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Supplementing the diet of Nile tilapia (*Oreochromis niloticus*) with *Amphora coffeaeformis* nanoparticles (Am NPs) enhances the growth performance, redox status, digestion, immune responses, and defense against *Aeromonas veronii*

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ABSTRACT

A frequent species of microalgae in alkaline brackish, marine, and freshwater is *Amphora coffeaeformis*. It has a high concentration of physiologically active chemicals with antibacterial, anti-obesity, and antioxidant properties, as well as pigments involved in photosynthetic respiration, including as carotenoids and chlorophyll, that are effective in a range of medical applications. The current study sought to determine the effects of varying dosages of *Amphora coffeaeformis* nanoparticles (Am-NPs) incorporated diet on Nile tilapia (*Oreochromis niloticus*), which were weighted at 15±0.5 g and 9±0.5 cm in length, on growth performance, immunological, digestive enzymes, lipid peroxidation, and antioxidant activities, as well as histological examination of the intestinal villi and resistance to *Aeromonas veronii* (*A. veronii*). Four experimental groups (each with 80 fish) were run in duplicate. For four weeks, *Amphora* nanoparticles (Am NPs) at 2.5, 5 and 7.5 g/kg diet were added to the following three groups, while the first additive-free basal diet functioned as the control group. After the feeding trial, fish were exposed to an infection of pathogenic bacteria (*A. veronii*) with 9×10^8 CFU/ml. Supplementing with (Am-NPs) during the experiment period, specifically 7.5 g/kg diet ($P<0.05$), significantly improved the following: immunological parameters such as liver Nitric oxide (NO) and plasma IgM and IgG; biochemical parameters such as liver tissue alanine aminotransaminase, aspartate aminotransaminase, plasma lipase, and amylase; and growth performance (weight gain, specific growth rate, feed conversion rate, and length gain rate). The villus height, the villus height to crypt depth ratio and and the number of goblet cells were significantly ($P<0.05$) increase in the fish fed Am Nps especially at 7.5 g/ kg diet when compared with control group which had the lowest values. Additionally the supplemented groups showed a substantial ($P<0.05$) decrease in the crypt depth of the villus when compared to control group. Our results showed that the groups treated with Am-NPs had a relative percent survival (RPS) of 65-85% against the infection of pathogenic *Aeromonas veronii* bacteria, compared to a control group that had an RPS of 0%. In conclusion, *Amphora coffeaeformis* offers several advantages, the chief among them being that it is a significant food source. Additionally, it has a range of physiologically active substances with antibacterial, immunological, antioxidant, and biochemical properties that are used in numerous medicinal applications.

Introduction

Aquaculture plays a crucial role in ensuring food security and reducing poverty by providing nutrition and livelihood opportunities to millions of individuals worldwide. Aquaculture has gained significant popularity and has become a thriving business sector. In fact, approximately 50% of the world's seafood is now produced through aquaculture. This industry includes various practices like algaculture, mariculture, shrimp farming, oyster farming, fish farming, and ornamental fish culture. Aquaculture is the fastest-growing sector in the field of fishery sciences (Tacon and Metian, 2013). Over the last seven years, Egypt has seen a remarkable and quick expansion in the aquaculture industry. Egypt is presently the largest producer of grey mullet in the world, but it is also the top producer in Africa, ranking sixth internationally in aquaculture and third in tilapia production, behind only China and Indonesia. Aquaculture is becoming an important contributor to Egypt's food security and economy, with approximately farmed fish is represented as 81% from total fish production (USDA, 2022). According to Dong *et al*. (2017), *A. veronii* is known to have caused high mortalities in cultured Nile tilapia in Egypt. The mortality rate for the 8.9×10^6 CFU/ml dose that killed all the experimental fish reached 100% in less than 24 hours. A growing amount of focus is being given to the dietary combination of natural alternatives due to the hazards and restrictions that come with the use of antibiotics and chemical therapy in aquaculture (Song *et al*., 2014). Microalgae, especially *Amphora coffeaeformis*, is thought to be extremely valuable and adaptable organisms in a variety of sectors, including medicine, food, dietary supplements, and wastewater treatment. In the presence of a range of phytoconstituents produced by metabolic enzymes, microalgae exhibit a diversity of biological activities (Mansour *et al*., 2023). These metabolites are excellent alternatives to antibiotics, particularly in the treatment of illness. Several researchers improved the role of algae as immunostimulants. Numerous investigations have suggested that *Amphora* could be used as a preventative and antioxidant agent due to its antibacterial, antiviral, and anti-inflammatory properties. *Amphora* supplements appear to be a promising alternative to antibiotics for illness prevention in Nile tilapia culture (Ayoub *et al*., 2019). *Amphora coffeaeformis* has been widely employed as a natural antioxidant in recent years due to its safety and effectiveness in replacing current and commercial synthetic antioxidants. *Amphora coffeaeformis* is a microalgae that contains vitamins E and C, sulfated polysaccharides, polyunsaturated fatty acids, tocopherol, glucansin, and carotenoids (canthaxanthin and astaxanthin) (Glodde *et al*., 2018). Furthermore, include polyphenolic compounds including methyl gallate, naringenin, kaempferol, taxifolin, gallic acid, cinnamic acid, and syringic acid. The phenolic chemicals that provide *A. coffeaeformis* its larvicidal activity are gallic acid, vanillic acid, caffeic acid, syringic acid, cinnamic acid, coumaric acid, chlorogenic acid, quercetin, rutin, and benzoic acid, rosmarinic acid and unsaturated fatty acids. It contained polyphenolic antioxidant ingredients like syringic acid, taxifolin, kaempferol, cinnamic

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acid, caffeic acid, methyl gallate, naringenin, and gallic acid (Hassan *et al*., 2021). So, *Amphora* has been utilized for a variety of purposes, including food, dietary supplements, medicine, fuel, and wastewater treatment (Torres, 2016; Abdel-Wahab, 2018; Mekkawy *et al*., 2020). As a result, the current study aimed to investigate the role of *Amphora* nanoparticles in the protection of *Oreochromis niloticus* against infection by *A. veronii* through strengthening the immune response, improving growth performance, digestion, , intestinal morphometry and antioxidant biomarkers in Nile tilapia.

Materials and methods

Fish and diet preparation

The current study was conducted at Aquaculture Research Lab., Department of Physiology, Faculty of Veterinary Medicine (Moshtohor), Benha University, Egypt. All procedures utilized in this study were authorized by Benha University's Institutional Animal Care and Use Committee and followed the requirements of Egypt's National Institute of Health (NIH) (Ethical No. BUFVTM 18-09-22). Nile tilapia (*Oreochromis niloticus*) of average weight (15.0±0.5 g) and length (9.0±0.5 cm) were taken from a private fish farm in Abbassa (Sharkia Governorate, Egypt) and transported to the research lab according to Klemm *et al*. (1993). A total of 320 apparent healthy fish were distributed into 8 large fiber glass tanks (500 L capacity) to symbolize four groups (in duplicate), with each tank containing 40 fish and an additional stock tank to replace the dead ones. Before beginning this investigation, fish were acclimatized for two weeks in glass tanks (100 L capacity). A sophisticated suction pump removed organic matters and remaining food on a daily basis and water was partially replaced every day. To maintain a constant, the physical characteristics of water were monitored daily (dissolved O2 was 3.32±4.5 mg/L, pH was 7.6±0.34, and temperature was 28.8±3.0°C). The photoperiod remained consistent (12 hours of light and 12 hours of darkness).

Amphora coffeaeformis nanoparticles (Am NPs)

Amphora coffeaeformis powder was donated by the Department of Algal Biotechnology at the National Research Centre, Dokki, Giza, Egypt. It was prepared in nano form at the Egyptian Nanotechnology Center (EGNC), located in Sheikh Zayed City, an Egyptian suburb of Cairo, using the ball mill method in accordance with EGNC protocols and the top-down technique for green synthesis of *A. coffeaeformis* nanoparticles as presented in Fig. 1.

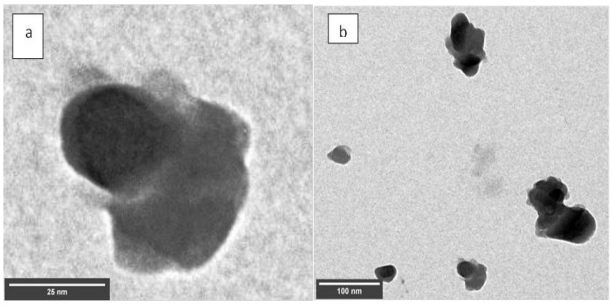

Fig. 1. *A. coffeaeformis* nanoparticles (A and B) used in the current study by transmission electron microscope (TEM).

Experimental design

Concentrations of *A. coffeaeformis* (Am NPs) of 2.5, 5 and 7.5 g/kg food were selected and added to the commercial fine basal diet for tilapia (Tilapia fish feed 30%, 6th October business, Egypt). After combining all the ingredients in a mixer (Mienta, France), the resulting pellets were allowed to air dry at room temperature before being kept refrigerated until needed. From the beginning of the experiment to its conclusion, fish were weighted once a week in order to determine needed amount of food (5% of their body weight). For duration of 4 weeks, fish were fed this well-balanced ration twice a day, at 9:00 a.m. and 17:00 p.m (Ayoub *et al*., 2019).

Growth performance parameters

Fish weight and growth were measured at the end of the experiment using digital electronic balance that had the following specifications: The following formulas were used to determine the specific growth rate (SGR%/day), feed conversion ratio (FCR), and length gain rate (LGR %):
SGR (%) =[(Ln Wt2-Ln Wt1)/((T))]X100

Ln Wt2 is the natural logarithm of final body weight in g; Ln Wt1is natural logarithm of initial body weight in g and T is number of trials days.
FCR= The amount of food ingested (air dry)/The increase in weight gain
LGR (%)=[(Final length (cm)-Initial length (cm))/(Initial length (cm))]X100

Sampling

After being put to euthanized using 250 ppm of tricaine methane sulfonate (MS222) (Syndel Laboratories, British Columbia) and the identical protocols previously outlined by Elabd *et al*. (2016), a 1-mL syringe was used. At the end of the four weeks following feeding, whole blood samples were taken from 10 fish per group (5 fish/ tank). After centrifuging the samples for 10 minutes at 3600 rpm, they were put in non-heparinized tubes to clot. The serum was then separated. Subsequently, the serum was refrigerated at -20°C, and utilized to determine serum levels of blood glucose, albumin, globulin, total protein, and digestive enzymes (lipase and amylase). After the serum was extracted, the fish were all killed, and small pieces of gastrointestinal and liver tissue were removed by dissecting the ventral body wall. For testing liver contents of enzymes (ALT and AST), nitric oxide (NO), MDA, and antioxidant enzymes (SOD, Gpx, and GSH), liver tissues were collected in phosphate-buffered saline (PBS) with a pH of 7.4. For histological analysis, intestinal specimens were kept in 10% buffered neutral formalin.

Liver oxidant- antioxidant biomarkers and Liver enzymes

According to Mansour *et al*. (2023), the levels of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver were determined using HPLC (Agilent HP 1200 Series Apparatus, USA). To identify the thiol compounds of reduced glutathione, the HPLC was equipped with a Bondapak column (30 cm x 3.9 mm C18µ) and loaded with a mobile phase consisting of 13% methanol, 0.005 M tetra-butylammonium phosphate, pH 3.5, and 0.0025M sodium phosphate buffer. The hepatic level of 8-OHdG was separated using successive C18 reversed-phase columns (Supelco, 5 p.m., I.D. 0.46 × 25 cm) at a wavelength of 245 nm and a flow rate of 0.68 mL/min. The eluting solution utilized was pH 5.5, H_2O/methanol (85:15 v/v), containing 50 mM KH3PO4. According to Aebi (1984), Using spectrophotometry, the liver's glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity were measured at 340 nm. This was carried out utilizing nitro blue tetrazolium dye as an indication for SOD estimate, based on the enzyme's ability to block phenazine methosulphate. The GPx measurement is based on the quantity of enzyme needed to oxidize 1.0 nmol of NADPH to NADP+ per minute at 25°C. According to Huang *et al*. (2006) and Liu *et al*. (2014), AST and ALT activities in liver were determined spectrophotometrically at 340 nm using commercial kits from Diamond Diagnostics Company, Egypt.

Serum immunological parameters, glucose level, protein profile and digestive enzymes

Using ELISA kits (My BioSource Inc.) and the company's procedure, serum immunoglobulin M and G (IgM and IgG) antibody titres were ascertained. Using Griess reagent (Sigma-Aldrich, USA), nitric oxide (NO)

was detected spectrophotometrically at 570 nm, as reported by Reda *et al*. (2018). Using commercial kits from Diamond Diagnostics Company, Egypt, the serum glucose level was determined spectrophotometrically at 340 nm. Henry (1964) described the calorimetric measurement of total protein, albumin, and globulin in fish serum. Furthermore, albumin was quantified in accordance with Doumas *et al.* (1971), and globulin was ascertained in accordance with Henry (1964). Using the diagnostic reagent kits and the company's procedure (Cusabio Biotech Co. Ltd., China), the levels of the digestive enzymes lipase and amylase in serum was determined spectrophotometrically.

Histological examination

Following the experiment, two fish from each group had their whole digestive tract removed and carefully cleaned in distilled water. A portion of the small intestine's midsection was used to remove selected gut fragments in order to examine the histological and photomicrograph alterations of the intestinal villi. Gut pieces were removed from the intestine fixed in 10% neutral buffer formalin, dehydrated in increasing series of ethanol, cleaned in xylene then embedded in paraffin block, and thinly sliced into 5 micrometer thick sections. Sections were stained using Eosin and Haematoxylin (H&E). Techniques for fixation and staining and techniques were carried out according to Abd El Latif *et al.* (2019). The stained sections were examined using a computerized light microscope (Leica DM 3000 LED).

Intestinal morphology was determined by measuring height of the villus, ratio of the villus height and depth of the crypt (height of villus/ depth of crypt) using image J software. Goblet cells were counted according to same - sized villi.

Challenge experiment

Following Abd El Latif *et al.* (2019), twenty fish per group were intraperitoneally injected with a pathogenic *A. veronii* isolate at a density of 0.2 ml ($9x10^8$) colony-forming units per ml (CFU/ml) using McFarland standard tubes at the conclusion of the experiment, which involved a four-week feeding trial. The precisely known *A. veronii* was isolated from sick fish at Benha University's Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine. For seven days, the death rate was publicized every day, and the relative percentage of survival (RPS) was calculated as follows:

RPS= [1−(mortality in vaccinated group/mortality in control group)] ×100%.

Statistical analysis

Using One-way ANOVA, the Statistical Package for the Social Sciences (SPSS) software (v# 22.0) was used to statistically test all of the numerical results. The differences between the groups were assessed using Duncan multiple tests at ($p < 0.05$) (Steel and Torrie, 1984). Every data point was statistically significant at $p < 0.05$ and is shown as means ± standard errors (SE).

Results

Growth performance

Table 1 illustrates how *Amphora coffeaeformis* nanoparticles (Am NPs) supplementation affects *O. niloticus* growth performance and feed

Table 1. Effect of dietary supplementation of *Amphora coffeaeformis* nanoparticles (Am NPs) on growth performance and feed efficiency parameters of *O. niloticus* (n=15)

Parameters	Am NPs g/ Kg feed			
	Control (0)	2.5	5	7.5
Initial weight (g)	15.12±0.15^{a}	15.12±0.15^{a}	15.07±0.16^{a}	14.93±0.15^{a}
Final weight (g)	21.74±1.29^{d}	21.74±1.29^{d}	39.74±1.46^{b}	45.16±1.77^{a}
Weight gain (g)	6.62±1.25^{d}	6.62±1.25^{d}	24.67±1.44^{b}	30.22±1.78^{a}
Weight gain %	43.71±8.16^{d}	43.71±8.16^{d}	163.80±9.40^{b}	202.54±12.28^{a}
Specific growth rate (SGR)(%/day)	0.51±0.08^{d}	0.51±0.08^{d}	1.40±0.05^{b}	1.60±0.06a
Feed conversion ratio (FCR)	4.16±1.52^{a}	4.16±1.52^{a}	1.65±0.10^{b}	1.35±0.10^{b}
Average daily gain ADG (g/day)	0.22±0.04d	0.22±0.04d	0.82±0.05b	1.01±0.06a
Initial length (g)	9.02±0.24^{a}	9.02±0.24^{a}	9.14±0.17^{a}	8.94±0.19^{a}
Final length (g)	9.96±0.12^{b}	9.96±0.12^{b}	13.08±0.36^{a}	13.62±0.24^{a}
Length gain (g)	0.94±0.32^{b}	0.94±0.32^{b}	3.94±0.33^{a}	4.68±0.29^{a}
Length gain rate(LGR)(%/30day)	10.83±3.92^{b}	10.83±3.92^{b}	43.18±3.62^{a}	52.60±4.00^{a}

Data are presented as Mean ±SE. Means with different letters in the same row are significantly different ($p < 0.05$).

Table 2. Effect of dietary supplementation of *Amphora coffeaeformis* nanoparticles (Am-NPs) on liver oxidant-antioxidant enzymes and liver function of *O. niloticus* (n=6).

Parameters	Am NPs g/ Kg feed			
	Control (0)	2.5	5	7.5
AST (nM/g tissue)	34.67±1.45^{a}	27.33±1.20^{b}	22.33±1.45^{c}	17.67±1.20^{d}
ALT (nM/g tissue)	56.67±2.02^{a}	49.33±2.02^{b}	42.33±1.20^{c}	33.67±2.02^{d}
MDA (nM/g)	55.17±1.01^{a}	42.06±0.83^{b}	36.56±1.40^{c}	32.27±1.33^{d}
GSH (nM/g)	2.05±0.33^{b}	3.58±0.31^{a}	3.81±0.38^{a}	4.72±0.36^{a}
GPx (U/g tissue)	32.22±1.40^{c}	47.76±1.30^{b}	51.76±1.81^{b}	57.25±1.18^{a}
SOD (U/g tissue)	41.78±1.31^{c}	49.72±1.81^{b}	56.57±1.13^{a}	60.28±1.57^{a}

Data are presented as Mean ±SE.
MDA: malondialdehyde; GSH: reduced glutathione; GPx: glutathione peroxidase; SOD: superoxide dismutase; nM: nanomole; Means with different letters in the same row are significantly different ($p < 0.05$).

efficiency metrics. When comparing the Am NPs groups to the control, the growth performance metrics (WG, WGR, SGR%, FCR, and LGR %) showed substantial improvements ($P < 0.05$). At the conclusion of the feeding trial, the Am NPs group that received 7.5 g/kg of food showed the greatest improvements ($P < 0.05$).

Liver oxidant- antioxidant biomarkers and Liver enzymes

The effects of *Amphora coffeaeformis* nanoparticle dietary supplementation on oxidant-antioxidant enzymes and liver function of *O. niloticus* are shown in Table 2. Dietary incorporation with 7.5 g/kg diet Am NPs showed the most ($P < 0.05$) marked increase in reduced glutathione (GSH), glutathione peroxidase (GOx), and superoxide dismutase (SOD) in comparison to the control and other treated groups at the end of feeding trial while MDA and liver enzyme (ALT and AST) showed significant decrease in dietary incorporation with 7.5 g/kg diet Am NPs in comparison to the control and other treated groups.

Serum immunological parameters, glucose level, protein profile and digestive enzymes

The effects of *Amphora coffeaeformis* nanoparticle dietary supplementation on serum immunological parameters, glucose level, protein profile and digestive enzymes of *O. niloticus* are shown in Table 3. Dietary incorporation with 7.5 g/kg diet Am NPs showed the most ($P < 0.05$) marked increase in protein profile (total protein, albumin and globulin), immunological parameters (IgM, IgG and NO) and digestive enzymes (lipase and amylase) in comparison to the control and other treated groups at the end of feeding trial while glucose level showed significant decrease in dietary incorporation with 7.5 g/kg diet Am NPs in comparison to the control and other treated groups.

Histological examination

The effects of *Amphora coffeaeformis* nanoparticle dietary supplementation on small intestine of *O. niloticus* as presented in Table 4 and Figure 2, as compared to the control group, dietary supplementation with Am NPs significantly altered the number of goblet cells, villus height, crypt depth, and the villus height to crypt depth ratio. Overall, in the supplemented groups, there was a significant ($P \leq 0.05$) increase in villus height and the ratio of villus height to crypt depth. The 7.5 g Am NPs/ kg diet group showed the highest values (587.56 ±1.17 µm, 12.55±1.00, and 69±1.00, respectively), while the control group had the lowest values (163.22±1.05, 2.19±0.06 and 12±1.20 µm, respectively). Additionally, the supplemented groups showed a substantial ($P < 0.05$) decrease in the crypt depth of the villus, with the 7.5 g Am NPs/ kg diet group exhibiting the greatest value of 74.45±1.00 µm. NPs group, while the control group had the lowest value (46.89±1.30 µm).

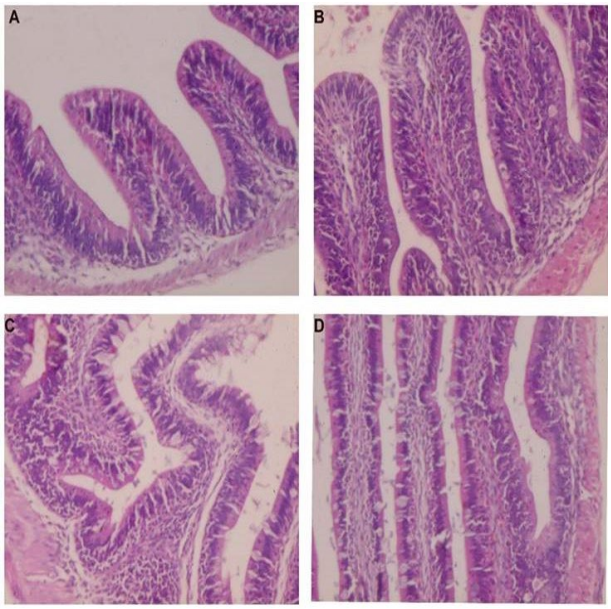

Fig. 2. Histological photomicrograph of the *O. niloticus* small intestine showed gradual increase in the length of the intestinal villi. (A): Small intestine from control group, (B): Am NPs 2.5 g/ kg diet group, (C): Am NPs 5 g/ kg diet group, (D): Am NPs 7.5 g/ kg diet group. H&E stain, Magnification: ×200.

Table 3. Effect of dietary supplementation of *Amphora coffeaeformis* nanoparticles (Am-NPs) on serum immunological parameters, glucose level, and protein profile and digestive enzymes of *O. niloticus* (n=6).

Parameters	Am NPs g/ Kg feed			
	Control (0)	2.5	5	7.5
Total protein (g/dl)	3.33±0.13^{c}	4.13±0.06^{b}	4.45±0.12^{b}	5.25±0.07^{a}
Albumin (g/dl)	1.39±0.14^{b}	2.01±0.10^{a}	1.88±0.09^{a}	2.11±0.12^{a}
Globulin (g/dl)	1.89±0.07^{b}	2.78±0.10^{a}	2.49±0.10^{a}	2.94±0.09^{a}
IgM (mg/dl)	37.75±2.70^{c}	45.33±2.19^{b}	50.15±1.60^{b}	69.63±1.90^{a}
IgG (mg/dl)	18.47±0.68^{b}	21.34±0.65^{a}	22.40±0.93^{a}	22.53±0.58^{a}
Glucose (mg/dl)	232.67±15.76^{a}	174.00±9.54^{b}	195.00±13.43^{ab}	100.67±12.19^{c}
Lipase (U/L)	6.40±0.95^{b}	7.81±1.00^{ab}	9.73±0.89^{ab}	11.26±1.40^{a}
Amylase (U/L)	18.18±1.41^{d}	28.33±1.20^{c}	52.33±1.20^{b}	58.67±1.20^{a}
NO (µM/L)	25.37±1.31^{d}	66.87±1.57^{b}	39.04±1.15^{c}	92.37±1.67^{a}

Data are presented as Mean ±SE. Means with different letters in the same row are significantly different ($p < 0.05$).

Table 4. Effect of dietary supplementation of *Amphora coffeaeformis* nanoparticles (Am-NPs) on photomicrograph of the intestinal villi of *O. niloticus* (n=3).

Parameters	Am NPs g/ Kg feed			
	Control (0)	2.5	5	7.5
Length of villi (µm)	163.22±1.05^{d}	293.46±1.00^{c}	372.72±1.53^{b}	587.56±1.17^{a}
Depth of crypt (µm)	74.45±1.00^{a}	68.38±1.42^{b}	57.49±1.04^{c}	46.89±1.30^{d}
Villi height/ crypts ratio	2.19±0.06^{d}	4.29±0.07^{c}	6.48±0.08^{b}	12.55±1.00^{a}
Number of goblet cells	12.00±1.20^{d}	17.00±1.45^{c}	44.00±1.00^{b}	69.00±1.00^{a}

Data are presented as Mean ±SE. Means with different letters in the same row are significantly different ($p < 0.05$).

Table 5. Effect of dietary supplementation of *Amphora coffeaeformis* nanoparticles (Am-NPs) on mortality rate and the relative percentage survival (RPS) of *O. niloticus* at 7 days post challenging by *A. veronii* isolate (n=20).

Item	Am NPs g/ Kg diet			
	Control (0)	2.5	5	7.5
Number of fish/group	20	20	20	20
1st day	8	4	2	2
2nd day	5	2	1	1
3rd day	4	1	1	0
4th day	2	1	0	0
5th day	0	0	0	0
6th day	1	0	0	0
7th day	0	0	0	0
Dead	20	7	4	3
Survival	0	13	16	17
Mortality %	100	35	20	15
RPS %	0	65	80	85

Challenge test

In contrast to treatment groups supplemented with Am NPs, the control group challenged *O. niloticus* with *A. veronii* and displayed detached scales, abdominal distension, dark skin, hemorrhagic patches all over the body, and congestion of all internal organs, especially the kidney, liver, and spleen. Comparing the Am NPs-incorporated group to the control group, which displayed a 100% mortality rate with 0% RPS, the former demonstrated 65–85% protection as presented in Table 5.

Discussion

Due to their abundance in polyunsaturated fats (PUFAs), proteins, vitamins, pigments, and derivatives of polysaccharides, as well as other metabolites that have antibacterial, anti-inflammatory, and immunostimulant properties, microalgae have recently attracted a lot of attention in aquafeeds (Kiran and Ven Mohan, 2021). The purpose of the current study is to assess the impact of adding *Amphora coffeaeformis* nanoparticles (Am NPs) to Nile tilapia diets. The current study evaluated the impact on *O. niloticus* growth performance and feed efficiency metrics of dietary supplementation with *Amphora coffeaeformis* nanoparticles (Am NPs). The growth performance measures (WG, WGR, SGR%, FCR, and LGR %) showed a substantial improvement ($P < 0.05$) in the Am NPs groups when compared to the control. At the end of the study, the Am NPs group that consumed 7.5 g/kg of diet showed the most significant results ($P < 0.05$) at the end of feeding trial. These results are in line with those of Ayoub *et al*. (2019), who showed that *A. coffeaeformis* improved Nile tilapia performance by boosting growth. The fish with the best development and feed utilization were those fed a diet with 10 g *A. coffeaeformis*/kg. *A. coffeaeformis* supplementation raised the protein and fat contents of fish bodies when given 20 or 30 g per kg diet, with no appreciable difference between treatments. For the moisture and ash contents, there were no appreciable variations between the treatments. *A. coffeaeformis* has better nutritional digestibility and contains a variety of nutrients, including vitamins and minerals that support growth and enhance growt (Valente *et al*., 2006)

Oxidative stress research can benefit from the use of antioxidant biomarkers as CAT, T-SOD, and GPx (Storey, 1996). At the end of the feeding trial, the diet containing 7.5 g/kg Am NPs significantly increased ($P < 0.05$) the levels of reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) when compared to the control and other treated groups. However, the diet containing 7.5 g/kg Am NPs significantly decreased ($P < 0.05$) the levels of MDA and liver enzyme (ALT and AST) when compared to the control and other treated groups. These results corroborate the theory proposed by Mekkawy *et al*. (2020) that *A. coffeaeformis* extracts might restore normal levels of antioxidant enzymes. *A. coffeaeformis* is recognized as a natural antioxidant because to its antioxidative properties, which can initiate a specific set of physiological and biochemical reactions that mitigate the negative impacts of environmental pollutants. *Amphora* algae's inherent antioxidant qualities may be the cause of the enhancement in the liver's antioxidant capacity (El-Sayed *et al*., 2018).

Bacterial infections are fought off by fish using a range of non-specific and specific humoral- and cell-mediated defense mechanisms, while bacterial pathogens have to get past these defenses in order to infect the host and spread throughout its tissues. The fish immune system frequently has to work together to respond to infections (Ellis, 1999). Dietary incorporation with 7.5 g/kg diet Am NPs showed the most ($P < 0.05$) marked increase in protein profile (total protein, albumin and globulin), immunological parameters (IgM, IgG and NO) and digestive enzymes (lipase and amylase) in comparison to the control and other treated groups at the end of feeding trial while glucose level showed significant decrease in dietary incorporation with 7.5 g/kg diet Am NPs in comparison to the control and other treated groups. Importantly for our research, Alexander *et al*. (2010) and Wang *et al*. (2010) found that fish treated with immunostimulants had higher NO concentrations. There have been suggestions that the bioactive chemicals of *Amphora* algae may be the cause of the enhanced fish immune response observed in this study (Ragab *et al*., 2012; El-Sayed *et al*., 2018).

The mid intestine serves as the fundamental part for the complete digestion and absorption of the digested food. Morphometric measurement of the intestinal villi heights, crypt depth and villi heights crypt depth ratio and number of the goblet cells are significant marker for assessing of intestinal capacity for digestion and absorption as mentioned by Dawood et al (2019). Caspary (1992) was demonstrated that the expansion of the intestinal villi in height in corresponds to an increase in the mucosal surface area and expansion of the absorption area. This changes facilitate the entry of digestive enzymes and other substances into the intestine and improve intake digestion and absorption. In our study; the improved intestinal villi in Nile tilapia could be explained by Am NPs incorporated diet positively increase the intestinal villi heights, crypt depth and hight depth ratio that improve ability of digestion and absorption of the nutrients. The intestine goblet cells have the ability to secrete mucus which is the first line of intestinal defense systems which mucus can block the pathogenic microorganisms receptors and prevent toxines from contacting with epithelial cells so avoid damage of intestinal wall (Pirarat et al., 2015). These results also might be comparable to those of Qiang and Cheng (2021), who discovered that exposure to *Amphora coffeaeformis* caused changes in the structure of seminiferous lobules, a disintegration of the basal membrane, an increase in interstitial tissue, and a loose arrangement of spermatocytes in the testicular tissues. The testis basement membrane's thickness was also found to have decreased.

The fish fed *Amphora*-supplemented diets had a lower cumulative mortality rate seven days after being challenged with *A. veronii* than the control group, which challenged *O. niloticus* with *A. veronii*. The control group *O. niloticus* displayed detached scales, abdominal distension, dark skin, hemorrhagic patches all over the body, and congestion of all internal organs, especially the kidney, liver, and spleen, compared to the treated groups supplemented with Am NPs. Comparing the Am NPs-incorporated group to the control group; this displayed a 100% mortality rate with 0% RPS, the former demonstrated 65–85% protection. These results suggest that *A. coffeaeformis* supplements, when used in place of antibiotics, have potential as a disease-prevention method in Nile tilapia culture. According to Ayoub *et al*. (2019), the optimal concentration of *A. coffeaeformis* in a fish diet is between 10 and 20 g/kg. The bioactive compounds in the treated diet may have contributed to the decreased mortality in the *A. coffeaeformis* group. Certain natural antibacterial polysaccharides can finally stop pathogenic bacteria from growing by boosting macrophage phagocytic activity and pro-inflammatory cytokine gene

production. Furthermore, these compounds stimulate the body's own defense mechanisms (Mohan *et al*., 2019).

Conclusion

The current study's findings showed that, both in normal aquaculture conditions and following an injection of *Aeromonas veronii*, the addition of *Amphora coffeaeformis* nanoparticles at a rate of 7.5g/kg diet as a feed supplement promoted growth performance and increased fish immunity. Through inducing the innate immune system response, *Amphora coffeaeformis* NPs is a promising feed addition that has the potential to substitute antibiotics in the removal of pathogenic bacteria and prevention of disease breakout in Nile tilapia cultivation.

Conflict of interest

The authors declare that they have no competing interests.

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