11-1-2023

Growth Performance, Feed Utilization, Hematological Parameters, and Histological Features of Nile Tilapia (*Oreochromis niloticus*) Fed Diets with Supplementary Herbal Extracts Under Prolonged Water Exchange

Mohamed F.A. Abdel-Aziz
1 Department of Aquaculture and Biotechnology, Faculty of Aquaculture and Marine Fisheries , Arish University , Egypt

Mohammed F. El Basuini
2 Faculty of Agriculture , Tanta University , , Tanta , Egypt,3 Faculty of Desert Agriculture , King Salman International University , South Sinai , Egypt

Ibrahim Teiba
2 Faculty of Agriculture , Tanta University , , Tanta , Egypt

Mohamed M.M. Metwally
4 Department of Pathology, Faculty of Veterinary Medicine , Zagazig University , Zagazig , , Egypt

Ann. Anim. Sci., Vol. 23, No. 4 (2023) 1147–1157 *DOI: 10.2478/aoas-2023-0017*

sciendo

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

Mohamed F.A. Abdel-Aziz[1]•, Mohammed F. El Basuini[2,3], Islam I. Teiba[2], Mohamed M.M. Metwally[4], Ashraf Y El-Dakar[1], Amr M. Helal[5], Habib Ul Hassan[6], Abdel-Moniem M. Yones[5], Ahmed Shehab[7], Mahmoud A.O. Dawood[8,9]

[1]Department of Aquaculture and Biotechnology, Faculty of Aquaculture and Marine Fisheries, Arish University, Egypt
[2]Faculty of Agriculture, Tanta University, 31527, Tanta, Egypt
[3]Faculty of Desert Agriculture, King Salman International University, South Sinai 46618, Egypt
[4]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[5]Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), Egypt
[6]Department of Zoology (MRCC), University of Karachi, Karachi-75270, Pakistan
[7]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Benha, Egypt
[8]Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt
[9]The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, Cairo 11835, Egypt
•Corresponding author: m_fathy8789@yahoo.com
Mohamed F.A. Abdel-Aziz: ORCID: 0000-0001-6042-6183

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±0.05 g, n=1200) were distributed into five groups (15 fiberglass tanks, 2 m^3) 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 Basuini 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 systems, particularly the accretion of inorganic nitrogen wastes (NH_3 and NO_2) (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; Ahmadifar 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 Ethical 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°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°C and stored at –15°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^{-1} 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_1 = 1 mg; Vitamin B_{12} = 0.01 mg; Vitamin B_2 = 4 mg; Vitamin B_6 = 1 mg; Vitamin D = 1400 IU; Vitamin E = 10 mg; Vitamin K_3 = 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 Kjeltec 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^{-1} 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.; $AY_{2019\text{-}2020}$/ 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^3 $tank^{-1}$ 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^{-1}), were recorded every day using a multi-parameter water quality analyzer (MULP-8C), while total ammonia (TA, mg L^{-1}), and nitrite (NO_2, mg L^{-1}) 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^{-1}) = [(FBW, g – IBW, g) / time, days].

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

Specific growth rate (SGR, % day^{-1}) = [(Ln FBW - Ln IBW ×100) / time, days].

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

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

Feed intake (FI, g $fish^{-1}$) = 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^{-1}, 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; 10^9 L^{-1}, hemoglobin, HB g dL^{-1}, and hematocrit Hct, %) were determined by following Rawling et al. (2009). Biochemical indicators such as plasma glucose (RBCs, mg dL^{-1}, glucose-TR 1001191), serum aspartate aminotransferase (AST, U L^{-1}, Liquizyme (4+1) E.C.2.6.1.1.), alanine aminotransferase (ALT, U L^{-1}, Liquizyme (4+1) E.C.2.6.1.2.), and urea (mg dL^{-1}, 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_2 compared to the T1 group. No significant differences existed between the treated groups with herbs in DO, pH, unionized ammonia (NH_3, mg L^{-1}), and NO_2. 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^{-1}, 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^{-1})	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^{-1})	0.075 c	0.60 a	0.40 b	0.52 a	0.53 a	0.035	6890	0.001
Unionized ammonia (mg L^{-1})	0.012 b	0.085 a	0.018 b	0.023 b	0.029 b	0.011	17.16	0.004
NO_2 (mg L^{-1})	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^{-1})	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 $fish^{-1}$)	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 (10^9 L^{-1})	122.50 a	90.00 d	114.00 b	90.50 d	100.00 c	2.42	71.17	0.002
Hb (g dL^{-1})	8.70 a	5.200 d	7.50 b	6.10 d	6.9.00 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^{-1})	39.00 b	59.50 a	44.00 b	54.50 a	42.00 b	2.14	33.402	0.003
Urea (mg dL^{-1})	8.70	7.10	7.60	6.60	7.90	0.78	2.098	0.219
AST (U L^{-1})	34.00 d	65.41 a	34.10 d	54.70 b	45.90 c	1.11	295.47	0.001
ALT (U L^{-1})	7.15	7.70	7.3.00	7.60	7.20	0.46	0.568	0.698
Lysozyme (ng mL^{-1})	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).

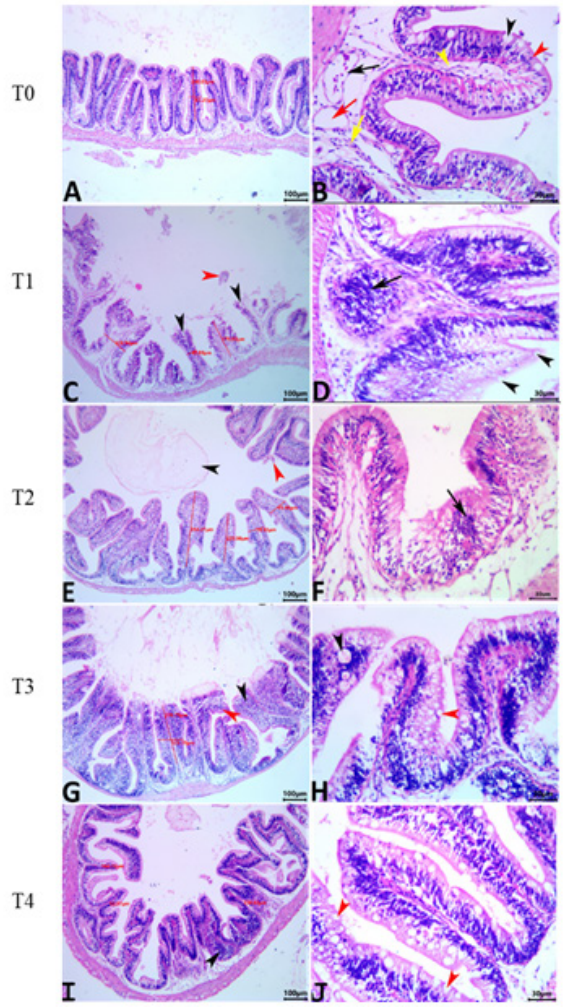

Figure 1. Photomicrograph of H&E-stained intestinal tissue sections at scale bar of 30 µm [A, C, E, G, I] and 100 µm [B, D, F, H, J]. T0 = normal control: [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile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

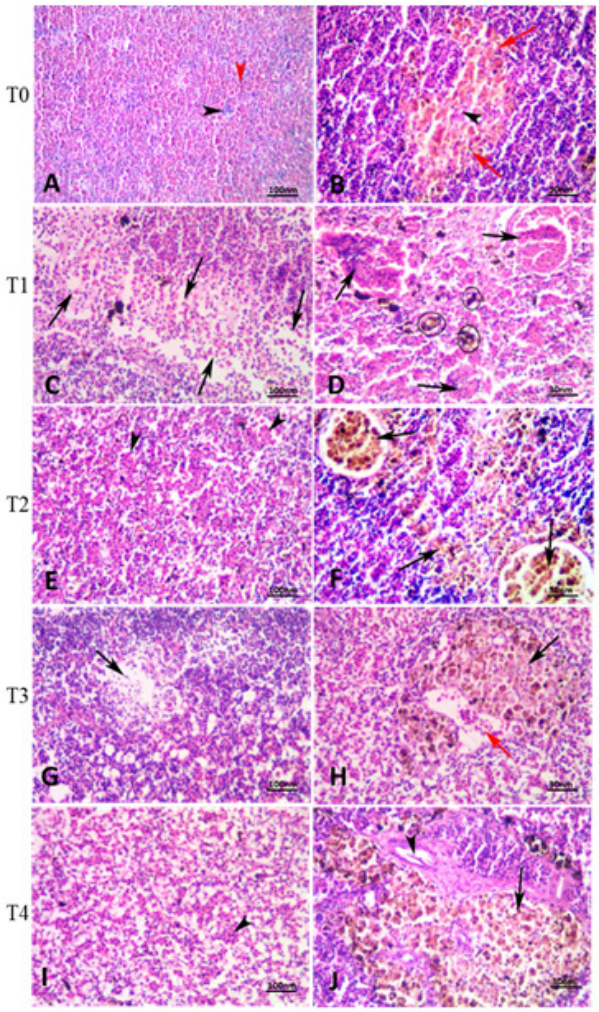

Figure 2. Photomicrograph of H&E-stained splenic tissue sections at scale bar of 30 µm [A, C, E, G, I] and 100 µm [B, D, F, H, J]. T0 = normal control: [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile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

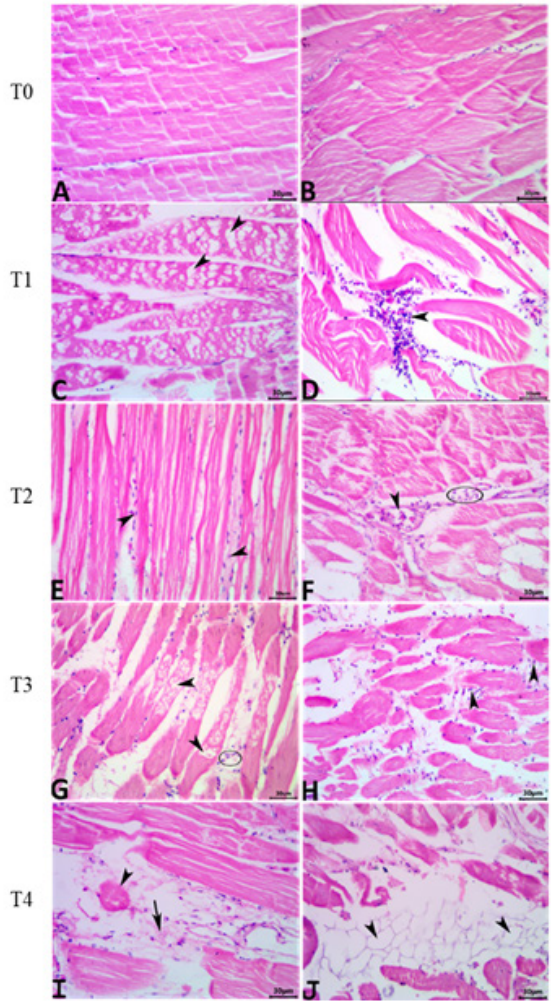

Figure 3. Photomicrograph of H&E-stained muscular tissue sections at scale bar of 30 µm. T0 = normal control: [A and B]; T1 = stressed control [C and D]; T2 = group of fish fed a 1% dietary *Aloe vera* (AVE) [E and F]; T3 = group of fish fed a 1% dietary chamomile flowers *Matricaria chamomilla* (CFE) [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^3). 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^{-1} (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^3 as changed water, while other groups consumed about 3 m^3.

It was evident from Table 2 that water temperature, DO mg L^{-1}, 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^{-1}) 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_3) or ionized ammonia (NH_4^+), nitrite (NO_2), and nitrate (NO_3) (Putra et al., 2020). Findings in Table 2 cleared that NO_2 of T2, T3, and T4

groups were 0.377, 0.465, and 0.470, respectively, while T1 recorded 0.507 mg L^{-1}. As a result, the NO_2 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_2 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_{12}, E, B_2, B_1, 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; Mzengereza 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.

Acknowledgment

The authors would like to thank all staff members of Aquaculture and Biotechnology Dep., Faculty of Aquaculture and Marine Fisheries, Arish University, Egypt and all staff members of Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), Egypt for supporting this research.

References

Abdel-Latif H.M.R., Hendam B.M., Nofal M.I., El-Son M.A. (2021). *Ginkgo biloba* leaf extract improves growth, intestinal histomorphometry, immunity, antioxidant status and modulates transcription of cytokine genes in hapa-reared *Oreochromis niloticus*. Fish Shellfish Immunol., 117: 339–349.

Abdel-Latif H.M.R., Shukry M., Noreldin A.E., Ahmed H.A., El-Bahrawy A., Ghetas H.A., Khalifa E. (2022). Milk thistle (*Silybum marianum*) extract improves growth, immunity, serum biochemical indices, antioxidant state, hepatic histoarchitecture, and intestinal histomorphometry of striped catfish, *Pangasianodon hypophthalmus*. Aquaculture, 738761.

Abdel-Tawwab M. (2012). Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). Int. Aquat. Res., 4:3.

Abdel-Tawwab M., Hagras A.E., Elbaghdady H.A.M., Monier M.N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity. Aquac. Int., 23: 1261–1274.

Abdelha Y.M., Saleh O.A., Sakr S.F. (2010). Study on the effect of wormseed plants; *Artemisia cina* L. and chamomile; *Matricaria chamomilla* L. on growth parameters and immune response of African catfish, *Clarias gariepinus*. J. Fish. Int., 5: 1–7.

Ahmadifar E., Yousefi M., Karimi M., Fadaei Raieni R., Dadar M., Yilmaz S., Abdel-Latif H. M.R. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquac., 29: 478–511.

Ahlawat K.S., Khatkar B.S. (2011). Processing, food applications and safety of *Aloe vera* products: a review. J. Food Sci. Technol., 48: 525–533.

Amirkolaie A.K. (2011). Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. Rev. Aquac., 3: 19–26.

Ani J.S., Manyala J.O., Masese F.O., Fitzsimmons K. (2022). Effect of stocking density on growth performance of monosex Nile tilapia (*Oreochromis niloticus*) in the aquaponic system integrated with lettuce (*Lactuca sativa*). Aquac. Fish., 7: 328–335.

AOAC (2000). Official Methods of Analysis, 16th ed, Association of Official Analysis Chemists. Washington DC.

APHA (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association; American Water Works Association; Water Environment Federation, Washington, DC.

Azaroual L., Liazid A., Barbero G.F., Brigui J., Palma M., Barroso C.G. (2012). Improved chromatographic methods for determination of bioactive compounds from *Aloe vera* leaves. ISRN Chromatogr., 2012: 1–7.

Bancroft J.D., Layton C. (2019). Bancroft's Theory and Practice of Histological Techniques, Elsevier.

Baruah A., Bordoloi M., Deka Baruah H.P. (2016). *Aloe vera*: A multipurpose industrial crop. Ind. Crops Prod., 94: 951–963.

Citarasu T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. Aquac. Int., 18: 403–414.

Darzi S., Paul K., Leitan S., Werkmeister J.A., Mukherjee S. (2021). Immunobiology and application of *Aloe vera*-based scaffolds in tissue engineering. Int. J. Mol. Sci., 22: 1708.

Dawood M.A.O., Basuini M.F. El, Yilmaz S., Abdel-Latif H.M.R., Kari Z.A., Abdul Razab M.K., Ahmed H.A., Alagawany M., Gewaily M.S. (2021). Selenium nanoparticles as a natural antioxidant and metabolic regulator in aquaculture: A review. Antioxidants, 10: 1364.

Dey P., Dutta S., Chowdhury A., Das A.P., Chaudhuri T.K. (2017). Variation in phytochemical composition reveals distinct divergence of *Aloe vera* (L.) Burm.f. from other aloe species: rationale behind selective preference of *Aloe vera* in nutritional and therapeutic use. J. Evid. Based. Complementary Altern. Med., 22: 624–631.

Dossou S., Dawood M.A.O., Zaineldin A.I., Abouelsaad I.A., Mzengereza K., Shadrack R.S., Zhang Y., El-Sharnouby M., Ahmed H.A., El Basuini M.F. (2021). Dynamical hybrid system for optimizing and controlling efficacy of plant-based protein in aquafeeds. Complexity, 2021: 1–7.

Effendi H., Widyatmoko Utomo B.A., Pratiwi N.T.M. (2020). Ammonia and orthophosphate removal of tilapia cultivation wastewater with *Vetiveria zizanioides*. J. King Saud Univ. - Sci., 32: 207–212.

El Basuini M.F., Teiba I.I., Shahin S.A., Mourad M.M., Zaki M.A.A., Labib E.M.H., Azra M.N., Sewilam H., El-Dakroury M.F., Dawood M.A.O. (2022). Dietary Guduchi (*Tinospora cordifolia*) enhanced the growth performance, antioxidative capacity, immune response and ameliorated stress-related markers induced by hypoxia stress in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol., 120: 337–344.

El-Sayed A.-F.M. (1999). Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture, 179:149–168.

El-Son M.A.M., Hendam B.M., Nofal M.I., Abdel-Latif H.M.R. (2022). Effects of *Moringa oleifera*-based diets on growth, immunological responses, liver antioxidant biomarkers and expression of immune-related genes in Nile tilapia (*Oreochromis niloticus*) raised in hapa-in-pond system. Aquac. Res., 53: 4338– 4352.

Engin K., Carter C.G. (2001). Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level. Aquaculture, 194: 123–136.

Fasiha Nordin S., Luqman Nordin M., Yusuf Osman A., Hayati Hamda R., Shaari R., Mokhtar Arshad M., Rahman Aziz A. (2017). The effect of *Matricaria chamomilla* L. on the growth performance of red hybrid tilapia. Biomed. Pharmacol. J., 10: 1905–1915.

Gabriel N.N., Qiang J., He J., Ma X.Y., Kpundeh M.D., Xu P. (2015 a) Dietary *Aloe vera* supplementation on growth performance, some

haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). Fish Shellfish Immunol., 44: 504–514.

Gabriel N.N., Qiang J., Ma X.Y., He J., Xu P., Liu K. (2015 b). Dietary *Aloe vera* improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (*Oreochromis niloticus*) after *Streptococcus iniae* challenge. Fish Physiol. Biochem., 41: 1321–32.

Gabriel N.N., Qiang J., Ma X.Y., He J., Xu P., Omoregie E. (2017). Sex-reversal effect of dietary *Aloe vera* (Liliaceae) on genetically improved farmed Nile tilapia fry. N. Am. J. Aquac., 79: 100–105.

Gabriel N.N., Wilhelm M.R., Habte-Tsion H.-M., Chimwamurombe P., Omoregie E., Iipinge L.N., Shimooshili K. (2019). Effect of dietary *Aloe vera* polysaccharides supplementation on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. Int. Aquat. Res., 11: 57–72.

Gewaily M.S., Abdo S.E., Moustafa E.M., AbdEl-kader M.F., Abd El-Razek I.M., El-Sharnouby M., Alkafafy M., Raza S.H., El Basuini M.F., Van Doan H., Dawood M.A.O. (2021). Dietary synbiotics can help relieve the impacts of deltamethrin toxicity of Nile tilapia reared at low temperatures. Animals, 11: 1790.

Haghi G., Hatami A., Safaei A., Mehran M. (2014). Analysis of phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV. Res. Pharm. Sci., 9: 31–37.

Hassan B., El-Salhia M., Khalifa A., Assem H., Al Basomy A., El-Sayed M. (2013). Environmental isotonicity improves cold tolerance of Nile tilapia, *Oreochromis niloticus*, in Egypt. Egypt. J. Aquat. Res., 39: 59–65.

Ip Y.K., Chew S.F. (2010). Ammonia production, excretion, toxicity, and defense in fish: a review. Front. Physiol., 1: 134.

Kayode O.A. (2016). Effects of *Aloe vera* gel application on epidermal wound healing in the domestic rabbit. Int. J. Res. Med. Sci., 5: 101–105.

Khalafalla M. (2009). Utilization of some medical plants as feed additives for Nile tilapia, *Oreochromis niloticus*, Feeds. Mediterr. Aquac. J., 2: 9–18.

Khanal M., Lamichhane S., Bhattarai A., Kayastha B.L., Labh S.N. (2021). Extract of *Aloe vera* (*Aloe barbadensis* Miller) enhances the growth, protein contents, and gastrosomatic index (GaSI) of common carp *Cyprinus carpio*. J. Nutr. Metab., 2021: 8029413.

Kim J.-H., Park H.-J., Hwang I.-K., Han J.-M., Kim D.-H., Oh C.W., Lee J.S., Kang J.-C. (2017). Alterations of growth performance, hematological parameters, and plasma constituents in the sablefish, *Anoplopoma fimbria* depending on ammonia concentrations. Fish. Aquat. Sci., 20: 4.

Kord M.I., Maulu S., Srour T.M., Omar E.A., Farag A.A., Nour A.A.M., Hasimuna O.J., Abdel-Tawwab M., Khalil H.S. (2022). Impacts of water additives on water quality, production efficiency, intestinal morphology, gut microbiota, and immunological responses of Nile tilapia fingerlings under a zero-water-exchange system. Aquaculture, 547: 737503.

Kroupova H., Machova J., Svobodova Z. (2012). Nitrite influence on fish: a review. Vet. Med. (Praha), 50: 461–471.

Kuebutornye F.K.A., Abarike E.D. (2020). The contribution of medicinal plants to tilapia aquaculture: a review. Aquac. Int., 28: 965–983.

Lee J.K., Lee M.K., Yun Y.P., Kim Y., Kim J.S., Kim Y.S., Kim K., Han S.S., Lee C.K. (2001). Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. Int. Immunopharmacol., 1: 1275–1284.

Lind C.E., Agyakwah S.K., Attipoe F.Y., Nugent C., Crooijmans R.P.M.A., Toguyeni A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. Sci. Rep., 9: 16767.

Liu C., Cui Y., Pi F., Cheng Y., Guo Y., Qian H. (2019). Extraction, purification, structural characteristics, biological activities and pharmacological applications of acemannan, a polysaccharide from *Aloe vera*: A Review. Molecules., 24: 1554.

Lygren B., Sveier H., Hjeltness B., Waagbø R. (1999). Examination of the immunomodulatory properties and the effect on disease resistance of dietary bovine lactoferrin and vitamin C fed to Atlantic salmon (*Salmo salar*) for a short-term period. Fish Shellfish Immunol., 9: 95–107.

Makori A.J., Abuom P.O., Kapiyo R., Anyona D.N., Dida G.O. (2017). Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. Fish. Aquat. Sci., 20: 30.

Meyers T. (2009). Standard necropsy procedures for finfish. In: National wild fish health survey – laboratory procedures manual, N. Heil (ed.). 5 Edition, Chapter: 4. United States Fish and Wildlife Service, Warmsprings, GA, pp. 4.1–4.10.

Mohammadi G., Rafiee G., El Basuini M.F., Van Doan H., Ahmed H.A., Dawood M.A.O., Abdel-Latif H.M.R. (2020). Oregano (*Origanum vulgare*), St John's-wort (*Hypericum perforatum*), and lemon balm (*Melissa officinalis*) extracts improved the growth rate, antioxidative, and immunological responses in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquac. Rep., 18: 100445.

Mohapatra S., Chakraborty T., Prusty A.K., PaniPrasad K., Mohanta K.N. (2014). Beneficial effects of dietary probiotics mixture on hemato-immunology and cell apoptosis of *Labeo rohita* fingerlings reared at higher water temperatures. PLoS One, 9:e100929.

Molnar M., Mendešević N., Šubarić D., Banjari I., Jokić S. (2017). Comparison of various techniques for the extraction of umbelliferone and herniarin in *Matricaria chamomilla* processing fractions. Chem. Cent. J., 11: 78.

Mzengereza K., Ishikawa M., Koshio S., Yokoyama S., Yukun Z., Shadrack R.S., Seo S., Kotani T., Dossou S., Basuini M.F. El, Dawood M.A.O. (2021). Growth performance, growth-related genes, digestibility, digestive enzyme activity, immune and stress responses of de novo camelina meal in diets of red seabream (*Pagrus major*). Animals, 11: 3118.

Ni Y., Turner D., Yates K.M., Tizard I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. Int. Immunopharmacol., 4: 1745–1755.

Ojha N.K., Kumar A. (2012). HPTLC profile of aqueous extract of different chromatographic fractions of *Aloe barbadensis* Miller. Asian Pacific J. Trop. Dis., 2: S104–S108.

Okomoda V.T., Tiamiyu L.O., Uma S.G. (2016). Effects of hydrothermal processing on nutritional value of *Canavalia ensiformis* and its utilization by *Clarias gariepinus* (Burchell, 1822) fingerlings. Aquac. Rep., 3: 214–219.

Paray B.A., El-Basuini M.F., Alagawany M., Albeshr M.F., Farah M.A., Dawood M.A.O. (2021). *Yucca schidigera* usage for healthy aquatic animals: potential roles for sustainability. Animals, 11: 93.

Putra I., Effendi I., Lukistyowati I., Tang U.M., Fauzi M., Suharman I., Muchlisin Z.A. (2020). Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. F1000Res., 9: 293.

Rawling M.D., Merrifield D.L., Davies S.J. (2009). Preliminary assessment of dietary supplementation of Sangrovit® on red tilapia (*Oreochromis niloticus*) growth performance and health. Aquaculture, 294: 118–122.

Reverter M., Tapissier-Bontemps N., Sasal P., Saulnier D. (2017). Use of medicinal plants in aquaculture. Diagnosis Control Dis. Fish Shellfish, Wiley Online Books. https://doi.org/https://doi.org/10.1002/9781119152125.ch9

Sánchez-Machado D.I., López-Cervantes J., Mariscal-Domínguez M.F., Cruz-Flores P., Campas-Baypoli O.N., Cantú-Soto E.U., Sanches-Silva A. (2017). An HPLC procedure for the quantification of aloin in latex and gel from *Aloe barbadensis* leaves. J. Chromatogr. Sci., 55: 251–257.

Schar D., Zhao C., Wang Y., Larsson D.G.J., Gilbert M., Van Boeckel T.P. (2021). Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. Nat. Commun., 12: 5384.

Selvaraj V., Sampath K., Sekar V. (2005). Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Fish Shellfish Immunol., 19: 293–306.

Shadrack R.S., Manabu I., Koshio S., Yokoyama S., Zhang Y., Mzengereza K., El Basuini M.F., Dawood M.A.O. (2022). Effects of single and mixture probiotic supplements on growth, digestive activity, antioxidative status, immune and growth-related genes, and

stress response of juvenile red sea bream (*Pagrus major*). Aquac. Nutr., 2022: 8968494.

Šibul F., Orčić D., Berežni S., Anačkov G., Mimica-Dukić N. (2020). HPLC–MS/MS profiling of wild-growing scentless chamomile. Acta Chromatogr., 32: 86–94.

Srivastava J.K., Shankar E., Gupta S. (2010). Chamomile: A herbal medicine of the past with bright future. Mol. Med. Rep., 3: 895–901.

Sukhithasri V., Nisha N., Biswas L., Anil Kumar V., Biswas R. (2013). Innate immune recognition of microbial cell wall components and microbial strategies to evade such recognitions. Microbiol. Res., 168: 396–406.

Tafi A.A., Meshkini S., Tukmechi A., Alishahi M., Noori F. (2020). Therapeutic and histopathological effect of *Aloe vera* and *Salvia officinalis* hydroethanolic extracts against *Streptococcus iniae* in rainbow trout. Arch. Razi Inst., 75: 257–287.

Tran-Duy A., Schrama J.W., van Dam A.A., Verreth J.A.J. (2008). Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*. Aquaculture, 275: 152–162.

Tran-Ngoc K.T., Dinh N.T., Nguyen T.H., Roem A.J., Schrama J.W., Verreth J.A.J. (2016). Interaction between dissolved oxygen concentration and diet composition on growth, digestibility and intestinal health of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 462: 101–108.

Trang N.T.D., Konnerup D., Brix H. (2017). Effects of recirculation rates on water quality and *Oreochromis niloticus* growth in aquaponic systems. Aquac. Eng., 78: 95–104.

Viapiana A., Struck-Lewicka W., Konieczynski P., Wesolowski M., Kaliszan R. (2016). An approach based on HPLC-fingerprint and chemometrics to quality consistency evaluation of *Matricaria chamomilla* L. commercial samples. Front. Plant Sci., 7: 1561.

Yacoub A.M., Sabra S., Al-Kourashi M. (2017). Pathological changes in liver structure and function of *Oreochromis niloticus* experimentally exposed to *Escherichia coli*. Int. J. Biotechnol. Bioeng., 3: 95–106.

Yavuzcan Y., H., Robaina L., Pirhonen J., Mente E., Domínguez D., Parisi G. (2017). Fish welfare in aquaponic systems: Its relation to water quality with an emphasis on feed and faeces – a review. Water, 9: 13.

Yu Y., Shen M., Song Q., Xie J. (2018). Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. Carbohydr. Polym., 183: 91–101.

Zaki M.A., Labib E.M., Nour A.M., Tonsy H.D., Mahmoud S.H. (2012). Effect some medicinal plants diets on mono sex Nile tilapia (*Oreochromis niloticus*), growth performance, feed utilization and physiological parameters. APCBEE Procedia, 4: 220–227.

Zaki M A A, Alabssawy A.N., Nour A.E.-A.M., El Basuini M.F., Dawood M.A.O., Alkahtani S., Abdel-Daim M.M. (2020). The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Aquac. Rep., 16: 100282.

Received: 9 VIII 2022
Accepted: 24 I 2023