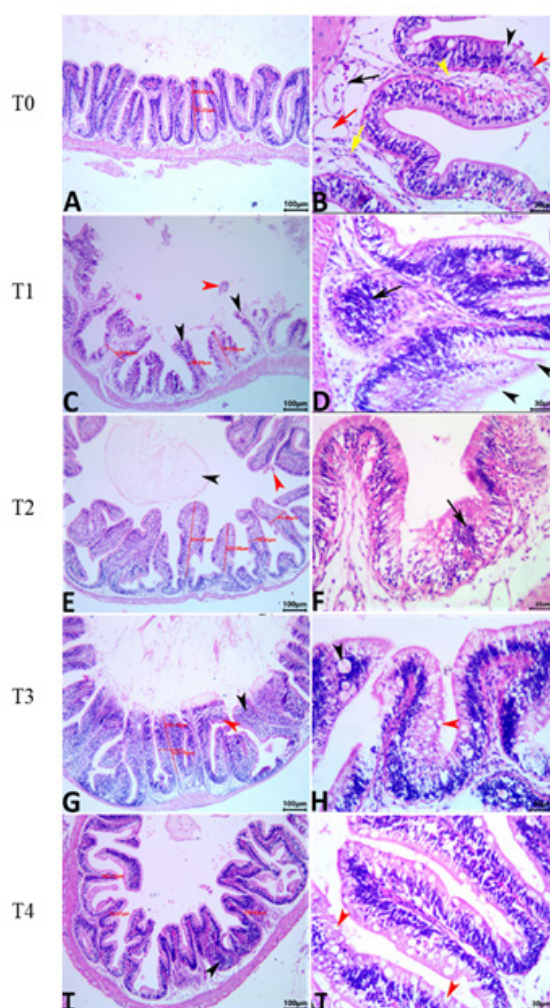


Figure 1. Photomicrograph of H&E-stained intestinal tissue sections at scale bar of 30  $\mu$ m [A, C, E, G, I] and 100  $\mu$ m [B, D, F, H, J]. T0 = normal control: [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE) [G and H]; T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE) [I and J]

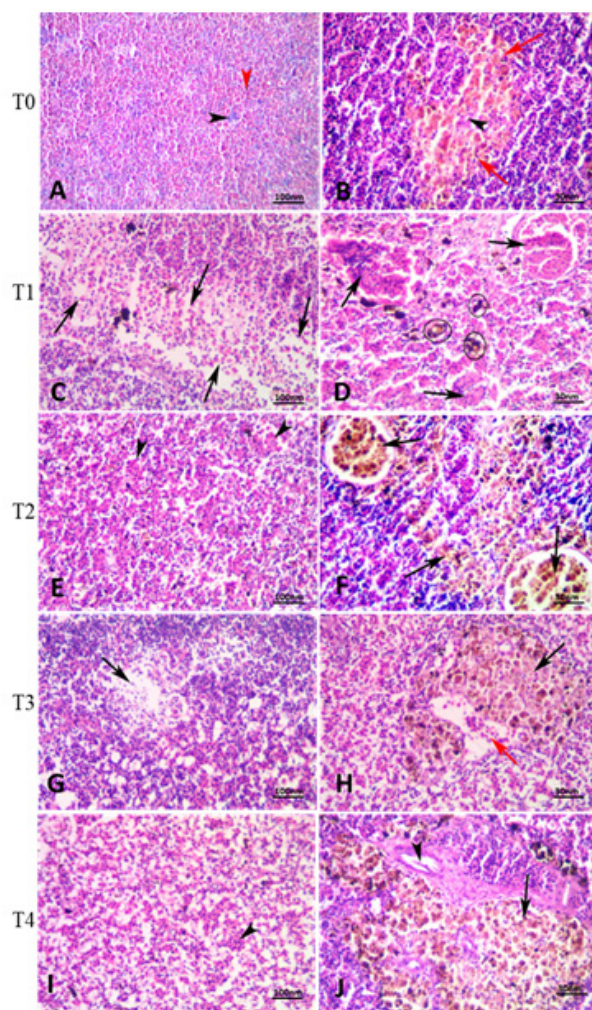


Figure 2. Photomicrograph of H&E-stained splenic tissue sections at scale bar of 30 µm [A, C, E, G, I] and 100 µm [B, D, F, H, J]. T0 = normal control [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE) [G and H]; T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE) [I and J]

### Spleen

Figure 2 is a representative photomicrograph of H&E-stained splenic tissue sections showing normal mixed red (red arrowhead) and white (black arrowhead) pulps (A) and ellipsoid [(thick-walled arteriole (BLACK arrowhead) surrounded by melanomacrophage centers (MMCs) (red arrows)] (B) in the normal control fish (T0). The stressed control (T1) fish showed a necrotic area infiltrated with numerous erythrocytes and edematous fluid (black arrows) (C), significant vascular congestion (black arrows), and necrotic MMCs (ellipses) (D). The AVE-treated fish (T2) showed very mild depletion of the lymphoid elements with expanded erythropoietic elements (black arrowheads) (E), with MMCs hyperplasia (black arrows) (F). The CFE-treated fish (T3) showed focal necrotic area (black arrow) (G), vascular congestion (red arrow), and hyperplastic MMCs (black arrow) (H). The AVE+CFE treated fish (T4) showed erythroid hyperplasia (black arrowhead) (I), endothelial

hypertrophy (black arrowhead), and MMCs hyperplasia (black arrow) (J).

### Muscle

Figure 3 is a representative photomicrograph of H&E-stained muscular tissue sections showing a normal histological picture in the normal control fish; longitudinal (A) and cross (B) sections. The negative control fish (T1) showed notable fatty change (arrowheads) (C) and intramuscular inflammatory cell infiltrate (arrowhead) (D). The AVE-treated fish (T2) showed almost normal myofibers except for mild intermuscular edema (arrowheads) (E), mild congestion (arrowhead), and a few intramuscular lymphocytic infiltrates (F). The CFE-treated fish (T3) showed focal fatty change (arrowheads), intermuscular edema with few lymphocytes (ellipse) (G) and increased mononuclear cells in the endomysium (arrowheads) (H). The AVE+CFE-treated fish (T4) showed single-cell necrosis (arrowhead), intermuscular edema (arrow) (I), and fatty infiltration (arrowheads) (J).

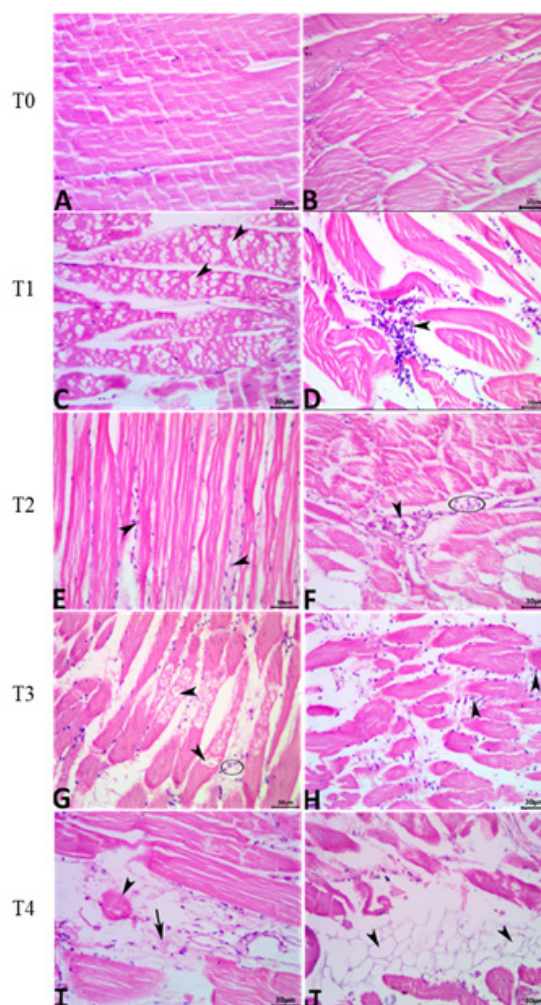


Figure 3. Photomicrograph of H&E-stained muscular tissue sections at scale bar of 30  $\mu\text{m}$ . T0 = normal control: [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE) [G and H]; T4 = group of fish fed 0.5% (AVE) + 0.5% (CFE) [I and J]

## Discussion

There is no doubt that the continuous water change improves the performance and well-being of the fish reflecting a positive effect on the parameters of growth, feed efficiency, and hematological, immunological, and histological indicators (Abdel-Latif et al., 2022; El-Son et al., 2022). *A. vera* is one of the candidate medicinal plants in the aquaculture feed industry as a feed supplement. Fish of normal control (T0) was the best in water quality, performance, and well-being compared with fish in other groups, whereas fish were more suffering from extending the water change period considering that the fish were reared under high density (40/m<sup>3</sup>). Thus constantly changing the water reduces the stressful effects of a high density, improves DO level, and decreases the harmful substances, such as uneaten feed, feces, and turbidity level, also other metabolites, such as nitrogen compounds (Okomoda et al., 2016; Zaki et al., 2020). Under a long-term water exchange period or in recirculating systems, a natural decrease in the percentage of

dissolved oxygen level occurs (Trang et al., 2017; Zaki et al., 2020). The previous researchers marked the DO concentration with adverse impacts on Nile tilapia growth performance and feed efficiency to be from less than 0.8 to 3 mg L<sup>-1</sup> (Abdel-Tawwab et al., 2015; Ani et al., 2022; Tran-Duy et al., 2008; Tran-Ngoc et al., 2016). Despite the high growth of fish in T0, it consumed about 20 m<sup>3</sup> as changed water, while other groups consumed about 3 m<sup>3</sup>.

It was evident from Table 2 that water temperature, DO mg L<sup>-1</sup>, and pH values were within the acceptable ranges conducive for tilapia growth. Tilapia is known to tolerate a wide range of harsh circumstances. The optimum temperature range for normal development, reproduction, and growth is 20 to 35°C (Hassan et al., 2013). Tilapia species normal growth requires a minimum of dissolved oxygen (3 mg L<sup>-1</sup>) and a pH range of 5.5–9.5 (Effendi et al., 2020; Makori et al., 2017). Nitrogen major compounds in water are total ammonia (TA), either unionized ammonia (NH<sub>3</sub>) or ionized ammonia (NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub>), and nitrate (NO<sub>3</sub>) (Putra et al., 2020). Findings in Table 2 cleared that NO<sub>2</sub> of T2, T3, and T4

groups were 0.377, 0.465, and 0.470, respectively, while T1 recorded 0.507 mg L<sup>-1</sup>. As a result, the NO<sub>2</sub> levels in aquariums supplied with a diet containing herbal extract supplements are within a permissible range for freshwater fish, as reported by Kroupova et al. (2012). Moreover, all groups fed a diet containing herb extract recorded a lower level of TA than T1. Furthermore, fish in T2 fed a diet containing 1% *A. vera* extract (AVE) had the lowest TA and NO<sub>2</sub> compared to all treated fish. These results corroborate with that observed by Yavuzcan et al. (2017).

Feeding in aquaculture contributes directly and indirectly to the water quality and, ultimately, fish welfare. Moreover, increasing the concentrations of nutrients increases the risk of their potentially harmful solutes in combination with the environmental conditions and hence can affect survival and needs to be optimized. Artificial tilapia diets always contain crude protein levels of 25 as a minimum limit to 34% as a maximum limit, and most of their ingredients are plant sources such as corn, wheat bran, corn gluten, and soybean as sources of carbohydrates and crude protein, this lead to an increase in nitrogen loss (Abdel-Tawwab, 2012; El-Sayed, 1999). Decreasing the PER of fish leads to increased ammonia excretion through the gills into the rearing water (Engin and Carter, 2001; Ip and Chew, 2010). However, using some substances which stimulate and boost the excretion of digestive enzymes led to more efficiency in feed utilization and decreased nitrogen loss in water, as confirmed by Amirkolaie (2011).

Implementing AVE as an additive at the optimum dose stimulates lipase, amylase, and trypsin excretion of the fish gut, so it does not use the amino acids as an energy source and is directed to muscle-building (Gabriel et al., 2017). *A. vera* is a good source of thiamine, riboflavin, folic acid, and essential and non-essential amino acids (Ahlawat and Khatkar, 2011; Darzi et al., 2021). Also, polysaccharides are the major component of *A. vera* and have been reported to stimulate erythropoiesis (Ni et al., 2004), which can boost the gut microbial community and also improve feed digestibility and availability of nutrients from feedstuffs, and shorten the feed transit time, hence there is a beneficial effect on digestive enzymes (Citarasu, 2010; Yu et al., 2018). Also, the valuable impact of *A. vera* for being an excellent source of fatty acids, vitamins (C, B<sub>12</sub>, E, B<sub>2</sub>, B<sub>1</sub>, and folic) as well as minerals (Ca, Cr, Cu, Se, Mn, K, Na, and Zn) as reported by Kayode (2016).

Experimental findings demonstrated that dietary extract of AVE supplementation in general and at the level of 1% could improve growth rate and maintain the water quality, improving feed utilization efficiency. The improvement of feed utilization for tilapia fed a diet containing AVE compared to the control group and dietary CFE supplement may be returned to AVE's ability to enhance appetite, increase nutrient digestibility, absorption, and assimilation capacity, partly through improved digestive enzymes and healthy intestinal microflora. On the other hand, the chamomile flower in the fish feed had

a positive effect on the performance and well-being of Nile tilapia (Khalafalla, 2009; Zaki et al., 2012), red hybrid tilapia (Fasiha Nordin et al., 2017), and African catfish (*Clarias gariepinus*) (Abdelha et al., 2010). Despite these positive results of dietary chamomile as aforementioned, the conflicting results in the present study with these authors may be attributed to the effectiveness of the used herbs depending on several factors such as the used part of herb (leaves or flowers), form (extract or powder), dose, supplementation level (feed additive or main ingredient), fish species, and rearing conditions.

The survival rate is an important indicator that reflects fish's health status and water quality. Generally, fish fed with a diet containing 1% AVE was better in SR and similar to the normal control (T0). Owing to *A. vera*'s beneficial components, adding AVE to the fish diet increased and reinforced the immune responses, health status, resistance to stress, and bacterial infection (Gabriel et al., 2019).

Hematological indicators (WBC, Hb, and HCT) showed an increase with fish-fed dietary herbs, especially fish of T2 and T4 that were fed 1% and 0.5 dietary AVE supplementation. This result agreed with Gabriel et al. (2015 a), who suggested that hematological parameters, including WBC, were increased in fish blood fed a diet containing *A. vera*. Also, using *A. vera* as an additive at a rate of 0.5 to 2% in the African catfish diet leads to increasing hematological indices (Gabriel et al., 2019). Increasing WBC, Hb, and HCT in blood fish can be caused by non-specific immune stimulation, whereas Selvaraj et al. (2005) declared increasing WBC count when using immune-stimulant substances in fish diet. Increasing leukocytes of fish fed dietary AVE and their high resistance against the stress of decreasing the water quality because of extending its period of change. This refers to the ability of herbs or their essential oil at the optimum dose to stimulate leucopoiesis (formation of WBC) and erythropoiesis, increasing oxygen-carrying capacity and strengthening of defense mechanism against physiological stress (Gabriel et al., 2019; Ni et al., 2004). Glucose (RBCs) is an accurate sign of fish exposure to stress conditions (Dossou et al., 2021; Mohapatra et al., 2014; Mzengeza et al., 2021).

Furthermore, increasing the levels of liver enzymes (AST and ALT) is considered an indication of the amount of liver damage as a result of exposing the fish to stressful conditions such as a high density and deterioration of water quality (Kim et al., 2017; Yacoub et al., 2017). In the present trial, the lowest levels of RBCs and AST were obtained with T2 and T0 and subsequently T4, T3, and T1, respectively. These results confirm the influential role of *Aloe vera* in relieving stress conditions resulting from the deterioration of water characteristics. Lysozyme is an essential component of non-specific immunity; it destroys the peptide glycan layer of gram bacteria (Sukhithasri et al., 2013). Fish in the T2 group exhibited the highest lysozyme activity, followed by T4, while lysozyme levels did not significantly change between T3,

T0, and T1. This outcome may be linked to the alteration in WBCs synthesis in response to AVE herbal treatment.

Results of histological examination showed that tissue specimens from the intestines, spleens, and musculatures of fish fed a diet containing AVE (1% or 5%) were less affected than other stressed groups. In line with the present results, Tafi et al. (2020) reported a therapeutic effect of *A. vera* extracts at the level of 1.5% against *Streptococcus iniae* and histopathological lesions in rainbow trout (*Oncorhynchus mykiss*). Similar findings were reported in common carp (*Cyprinus carpio* L.) (Khanal et al., 2021).

### Conclusions

Eventually, rearing fish under a long period of water exchange stress resulted in deteriorated performance and physiological indices. Under the present trial circumstances, feeding 1% *A. vera* to the stressed fish significantly restored their performance, survival rate, and preserved their histological characteristics almost to the level of their counterparts under normal conditions.

### Conflict of interest

The authors declare no conflict of interest.

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