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Effects of Dietary Silica Nanoparticle on Growth Performance, Protein Digestibility, Hematology, Digestive Morphology, and Muscle Composition of Nile Tilapia, *Oreochromis Niloticus*

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The use of nanotechnology in food production systems is being investigated globally, though there is limited research on its effect on fish nutrition. Therefore, this study aimed to identify the effects of silica nanoparticles (NPs) on the nutrition and physiology of tilapia, *Oreochromis niloticus*. Four isonitrogenous diets (300 g/kg crude protein) with NPs (0, 1, 2, and 3 mg/kg diet) were fed to fish (6.52 ± 0.20 g) in a recirculatory aquaculture system for 56 days. Throughout the study period, the effects of silica NP on survival rate, blood cell count, hemoglobin (Hb) level, condition factor (CF), and final product composition (except lipid content) were insignificant. However, growth performance and feed efficiency increased with an increasing level of silica NP, up to 2 mg/kg, and then decreased. This increase was due to the highest apparent protein digestibility and dry matter digestibility when fish were fed silica NP at 2 mg/kg. However, fish at the early stage showed better performance in all dietary groups than in later. Blood glucose (BG) content and histology of the kidney revealed that fish were stressed when a 3 mg/kg silica NP was used and they adapted through excessive excretion *via* expanded glomeruli. Though no significant effect on villi length was observed, silica NP increased the surface area widening the villi of the gut along with the number of goblet cells in the intestine significantly, when supplemented at a level of 2 mg/kg. The bioaccumulation of silica shows that incorporating silica NP in the fish feed will not compromise human health safety upon consumption. Although silica NP at 1 mg/kg and 3 mg/kg yielded some improvements to growth and final product quality, a 2 mg/kg silica NP generated the best results in all measured parameters.

Keywords: silica nanoparticle, tilapia (*Oreochromis Nilotica*), recirculatory aquaculture system, digestibility, nanotechnology in fish nutrition, bioaccumulation

INTRODUCTION

Increased access to multidisciplinary knowledge and the worldwide availability of low-cost compliances have made the global aquaculture industry one of the fastest-growing and irreplaceable animal protein sectors (Belton and Thilsted, 2014; FAO, 2016; Abd El-Naby et al., 2019). This intensified sector has been proved to be a significant contributor to food security, especially for developing Asian and African countries including Bangladesh (Gui et al., 2018; Chan et al., 2019; Hasan et al., 2021c). Inputs for aquaculture include feed, seed, and water, of which feed accounts for nearly 60–70% of the total production costs (Yuan et al., 2017; Yang et al., 2019; Nguyen et al., 2020a). In aquaculture operations, the quality of feed is a fundamental requirement that must be met (Ahmed, 2007; Singha et al., 2020). Besides the quality, digestibility of nutrients also impacts water quality, disease outbreak (Heal et al., 2021), total yield, and, consequently, the business profitability (Guo et al., 2020; Hassaan et al., 2020a; Kong et al., 2020; Nguyen et al., 2020a). While the sustainable use of feed is a challenge to the aquaculture industry, many strategies have been implemented, including the replacement of fishmeal (Perez-Velazquez et al., 2019; Li et al., 2020), the use of byproducts (Irm et al., 2020), selective breeding (Carlberg et al., 2018), minimizing nutrient waste (de Verdal et al., 2018), and, most recently, the use of feed additives including probiotics (Haque et al., 2021b; Hasan et al., 2021a), and nanoparticles (NPs) (Abd El-Naby et al., 2019; Rathore et al., 2020).

Over the last decade, Tilapia (*Oreochromis niloticus*), the global aquatic chicken, has gained its popularity throughout the world (Abdel-Tawwab et al., 2020) for its compatible characteristics, such as easy to produce seed (Barman and Little, 2011), quick response to artificial feeds (Ahmed et al., 2014; Ogello et al., 2014), a wide range of environmental tolerance (Singha et al., 2020), short crop cycle (Francis and Esa, 2016), nutritive values and larger edible portion with no intermuscular bone (Moesch et al., 2016), and high resistance to physical and biological hazards (Al-Deriny et al., 2020; Chaput et al., 2020; Foysal et al., 2020; Naiel et al., 2020). In the context of the increased feed price because of the increasing cost against the limited protein source, such as fish meal (Nguyen et al., 2020a; Pianesso et al., 2020) and a recession in the farmgate price of tilapia due to supply outstripping the national demand and export barriers for developing countries like Bangladesh (Uddin et al., 2019; Bashar et al., 2021; Haque et al., 2021a; Hasan et al., 2021b), recently dealing with this species in semi-intensive, intensive, and super-intensive commercial culture systems have become a great challenge (Kabir et al., 2019). Moreover, due to the lower nutrient digestion capability of tilapia, nutrients, e.g., protein, end up as metabolic waste, like NH_3 (Crab et al., 2007). These waste metabolites not only increase feed costs by increasing the feed conversion ratio (FCR) but also make the fish more susceptible to pathogens (Kent et al., 2009; Hasan and Haque, 2020; Hasan et al., 2020) through deterioration of water quality. Therefore, finding a way of increasing nutrient digestibility and absorption in *O. niloticus* can be a multifaceted solution for a range of issues existing in aquaculture, which could help to make the industry sustainable.

Due to unique physicochemical properties, NPs are being acknowledged by the food production industries for their medical and nutritional uses (Vidya et al., 2016; Khosravi-Katuli et al., 2017; Kumar et al., 2018; Rodriguez et al., 2018; Bashar et al., 2019; Thangapandiyan and Monika, 2020). NPs can benefit aquaculture production by enhancing the bioactivity of molecules, including micronutrients (Xu et al., 2018; Shah and Mraz, 2020), and enabling tissue-specific applications of disease treatments with no consequences to human health (Jennings et al., 2016). The high specific surface area of NPs facilitates the absorption of micronutrients from the intestine to the bloodstream in terrestrial and aquatic animals and makes them suitable for use as feed additives (Huang et al., 2015; Khosravi-Katuli et al., 2017; Pieszka et al., 2019). Along with other NPs, silica (silicon dioxide) NP can be used for its outstanding optical properties, adsorption capacity, low toxicity, biocompatibility (Bitar et al., 2012), thermal stability, and low production cost (Priyadarsini et al., 2018). In the aquaculture industry, silica NP has been reported to favor fish welfare through easing drug administration and reducing the risk of disease outbreak even in case of huge crowding (Khosravi-Katuli et al., 2017). In addition, silica in nanoform is found to be effective in wastewater treatment (Jarvie et al., 2009), controlling microbial load (Huang et al., 2015), and stimulating diatom growth in aquaculture systems.

Despite the huge potential of silica NP in aquaculture, studies on its use as feed additives for finfish, like tilapia, have not been carried out. Therefore, to make the aquaculture production more sustainable from the nutritional consideration without averting human health safety upon consumption, the current experiment was carried out to investigate the effects of different levels of silica NPs on growth performance, feed utilization, blood physiology, histology, and muscle composition of and bioaccumulation in tilapia.

MATERIALS AND METHODS

Ethical Issues

The design and execution of the experiment were approved by the ethical committee of the Bangladesh Agricultural University Research System (Approval No.: 2021/44/BAU). Fish were fed, handled, sampled, harvested, and sacrificed, maintaining proper care and welfare by all the mentioned authors.

Experimental Site and Culture Design

To conduct the 8-week experiment, a Recirculatory Aquaculture System with 12 fiberglass tanks (0.8 × 0.5 × 0.5 m) arranged in a two-tier system was developed in the Faculty of Fisheries, BAU, Mymensingh, Bangladesh. Continuous aeration and a water depth of 0.4 m were maintained throughout the feeding trial. To avoid the circulation of silica NP leaching from the feed and fecal content of different treatments, the inlet and outlet pipes of every biofilter were fitted with a 1-mm thick ceramic filter (0.01 μm pore size) covered by cheesecloth (double layered) of the 1-μm mesh size. Three tanks were designated as control (T0) and every treatment (T1, T2, and T3) had three replications.

TABLE 1 | The inclusion level of main ingredients and proximate composition of the formulated diets.

Ingredients	Diets			
	Control (T0)	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)
Inclusion level (g/kg)				
Fishmeal[a]	263.400	263.399	263.398	263.397
Soyabean meal[b]	263.400	263.400	263.400	263.400
Mustard oil cake[c]	100.000	100.000	100.000	100.000
Wheat bran[d]	151.350	151.350	151.350	151.350
Rice bran[e]	151.350	151.350	151.350	151.350
Molasses[f]	50.000	50.000	50.000	50.000
Vitamin and mineral premix[g]	20.000	20.000	20.000	20.000
Cr_2O_3	0.500	0.500	0.500	0.500
Silica nanoparticle	0.000	0.001	0.002	0.003
Proximate composition of formulated diets (g/kg)				
Ash	114.3	115.4	113.1	114.6
Moisture	105.1	106.6	107.3	109.7
Crude protein	299.9	299.7	299.6	299.6
Crude lipid	122.4	121.2	119.9	121.1
Crude fiber	113.7	113.3	114.3	111.4
Nitrogen-free extract (NFE)[h]	244.1	243.3	245.3	243.1
Cr2O3	0.5	0.5	0.5	0.5

[a]*Crude protein is 55.28%, the crude lipid is 9.90% (Supplied by ACI Godrej Agrovet Pvt. Ltd., Bangladesh).*
[b]*Crude protein is 36.7%, the crude lipid is 19.53%.*
[c]*Crude protein is 25.74%, the crude lipid is 22.11%.*
[d]*Crude protein is 10.65%, the crude lipid is 7.50%.*
[e]*Crude protein is 10.47%, the crude lipid is 8.10%.*
[f]*Sugarcane is derived and collected from a local market.*
[g]*Composition of premix (per kg): Vitamin A: 50,000 IU; vitamin B1: 12 mg; vitamin B2: 25 mg; pantothenate: 200 mg; vitamin B6: 15 mg; biotin: 12 mg; vitamin B12: 0.04 mg; folic acid: 86 mg; vitamin C: 120 mg; vitamin D: 10,000 IU; vitamin E: 0.4 mg; vitamin K3: 10 mg; inositol: 330 mg; zinc: 4.0 g; iron: 80 gm; manganese: 15.3 mg; copper: 427 mg; calcium: 47 gm; iodine: 2 gm; selenium 42 mg; cobalt 1.3 mg; magnesium: 100 mg; sodium chloride: 20 gm. Supplied by the ACI Godrej Agrovet Pvt. Ltd.*
[h]*Calculated as 100–(crude protein + crude lipid + crude fiber + Ash).*

Silica NP

Highly pure, magnetically activated, and 100% natural silica NP, composed of more than 98% of silicon dioxide (SiO_2), 0.08% Al_2O_3, 0.05% Fe_2O_3, and CaO, 0.5% K_2O, and 0.10% TiO_2, was collected from Ceresco Nutrition Ltd., Canada. The size of the ethanol-extracted silica NP ranges from 100 nm to 400 nm with an average active density of 2.03 ± 0.56 g/cm^3 (3.08×10^{16} NP per gram).

Diet Formulation

Four isonitrogenous (300 g/kg crude protein) (Nguyen et al., 2020b) experimental diets containing different levels of silica NPs were formulated according to the square method of Pearson (Wagner and Stanton, 2012), with locally available fish feed ingredients (**Table 1**). Silica NP was incorporated into diets at levels of 0 mg/Kg (T0), 1 mg/Kg (T1), 2 mg/kg (T2), and 3 mg/Kg (T3). As an innate marker, 0.05% Cr_2O_3 was incorporated into diets for the further determination of the digestibility of tilapia according to Austreng (1978). The proximate compositions of the ingredients and diets were analyzed before and after the formulation of diets (**Table 1**), respectively, according to AOAC (2005) (for details, as shown in section Proximate Composition of Fish Muscle).

All feed ingredients were milled (in powder form) and mixed thoroughly using a mixer machine to ensure homogeneous mixing of ingredients and silica NP. Then, double distilled water was added to make the dough, and pellets were prepared through a pelletizer (0.5 mm diameter). After drying for 4 days, pellets were stored in polythene bags at −20°C until feeding tilapia.

Experimental Fish

Male *O. niloticus* (GIFT strain) fry, weighing 6.52 g (± 0.20 g), from the same breeding stock, were procured from Reliance Hatchery Ltd., Mymensingh, Bangladesh. There was no sign of diseases and/or abnormalities. After collection, the fry was acclimatized for 10 days in the experimental system, following the method adopted by Zhang et al. (2019). During the acclimation period, a constant oxygen supply was maintained and fish were fed with a control diet. After 10 days, fish were randomly assigned to the replication tanks, at a density of 188 per cubic meter (30 fish per 160 L tank). To understand how the age of fish affects silica NP utilization, two sampling stages were considered: the first stage (0–28 days) and the second stage (28–56 days). At the second stage, the number of fish in replications of different treatments reduced and varied between 22 and 24 because of different mortality rates and immolation for histological and

hematological studies after the first stage. To ensure evenness among the treatments, the number of fish was adjusted to 21 (132 per cubic meter) in each tank.

Feeding Trials and Data Collection

After final stocking, all groups of fish were fed with their respective diet, at a level close to the apparent satiation, as defined by Simon et al. (2019) twice daily (09:00 and 15:00). In each sampling trip (14 days), at least 50% of fish from each replication tank was sampled randomly and weighed individually with an analytical balance (Model: AS 310.X2 Plus). Fish were fasted for 6 h before and after sampling. Mortality was observed daily, and dead fish were removed to calculate the survival rate.

Growth and Feed Utilization Parameters

Growth indices were calculated according to the following formulae (Aanyu et al., 2018, 2020; Hassaan et al., 2020a):

1. *Weight gain* $(g) = final\ weight\ (g) - initial\ weight(g)$
2. *Percent weight gain* (%) $=$ (*weight gain* (*g*))/(*initial weight* (*g*)) $\times$ 100
3. *Specific growth rate* (% *per day*) $=$ (*Ln* (*final weight*) $-$ *Ln* (*initial weight*))/(*study period* (*day*)) $\times$ 100
4. *Daily growth coefficient* (% *per day*) $=$ (*final weight* $(g)\hat{}.33 -$ *initial weight*$(g)\hat{}.33$)/(*study period* (*day*)) $\times$ 100
5. *Condition factor* $=$ (*final weight* (*g*))/(*final length* $(cm)\hat{}3$)
6. *Survival rate* (%) $=$ (*final number*)/(*initial number*) $\times$ 100

Feed utilization parameters were calculated from the following formulae (Aanyu et al., 2018, 2020):

1. *Food conversion ratio* $=$ (*dry feed fed* (*kg*))/(*live weight gain* (*kg*))
2. *Protein efficiency ratio* $=$ (*total weight gain* (*g*))/(*protein intake* (*g*))
3. *Dry matter digestibility* (% *DMD*) $=$ 100 $-$ 100 ((% *Cr2O3 in diet*)/(% *Cr2O3 in feces*))
4. *Protein digestibility* (% *APD*) $=$ 100 $-$ 100 (% *Cr2O3 in Diet*)/(% *Cr2O3 in feces*) $\times$ (% *CP in feces*)/(% *CP in diet*))

For the estimation of %APD and %DMD, five fish from each replication tank were sacrificed after 28 days (first stage) and 56 days (second stage) of feeding. In both stages, to avoid nutrient and marker leaching into the water, the fecal content was collected from the marginal gut, close to the anal region, following the method of Austreng (1978). Feces were extracted from each of the five fish sacrificed, weighed, and dried individually (thereby 15 replications for each treatment) in a hot-air oven (106°C). Fifty milligrams of each dried sample was digested with 5 ml of concentrated nitric acid (20 min) and then 3 ml of perchloric acid (until they turned reddish) in a micro-Kjeldahl flask, keeping in an electrothermal heater at 170°C. After cooling, double distilled water was added to make the volume to be exactly 100 ml and transferred to a spectrophotometer cuvette. The Cr_2O_3 contents in diet and feces were measured by the optical density at 440 nm (Fenton and Fenton, 1979) using a spectrophotometer (T60UV, PG Instrument, UK) according to the following formula:

$$Amount\ of\ Cr2O3\ (mg) = (Y - 0.0032)/0.2089$$

where $Y =$ optical density

Cr_2O_3 % was calculated by the following formula:

$$\%\ Cr2O3 = (Amount\ of\ Cr2O3\ (mg))/(Amount\ of\ sample\ (mg)) \times 100$$

The crude protein contents of the feed and feces were estimated according to AOAC (2005) (as shown in section Proximate Composition of Fish Muscle for details).

Blood Physiology

In both stages of the experiment, five fish (later used for the digestibility study) from each replication were anesthetized randomly with MS-222 (15 μg/L) after sampling, and blood samples were collected from the caudal vein to determine the level of hemoglobin (Hb), blood glucose (BG), red blood cells (RBCs), and white blood cells (WBCs). Immediately after blood collection, the Hb level (g/dl) and BG level (mg/dl) were determined by a digital EasyMate® GHB meter (Model ET 232, Bioptic Technology Inc., Taiwan) using Hb and glucose strips, respectively. WBCs and RBCs were counted using a Neubauer hemocytometer (Blaxhall and Daisley, 1973) placed under a light microscope (Olympus IX71) fitted with a Zeiss camera (Axiocam ERc 5s).

Histology

At the end of the feeding trial, five fish from each replication were necropsied to pick out organs of interest: e.g., liver, kidney, and intestine in the Fish Disease Laboratory, Department of Aquaculture, BAU. Intestines were defined and divided into foregut, midgut, and hindgut, according to Giorgini et al. (2018) and only the hindgut was used for the histomorphological study. The organs were fixed in a 10% buffered formalin, and an automatic tissue processor was used for the histological process (Naiel et al., 2020). Gut (5 μm), liver (4 μm), and kidney (4 μm) sections were cut with a microtome machine. Histological slides stained with hematoxylin (CI 75290) and eosin (Y, CI 45380; 0.1% alcoholic solution) and mounted with Canada balsam (C-1795; Sigma-Aldrich) were observed under a light microscope (Olympus IX71) at X100 magnification. The images of the histological slides were prepared with a fixed Zeiss camera and were analyzed with Image J (version 14.0) software.

Proximate Composition of Fish Muscle

At the end of the feeding trial, six fish from each replication were sacrificed to determine the proximate composition of the muscle. Moisture, ash, crude protein, and crude lipid contents were determined according to AOAC (2005). The total nitrogen content was determined using the micro-Kjeldahl analysis (method 945.01) and multiplied by the conversion factor (6.25) to translate it into the total crude protein content. Crude fat, ash, and moisture content were estimated by Soxhlet extraction (method 920.39C), by calcination in a muffle furnace

TABLE 2 | The effects of silica nanoparticle (NP) on growth performance and feed efficiency of experimental tilapia, Oreochromis niloticus, after 28 and 56 days of feeding trial.

Parameters	Days	Control	T1	T2	T3	*p*-value
Growth parameters						
Initial weight (g)	0–28	6.57 ± 0.03	6.57 ± 0.03	6.52 ± 0.33	6.51 ± 0.31	0.976
	28–56	17.96 ± 0.95^{a}	22.73 ± 0.17^{b}	27.30 ± 0.55^{c}	21.33 ± 0.84^{b}	<0.001
Weight gain (g)	0–28	11.39 ± 0.92^{a}	16.16 ± 0.15^{b}	20.78 ± 0.58^{c}	14.82 ± 1.03^{b}	<0.001
	28–56	31.10 ± 1.22^{a}	37.38 ± 2.63^{b}	44.70 ± 1.77^{c}	31.32 ± 2.25^{a}	<0.001
Initial length (cm)	0–28	6.04 ± 0.12	5.98 ± 0.04	6.01 ± 0.05	6.02 ± 0.25	0.954
	28–56	8.14 ± 0.08^{a}	8.76 ± 0.14^{b}	9.40 ± 0.05^{c}	8.57 ± 0.04^{b}	<0.001
Length gain (cm)	0–28	2.10 ± 0.06^{a}	2.78 ± 0.10^{b}	3.38 ± 0.05^{c}	2.55 ± 0.27^{b}	<0.001
	28–56	2.19 ± 0.19^{a}	2.53 ± 0.37^{ab}	2.84 ± 0.10^{b}	2.25 ± 0.10^{a}	0.023
PWG[1]	0–28	173.37 ± 13.17^{a}	245.84 ± 2.00^{b}	319.35 ± 21.99^{c}	228.45 ± 24.27^{b}	<0.001
	28–56	173.31 ± 8.05	164.46 ± 12.05	163.86 ± 9.25	147.24 ± 16.19	0.129
SGR[2]	0–28	3.59 ± 0.18^{a}	4.43 ± 0.02^{b}	5.12 ± 0.19^{c}	4.24 ± 0.27^{b}	<0.001
	28–56	3.59 ± 0.1^{a}	3.47 ± 0.17^{b}	3.46 ± 0.13^{b}	3.23 ± 0.23^{c}	<0.001
DGC[3]	0–28	2.61 ± 0.15^{a}	3.36 ± 0.02^{b}	4.01 ± 0.12^{c}	3.18 ± 0.20^{b}	<0.001
	28–56	3.64 ± 0.09^{ab}	3.79 ± 0.20^{ab}	4.01 ± 0.15^{b}	3.41 ± 0.24^{a}	0.019
CF[4]	0–28	3.33 ± 0.14	3.39 ± 0.16	3.29 ± 0.05	3.39 ± 0.1	0.412
	28–56	4.44 ± 0.14^{c}	4.17 ± 0.09^{b}	3.93 ± 0.09^{a}	4.16 ± 0.05^{b}	<0.001
Survival rate	0–28	92.59 ± 3.70	92.84 ± 3.60	96.30 ± 3.71	92.59 ± 3.70	0.562
	28–56	100	100	100	100	
Feed efficiency parameters						
FCR[5]	0–28	1.47 ± 0.03^{b}	1.42 ± 0.03^{b}	1.27 ± 0.04^{a}	1.43 ± 0.04^{b}	<0.001
	28–56	1.56 ± 0.06^{b}	1.47 ± 0.01^{b}	1.33 ± 0.03^{a}	1.52 ± 0.04^{b}	<0.001
PER[7] (%)	0–28	2.19 ± 0.04^{a}	2.27 ± 0.02^{a}	2.42 ± 0.04^{b}	2.23 ± 0.02^{a}	<0.001
	28–56	2.07 ± 0.02^{a}	2.15 ± 0.03^{b}	2.33 ± 0.03^{c}	2.11 ± 0.03^{ab}	<0.001
APD[8] (%)	0–28	79.21 ± 1.81^{a}	87.44 ± 4.16^{bc}	94.80 ± 1.71^{c}	84.41 ± 2.58^{b}	0.001
	28–56	77.12 ± 1.41^{a}	85.39 ± 2.10^{bc}	92.35 ± 1.71^{c}	82.34 ± 2.50^{b}	<0.001
DMD[9] (%)	0–28	73.56 ± 1.42^{a}	77.94 ± 1.77^{a}	84.86 ± 3.50^{b}	76.37 ± 2.69^{a}	0.003
	28–56	65.50 ± 3.12^{a}	72.09 ± 2.41^{a}	83.41 ± 2.95^{b}	69.86 ± 3.15^{a}	0.001

Results are presented as mean ± SD. Means in the same row with different superscript letters are significant ($p < 0.05$). [a]Percentage weight gain (%); [b]specific growth rate (% per day); [c]daily growth coefficient (% per day); [d]condition factor; [e]feed conversion ratio; [f]feed conversion efficiency; [g]protein efficiency ratio; [h]apparent protein digestibility; [i]dry matter digestibility.

at 550°C for 5 h (method 942.05), and by drying in a hot-air oven at 105°C (method 950.01), respectively. The crude fiber content (only for feed samples) was estimated with Fiber Tech (Tulin equipment, India) following the calcination in a muffle furnace.

Silica Bioaccumulation in Fish Muscle

To quantify the amount of silica persisting in fish muscle, at the end of the feeding trial, six fish from each replication were analyzed in the Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory by inductively coupled plasma-optical emission spectrophotometry (ICP-OES) determination method, as defined by Hauptkorn et al. (2001). Samples were digested with 25% tetramethylammonium hydroxide and distilled water for 30 min at 120°C under an 800-W microwave power. ICP-OES measurements of silicon contents were conducted at a 251.66-nm wavelength. Silica in muscle was determined by multiplying the silicon content with a conversion factor of 2.142 [as silica contains 46.69% (Merry, 2017)]. The bioaccumulation factor of silica, from the feed to muscle, was calculated according to the following formula [adapted from Gobas (2001)]:

$$Bioaccumulation\ factor\ (BAF) = \frac{Concentration\ of\ silica\ in\ muscle\ (mg/kg)}{Concentration\ of\ silica\ in\ feed\ fed\ (mg/kg)}$$

Statistical Analysis

The data were analyzed using SPSS (Version 23.0, IBM, Armonk, NY) and presented as mean ± SD. A one-way ANOVA was used to determine the significance of different levels of silica NPs on the measured responses. To specify the differences among the treatments, a multiple range test of Tukey, at a 5% significance level, was performed when a significant influence

TABLE 3 | Hematological parameters of tilapia, O. niloticus, fed with control and experimental diets after 28 or 56 days of feeding trial.

Parameters	Days	Control	T1	T2	T3	*p*-value
Hb[1]	0–28	4.10 ± 0.26	4.27 ± 0.15	4.17 ± 0.31	4.23 ± 0.40	0.902
	28–56	3.87 ± 0.25	4.17 ± 0.32	3.97 ± 0.15	3.93 ± 0.29	0.562
BG[2]	0–28	68.40 ± 3.05[a]	73.60 ± 8.84[a]	76.63 ± 6.65[a]	114.80 ± 14.48[b]	0.001
	28–56	65.23 ± 7.11[a]	72.47 ± 7.49[a]	73.57 ± 9.71[a]	96.93 ± 7.18[b]	0.006
RBC[3] ($\times 10^6$)	0–28	1.97 ± 0.02	1.99 ± 0.04	1.95 ± 0.06	2.00 ± 0.05	0.555
	28–56	1.99 ± 0.03	2.03 ± 0.05	2.01 ± 0.07	2.00 ± 0.01	0.755
WBC[4] ($\times 10^4$)	0–28	8.26 ± 0.32	8.30 ± 0.43	8.52 ± 0.54	7.80 ± 0.63	0.395
	28–56	8.13 ± 0.23	7.99 ± 0.64	8.00 ± 0.32	7.62 ± 0.85	0.732

Results are presented as mean ± SD. Means in the same row indicated by different superscript letters are significant (P < 0.05). [1]Haemoglobin (g/dl); [2]blood glucose (mg/dl); [3]red blood cell count; [4]white blood cell count.

TABLE 4 | Histomorphological data of hindgut collected from tilapia, *O. niloticus*, after 56 days of feeding trial with silica NPs.

Observed factors	Control	T1	T2	T3	*p*-value
Villi width (μm)	45.8 ± 7.01[a]	55 ± 8.09[a]	77.8 ± 8.64[b]	50.8 ± 6.5[a]	<0.001
Villi length (μm)	169.6±16.5	161.6 ± 5.73	167 ± 13.69	163 ± 9.35	0.711
Villi surface area (μm^2)	7793.6 ± 1517.001[a]	8874.8 ± 1245.09[a]	12960 ± 1504.21[b]	8283.2 ± 1193.05[a]	< 0.001
Goblet cells (/10,000 μm^2)	22.8 ± 7.79[a]	28.8 ± 8.17[ab]	44.2 ± 8.41[b]	26.6 ± 10.5[a]	0.007

Results are presented as mean ± SD. Means in the same row indicated by different superscript letters are significantly different (p < 0.05).

of silica NP was observed. A $p < 0.05$ was considered to be statistically significant.

RESULTS

Growth Performance and Feed Efficiency

The growth performance of fish fed with experimental and control diets is presented in **Table 2**. The results show that silica had a significant effect ($P < 0.05$) on all growth parameters measured, except percent weight gain (PWG) in the second stage and CF in the first stage. T2 had the most significant effect ($p < 0.001$) on weight gain (WG), length gain (LG), and daily growth coefficient (DGC) in both stages, while T2 had the most significant effect on PWG and specific growth rate (SGR) in only the first stage. The influence of the silica NP supplementation at 3 mg/kg on WG, SGR, PWG, and DGC was highly significant ($P < 0.001$) for the first 28 days, but no significant influence was found after 56 days. The survival rate throughout the feeding trial, in all the treatments, was above 90% with no significant differences between treatments ($P > 0.05$).

The lowest FCR was found in fish from T2 ($P < 0.001$) in both stages (**Table 2**). PER in T2 was the highest in both stages ($P < 0.001$), while in T3, a significant effect on PER was observed only in the second stage. Though the effects of silica NP on DMD on both sampling days were not significant, except T2 ($P = 0.003, 0.001$), a significant difference was found between all the treatments for APD ($P < 0.001$), with T2 causing the most significant increase in both stages.

Blood Physiology

Silica NP, at all treatment levels, had no significant effect on Hb, RBC, and WBC on either sampling date. Silica NP supplementation at 3 mg/kg (T3) showed a significant effect on the BG level of tilapia in both sampling stages ($P = 0.001$ and 0.006, respectively) (**Table 3**). The BG level was found to increase with the increasing dietary concentration of silica NP.

Histomorphology of Midgut, Liver, and Kidney

Data generated from the histological observations of the hindgut revealed that the villi width and villi surface area increased significantly ($P < 0.001$) by silica NP at 2 mg/kg (**Table 4**). Dietary silica NP had no significant effect on villi length ($P > 0.05$); however, the longest villi were observed in the tilapia from control. Silica NP at 2 mg/kg significantly increased the number of goblet cells ($P = 0.007$), ∼2-folds compared to the control (**Table 4**).

The normal kidney structure was observed in tilapia fed with silica NP at 0 mg/kg (control) and 1 mg/kg (T1), while slightly widened and extremely widened glomeruli were found in tilapia fed with silica NP at 2 mg/kg (T2) and 3 mg/kg (T3), respectively (**Figures 1A–D**). No structural aberration was found in liver histology in all treatments. However, with the increase of dietary silica NP, a substantial amount of fat deposition was observed in liver sections (**Figures 1F–H**), with the highest amount found in T3.

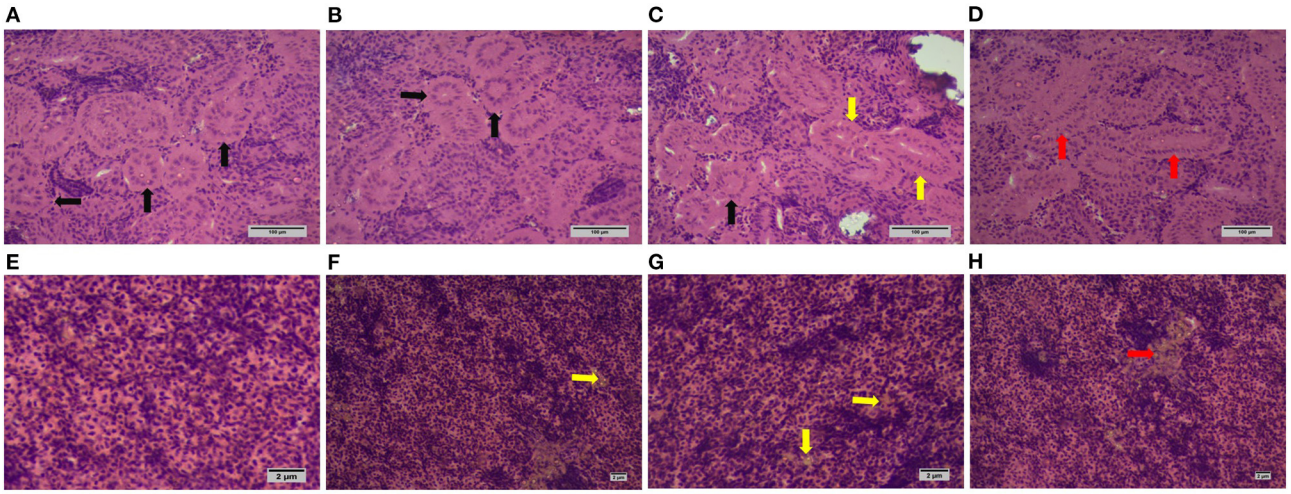

FIGURE 1 | Histoarchitecture of kidney and liver of experimental *Oreochromis niloticus* (H & E; X100 magnification; scale bar: 100 μm for kidney and 1 μm for liver samples). The normal glomerular structure is indicated by black arrows observed in control **(A)** and T1 **(B)**; both normal (black arrow) and slight widened (yellow arrow) structures are found in T2 **(C)**; extremely widened glomerulus is indicated by red arrows observed in T3 **(D)**. **(E)** indicates normal liver histology from control fish with no fat deposition; liver sections from T1 **(F)** and T2 **(G)** showed a slight fat deposition (yellow arrows), while T3 **(H)** experienced a higher deposition (red arrow).

TABLE 5 | Final muscle composition of experimental tilapia in the recirculatory aquaculture system (RAS) and bioaccumulation of silica NPs.

Parameters	Control	T1	T2	T3	*p*-value
Muscle composition (g/kg)					
Moisture	751.1 ± 28.4	725.4 ± 34.8	730.9 ± 27.7	722.1 ± 20.8	0.617
Ash	15.6 ± 1.0	17.3 ± 0.4	16.5 ± 1.3	15.7 ± 1.3	0.228
Crude protein	155.5 ± 14.4	158.1 ± 3.5	148.0 ± 12.5	147.1 ± 11.3	0.573
Crude lipid	25.6 ± 1.4^{a}	28.6 ± 0.9^{b}	28.7 ± 1.0^{b}	28.9 ± 2.2^{b}	0.008
Silica content (mg/kg)					
Silica in muscle	0^{a}	12.16 ± 1.84^{b}	29.27 ± 4.22^{c}	43.76 ± 5.70^{d}	< 0.001
BAF[1]	0^{a}	0.12 ± 0.02^{b}	0.12 ± 0.02^{b}	0.15 ± 0.02^{b}	< 0.001

Results are presented as mean ± SD. Means in the same row indicated by different superscript letters are significant ($P < 0.05$). [a]Bioaccumulation factor.

Proximate Composition of Tilapia Muscle and Silica NP Bioaccumulation

Moisture, ash, and crude protein contents of experimental tilapia at the final harvest showed no significant differences between the dietary groups (**Table 5**). However, the crude lipid content increased with increasing levels of silica NP supplementation. The effect of silica NP on the crude lipid content was significant, irrespective of the incorporation rate ($P < 0.05$), and the highest content was observed in T3 (**Table 5**). The highest silica levels and bioaccumulation of silica NP were found in T3, where the experimental diet was supplemented with 3 mg/kg silica NP.

DISCUSSION

To the best of the knowledge, this is the first study to investigate the effects of silica NP on feed utilization, growth performance, blood physiology, and digestive morphology of *O. niloticus*. The notable outcomes have been achieved in terms of growth performance and feed utilization without compromising human health safety.

Control fish reared with a low proteinous basal diet at a higher stocking density (almost 6-folds than the traditional pond farming system practiced in Bangladesh) showed better growth performance compared to other culture systems like pond-based intensive and semi-intensive cultures (Kabir et al., 2019; Dawood et al., 2020f), raceway culture, tank and aquaria cultures (El-Naby et al., 2019; Abd El-Naby et al., 2020), and even biofloc culture (da Silva et al., 2018; Martins et al., 2019). Controlled environmental conditions and nitrogenous waste maintenance in RAS might ensure optimum welfare for fish and ensure larger spillover advantages of surpassed yield. The growth performances favored from silica NP experienced in this study are far superior to the available findings in the growing literature (Aanyu et al., 2020; Amer et al., 2020; Dawood et al., 2020a,f; Jiang et al., 2020; Wardani et al., 2020) on tilapia, irrespective of culture systems and diets. The supplementation of NPs like nanochitosan (Abd El-Naby et al., 2019, 2020), nanoselenium (Dawood et al.,

2020f; Rathore et al., 2020), nanoselenium with Vit-C (Dawood et al., 2020e), and nanozeolite (Hassaan et al., 2020b) in tilapia have resulted in poorer growth performances than the findings presented in this study. The improved growth performance as seen in this study occurred because silica in nanoform may function as the nutrient carrier (especially for amino acids). These hauled nutrients could have further contributed to increasing the digestion and absorption of nutrient molecules through the controlled encapsulation and release of nutrients from the gastrointestinal tract to the bloodstream (Bahabadi et al., 2017). This increase could also be correlated with the amelioration of DNA and RNA syntheses and an improvement in gut microorganisms, which are supported by NPs (Onuegbu et al., 2018); however, this warrants a further investigation with silica NPs. The survival rate corresponds to those shown in tilapia fed with chitosan NP (Abd El-Naby et al., 2019) and nanoselenium (Dawood et al., 2020a) for the first 28 days. In the later stage, no mortality in any of the treatments, including the control, is in agreement with Abd El-Naby et al. (2019) and Rathore et al. (2020).

The FCR found in the present study is far lower than the results obtained from feeding tilapia with nanoselenium and vitamin E (Dawood et al., 2020e), nanoselenium (Abd El-Kader et al., 2020; Rathore et al., 2020), and nanozeolite (Hassaan et al., 2020b). Similar results were obtained in tilapia fed with chitosan NP supplemented with vitamin C (Naiel et al., 2020) and with only chitosan NP (Abd El-Naby et al., 2019). In the case of T1 and T3, although the growth parameters are significantly different, the FCR was not significant compared to the control. This indicates a high level of feed intake by fish in the T1 and T3 treatments but poorer growth outcomes following the ingestion. The highest PER in this study is similar to those seen in chitosan NP-treated tilapia (Abd El-Naby et al., 2019, 2020); however, it is greater than the findings of PER documented by Rathore et al. (2020) from nanoselenium in tilapia. Fish fed with silica NP demonstrated improved growth performance in tilapia, compared to the control of the current experiment and to other previously mentioned studies. This supports the recommendation of silica NP as one of the best feed additives for *O. niloticus*.

Digestibility data also demonstrate the effect of silica NP on improving growth performance. Digestive enzymes hydrolyze proteins, carbohydrates, and lipids into smaller parts for absorption through the microvilli of the fish intestine (García-Meilán et al., 2016). Tilapia, an omnivorous fish, comparatively possesses an inactive stomach for protein digestion. Digestion

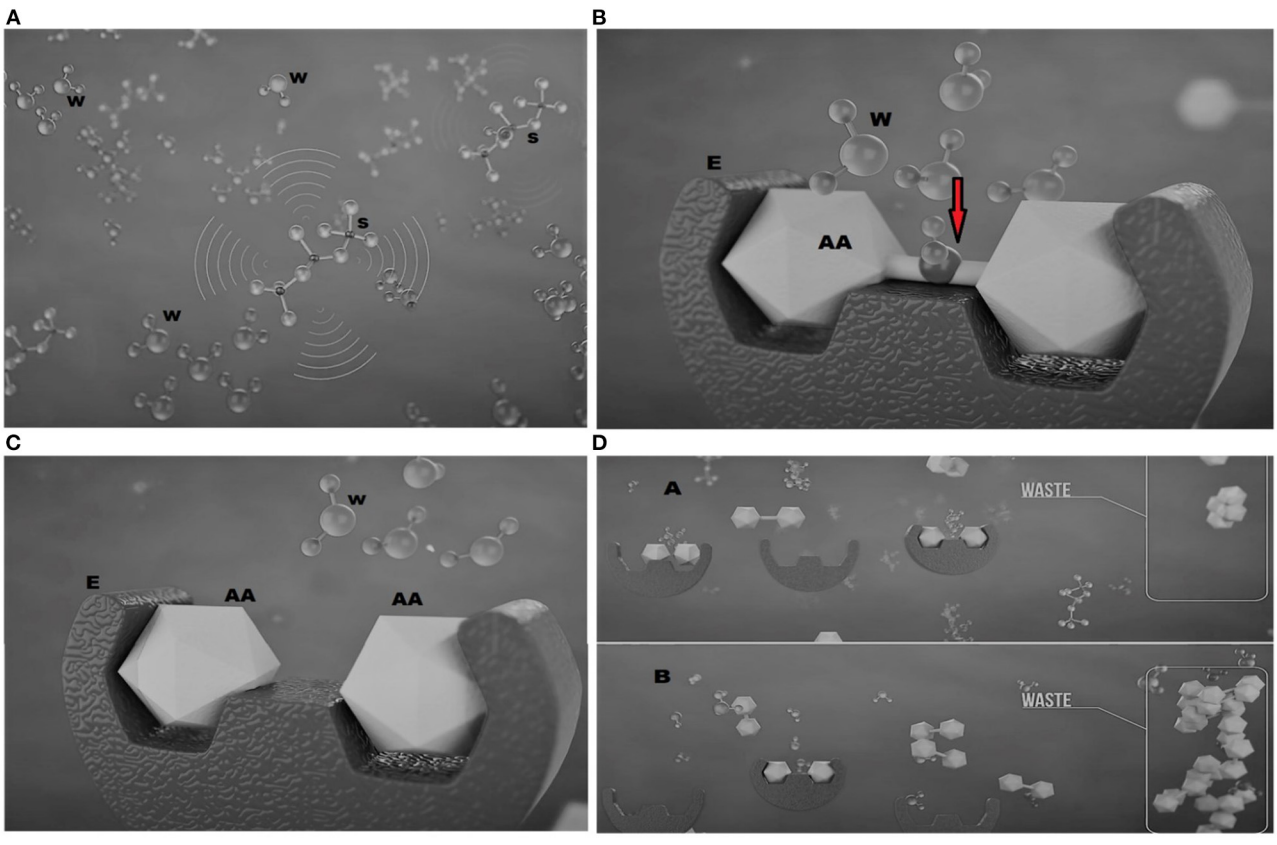

FIGURE 2 | Mechanisms of silica nanoparticles (NPs) responsible for the increased digestibility in tilapia fish (source: Ceresco Nutrition Ltd.) showing **(A)** silica NP(S) activating water molecules (W) inside the gut of fish; **(B)** activated water molecules (W) splitting (red arrow) the amino acid (AA) when it binds to a specific enzyme (E); **(C)** cleaved amino acids (AAs) suitable for absorption through the gut; and **(D)** the reduction in waste in the presence of silica NP **(A)** compared to that without **(B)**.

of protein eminently takes place in the gut just after the stomach, with the action of hydrochloric acid, and due to size limitations, only smaller peptides and amino acids are permitted to access through the gut wall to be absorbed and become available for growth (Wu et al., 2009). The water inside the gut remains inactive and, in general, has no direct role, except facilitating gut microbiota and maintaining homeostasis (Laforenza, 2012; Giatsis et al., 2015); however, in the presence of silica NP, water molecules within the gut may become activated (**Figure 2A**) with the influence of a higher infrared emissivity of silica NP (Faisal et al., 2021). A similar indication with Tourmaline (Borosilicate minerals) NP concluded that radiation emissivity from NP can modulate the structure of water clusters into smaller molecules through breaking hydrogen bonds (Sun et al., 2010). Furthermore, the magnetic treatment of silica NP may have a role in the activation of water molecules through creating magnetic resonance. These activated molecules are reported to facilitate a multitude of reactions, including protein metabolism and immune response (Sun et al., 2010). In the digestion process, ionized water molecules as nucleophiles act on the peptide carbonyl group of the ingested protein (Berg et al., 2002), resulting in smaller peptides and free amino acids, suitable for absorption through the gut epithelium into the blood (**Figures 2B,C**). Enzymatic hydrolysis of protein is largely influenced by the availability of water required for hydration (Butré et al., 2014). Furthermore, the presence of catalytic water is assumed to pledge the enzyme-substrate inhibition through cleaving the peptide bonds of the substrate tying to outside the active sites (Butré et al., 2014). Activated water molecules may also regenerate the enzymes, enabling them to work again within the shortest possible time, as clued by Berg et al. (2002). These evidences clearly disclose that silica NP might accelerate the digestibility of feed and, hence, the growth performance of fish, while the subsequent decrease in nutrient loss results in lower FCR (**Figure 2D**).

Blood physiology data of the experimental fish explain why the growth performance of experimental *O. niloticus* decreased while we incorporated silica at a level of 3 mg/Kg diet. BG is an indicator of stress syndrome, with increased levels providing a biomarker of stress levels in fish (Dawood et al., 2020b,c,d). The BG level of fish fed with 3 mg/kg silica was significantly high, indicating physiological stress due to the high level of silica and rendering that they are not able to use the nutrients properly to acquire growth. Generally, in this state of stress, fish continuously try to respond physiologically, be resistant to the stress, and/or restore homeostasis. These stress responses are energetically costly (Rodnick and Planas, 2016; Schreck and Tort, 2016): consequently, energy might be diverted to maintenance, rather than being available for growth. This explains the lower growth performance in T3, even though the digestibility of protein was still significantly greater than the control. As a defensive agent, WBCs circulate in the bloodstream searching for foreign particles and proliferating when an exogenous particle is identified. There were no significant changes in the Hb level and WBC count throughout the study, indicating that silica NP was not present in the blood and, therefore, no residual effect.

A larger surface area of intestinal villi facilitates nutrient absorption in fish, providing a greater surface area for enzymes for reactions to occur (Dawood et al., 2020a). Though silica NP did not enlarge the villi significantly, it did widen the villi, therefore significantly increasing the surface area. The enhanced surface area enabled the increased absorption of nutrients. Furthermore, the mucus-producing goblet cells have numerous roles in the digestive system, including prevention of gut wall damage and antibacterial action (Pirarat et al., 2015) and maintaining intestinal homeostasis (Junqueira and Carneiro, 2013). Increasing numbers of goblet cells with silica NP treatment may confirm the advantages of silica NP to intestinal health and the gut microbiota of fish that promotes the activities of mucus-secreting goblet cells. The kidney of fish plays an important role in RBC production, osmoregulation, and excretion of waste metabolites. The widened glomerular structure in freshwater fish indicates a high filtration rate for maintaining osmoregulation and excreting detrimental and ionic substances (Oguz, 2015). This reveals the adaptation measures of experimental tilapia against the stress when challenged with silica NP at 3 mg/kg. This adaptation might be accomplished through the excretion of silica NP *via* the enlarged kidney tubules, and this broadening of glomeruli was associated with the increasing level of silica NP in feed. This is also in line with the expansion of glomeruli observed by Hussain et al. (2019) in freshwater fishes coping against different environmental pollutants. Similar adaptation measures against arsenic in *Channa punctata* (Roy and Bhattacharya, 2006) also corroborate the reasoning for enlarged kidney tubules. However, the normal kidney structure in the control and T1 and the nearly normal and slight widening in T2 indicate that there was no such extreme pressure on the fish excretory system below 2 mg/kg dietary silica NP.

Lipids in fish play a crucial role as a source of energy and for the provision of essential fatty acids, necessary for fish growth and development (Kim et al., 2012). However, fish prefer to consume energy from protein, more specifically from the amino acids (Walton and Cowey, 1982; Wu et al., 2020), while lipids are known to be stored in the liver and muscle by fish and to spare the role of proteins (Kim et al., 2012; Zhang et al., 2019) when they are sourced inadequately or fish need to adapt physiologically through energetic cost (McCue, 2013). Silica NP undoubtedly increased digestibility and absorption of nutrients including proteins and lipids. However, due to proper feeding regimes, very little sparing might be required by lipids throughout the study period, and the higher growth performance could have resulted mainly from the absorption of proteins (amino acids). This allowed a greater portion of lipids to remain unused and deposited in the muscle.

Though crystalline silica is considered as a class-1 carcinogenic, amorphous silica in nanoform was non-carcinogenic in rats and mice up to 2,500 mg/kg and 7,500 mg/kg body weights, respectively (Younes et al., 2018). When it is available in food, drugs, and beverages, more than 50% silica in the form of silicon is filtered by the kidney in humans (Kelsay et al., 1979) and the remaining residue disperses through the skin, aorta, bone, and other parts of the body (Carlisle, 1981).

Moreover, if it exists in the blood, as silicic acid, it has no adverse effect on human physiology as it does not bind to proteins (De Araújo et al., 2016). In fact, in favorable concentrations, silica plays an important biological role in bone, brain, nerve, skin, and memory health. It also benefits patients with diabetes by stimulating insulin secretion from the pancreas (Jugdaohsingh, 2007). However, the US Food and Drug Administration has defined 2% by the weight of food as the maximum limit for human consumption, when silica is used as additives (FDA, 2019). The results presented in this study show that only a low level of silica NP accumulated in fish muscle, and therefore, the BAF data show that incorporating silica NP into fish diets will not adversely impact human health upon consumption of the fish.

CONCLUSION

These results have verified the clear advantages of silica NP on growth performance, feed utilization, and the final product quality in the experimental fish. Bioaccumulation study strongly approved a much higher amount of silica NP to be incorporated into feed without averting human health safety; however, growth performance and hematological and histological findings apprehend the limit to 2 mg/Kg for obtaining the highest possible payback from tilapia. On the other hand, the question of maintaining proper dose during manufacturing and feeding is apposite because of the narrow effective range as suggested in this study. The industrial bulk production of feed using appropriate binders to minimize the leaching of silica NP, paradoxically, could knock down the contradiction of accommodating the required dose. Conclusively, silica NP as an input of nanotechnology can be applied as novel feed additives to improve the rate of digestion, as well as improve absorption in the production of *O. niloticus* without making human health safety questioned. Much progress has been made through this experiment, and hopefully, it will make a big sense to widen the gateway of future investigation. It still possesses some limitations on which basis, this publication warrants furthermore studies delving the insights from molecular and physiological prospects.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the first author (AB), without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Bangladesh Agricultural University Research System.

AUTHOR CONTRIBUTIONS

AB: conceptualization, methodology, investigation, writing—original draft, visualization, and fund acquisition. NH: formal analysis, data curation, writing—original draft, and visualization. MHa: conceptualization, validation, writing—review, and editing. MR: methodology, investigation, and visualization. MHo: methodology, validation, resources, and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.706179/full#supplementary-material

Figure 1 | Kidney of control fish (T0).

Figure 2 | Kidney of fish treated with 1 mg/kg SiNP (T1).

Figure 3 | Kidney of fish treated with 2 mg/kg SiNP (T2).

Figure 4 | Kidney of fish treated with 3 mg/kg SiNP (T4).

Figure 5 | Liver of control fish (T0).

Figure 6 | Liver of fish treated with 1 mg/kg SiNP (T1).

Figure 7 | Liver of fish treated with 2 mg/kg SiNP (T2).

Figure 8 | Liver of fish treated with 3 mg/kg SiNP (T3).

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