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Chitosan Nanoparticles as Nutritional Stimulus in the Early Feeding of the Nile Tilapia Fry (*Oreochromis niloticus*)

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ABSTRACT
Chitosan nanoparticles have received a lot of attention in recent years due to their beneficial properties as healthy supplement to supply bioactive substances in the food industry and nutrition. The purpose of this study was to investigate the influence of chitosan nanoparticles (CsNPs) as nutritional stimulus at the early feeding for the Nile tilapia (*Oreochromis niloticus*) fry on the growth performance, feed efficiency, and histomorphological health of the liver and intestine. Graded levels of CsNPs (0.05, 0.1, 0.2, 0.4, 0.8, and 1.6g kg^{-1} diet) were added to the basal diet, and each experimental diet was fed to three groups of fish with an approximate weight (0.86± 0.04g) for 70 days. The experimental study concluded that CsNPs 0.1 and 0.2g kg^{-1} diets resulted in significantly higher final weight (FW), weight gain (WG), specific growth rate (SGR), energy efficiency ratio (EER), and survival (S%) compared to the control group (P <0.05). However, the protein efficiency ratio (PER) increased but did not differ substantially (P> 0.05) between the control and other treatments. The intestine of the Nile tilapia fry showed that the villi height increased in the diet-fed groups of 0.1, 0.2, and 0.4g CsNPs kg^{-1} diet, while the highest villi length was recorded in the group of 0.2g CsNPs kg^{-1} diet. The group fed on 0.2g CsNPs kg^{-1} diet demonstrated normal healthy intestinal walls and branched villi with intact enterocytes comprising the intestinal villi. Histopathological structure of the Nile tilapia liver in fry fed with CsNPs revealed that the control group had congested blood sinusoids and fatty vacuolization of hepatocytes, whereas the 0.2g CsNPs kg^{-1} diet group displayed normal hepatocytes with slight lymphocyte infiltration. Findings of the current study illustrated the positive effect of dietary supplementation of 0.2g kg^{-1} of chitosan nanoparticles which is considered as a promising compound in improving the growth performance, feed efficiencies, and an increase in villi length to facilitate the absorption process in the intestinal Nile tilapia (*Oreochromis niloticus*) fry.

INTRODUCTION

Throughout the past few decades, the emergence of new scientific challenges to improve fish nutrition and the rapid development of aquaculture production has been observed. Moreover, fish nutrition is vital for the development of a sustainable aquaculture **(FAO, 2018)**. The diet formulations used for farmed fish have been largely modified in the past few years. However, bottlenecks still exist in being able to suppress

totally marine resources in diets without negatively affecting growth performance and flesh quality. In this context, understanding the biological mechanisms that regulate intermediary nutritional metabolism is crucial. This understanding can lead to the development of more efficient fish capable of utilizing new diets, ultimately improving fish nutrition in the future. By focusing on the nutritional programming of metabolism linked to the early life stages of fish, we can also contribute to the development of sustainable aquaculture practices **(Panserat *et al*., 2019; Aboseif *et al*., 2022a, 2022b; Aboseif & Goda, 2022)**. Furthermore, the nutritional programming could be employed as a strategy in aquaculture to promote sustainable feeding strategies **(Hou & Fuiman, 2020)**. Additionally, nutritional programming is based on the fact that early feeding may have a long-term impact on metabolic processes in later life, and this critical window overlaps with high mortality during the early life stages **(Lucas, 1998; Fernandez-Twinn & Ozanne, 2010)**.

The ongoing development of this industry is a key factor in the strategy to guarantee the global nutritional safety. Nowadays, different types of nanotechnology-based systems have been employed to increase its production, efficiency and sustainability. Therefore, nanotechnology has a significant role to play in the improvement of the efficiency and the environmental impact of this industry **(Fajardo *et al*., 2022)**. **Rathore and Mahesh (2021)** corroborated that nanotechnology is an emerging technology influencing the material division at a nanoscale of 1- 100 nm and can change properties of materials and increase its potential to be utilized due to its increasing surface area that allows using nanoparticles in various applications. Nanotechnologies have wide range applications in the fishery industry, such as water treatment, sterilization, nano-feed for feeding the fish, and the control of aquatic diseases **(Fath El-Bab *et al*., 2020)**.

Moreover, **Can *et al.* (2011)** showed that nanoparticles of some elements like selenium and iron supplemented in diet could enhance the growth of fish and crustacean, as well as it can be practically used to create fish ponds that are safe from disease and pollution. Nanoparticles are seen to be effective for: (i) Growth promotion (e.g., nFe, nSe, and ZnO), (ii) Delivery of micronutrients (e.g., chitosan NPs), and (iii) Production of feed amount per unit time (e.g., fullerenes (C60), and nTiO2), during aquafeed development **(George *et al*., 2023)**. Chitosan is a cationic polymer obtained from the deacetylation of chitin, a naturally occurring and the second abundant polysaccharide after cellulose **(Jolles & Muzzarelli, 1999)**. Chitosan has excellent features due to its non-toxicity, biodegradability, biocompatibility, and bio-adhesion **(Muzzarelli, 2010)**. **Aranaz *et al*. (2009)** found that encapsulated active compounds, such as chitosan, are shielded from harsh conditions in the gastrointestinal tract, thereby enhancing their absorption. Chitosan nanoparticles (CsNPs) are biodegradable, biocompatible, and non-toxic materials which can be used in drug delivery, gene delivery and therapeutics, in agriculture as

biopesticides, and they have been proved to act as effective feed additives for fish **(Felt, *et al.*, 1998; Augustine *et al.*, 2019)**. **Alishahi *et al.* (2011)** demonstrated that CsNPs boost the bioavailability of essential compounds that enable efficient uptake of the body cells, moreover they deeply penetrate into the targeted sites due to their small size, thereby increasing the available surface area to interact with biological support. Dietary chitosan-incorporated diets help improve the growth performance and reduce the mortality of fish **(Zaki *et al.*, 2015; Akbary & Younesi, 2017)**. The application of dietary chitosan with formulated feeds also enhances protein and decreases lipid and moisture contents in fish **(Yıldız, 2017; Thilagar & Samuthirapandian, 2020)**. Dietary chitosan-treated formulated feed also affects lipid metabolism in fish **(Yan *et al.*, 2017)**.

Interestingly, dietary chitosan-incorporated diets also affect the intestinal histology **(Najafabad *et al.*, 2016)** and blood parameters, gut enzyme activity, and innate immunity **(Zhang, 2019)** of different fish at different dosages. **El-Naggar *et al.* (2022)** postulated that CS and CSNP act as safe feed additives, drug carriers, and are widely used in water treatment as well. **Niu *et al.* (2011)** reported that chitosan is an active growth promoter and an essential element for aquatic species' growth. Moreover, **Qi *et al.* (2004)** conveyed that chitosan nanoparticles significantly affect antimicrobial activities compared to chitosan particles of big size. The iInfluence of chitosan nanoparticles (CsNPs) supplementation on the growth, whole body composition, intestinal bacterial count and histomorphology, digestive enzymes, hematology, immune response, and liver status of the Nile tilapia (*O. niloticus*) was investigated in the study of **Abd El-Naby *et al.* (2019)**. The results exhibited an improved the growth performance and an increase in the crude lipid levels of the whole body in fish fed on 3 or 5g ChNP kg^{-1} diet. Further, the activities of enzymes amylase and lipase were notably enhanced in fish that were fed on the diets fortified with 5g kg^{-1} CsNPs. In *Oncorhyncus mykiss*, CS supplementation at a concentration of 2.5g kg^{-1} diet could significantly improve the survival rate of *O. mykiss* against stress conditions **(Abdel-Ghany & Salem, 2020)**. **Ranjan *et al.* (2014)** elucidated that the group fed a diet containing 10g of CS kg^{-1} exhibited the highest hematological and innate immune parameters on day 45 of the experiment. **Meshkini *et al.* (2012)** detected that 10 and 15g CS kg^{-1} diet supplemented groups recorded the highest innate immune response. Considering the effect of chitosan on both growth and survival of postlarval *Litopenaeus vannamei*, **Niu *et al.* (2011)** indicated, by second-degree polynomial regression of SGR and survival, that the optimum supplement of dietary chitosan should be between 2.13 and 2.67g kg^{-1}.

Tilapia fish are prolific breeders and constitute an economically important fish species supplying higher-quality protein. Moreover, they may play a vital role with respect to the possibility for sustainability in the nutrition and aquaculture sectors **(Arumugam *et al.*, 2023)**. **Ng *et al.* (2013)** stated that tilapia fish are one of the most important species of fish in aquaculture, which is capable of filling the gap of the increasing worldwide demand for protein sources. Production is increasing due to

tilapia's large size, fast growth, prolific breeding characteristics, palatability, and relatively low cost for production **(Prabu *et al*., 2019)**. Although tilapia is a freshwater species, it can tolerate osmotic and alkalinity stresses up to a particular range **(Suresh & Lin, 1992**) as well as low dissolved oxygen concentrations and osmotic and alkalinity stress **(Zhao *et al.*, 2020)**. Considering the advantages of the nutritional programming of metabolism linked to the early life of fish, the current study aimed to assess the beneficial impacts of CsNPs on growth performance, feed efficiency, and histomorphological structure of the liver and gut of the Nile tilapia *(O. niloticus*) fry.

MATERIALS AND METHODS

Experimental set-up

The present practical study was conducted at the Wet Laboratory of Freshwater in El-Qanater El-Khairiya Fish Station-Qalyubia, Egypt. Twenty-one circular fiber glass tanks (a 100-liter water capacity) were set at the Wet Laboratory of Freshwater, following the completely randomized design for seven treatments in triplicate.

Spawning and monosex tilapia culture

Brooders of the Nile tilapia, *O. niloticus*, were spawned at the National Institute of Oceanography and Fisheries (El-Qanater El-Khaireya, Fish Station) according to **Helal *et al.* (2020)**. Subsequently, the newly hatched larvae were collected and acclimated to the exploratory conditions for one week before the feeding trials began. Once the yolk sac had been totally retained, the newly hatched larvae were arbitrarily set in an $8m^3$ cement lake. The Nile tilapia monosex were fed commercial diets containing 35.0% CP and 19.0Kj kg^{-1} gross energy **(Bhujel, 2000)** during a 21-day hatching period.

The hormone, 17α-MT (17α-methyldihydrotestosterone) was dissolved in a solvent (methanol or ethanol) at a rate of 60- 70mg of hormone/ L solvent. The prepared mixture was added to the feed at a rate of 60- 70 mg kg^{-1} feed, hand-mixed thoroughly and allowed to air dry. This mixture was stored for less than 4 days, as indicated by **Chakraborty and Baneerjee (2009)** and **Chakraborty *et al.* (2011)**. They were fed 7% of their body weight seven days a week, with the everyday proportion isolated into five times a day (09:00, 11:00, 15:00, and 17:00h). After 21 days, a random sample (25% of the fish from the cement pond) was weighed to calculate the initial body weight (IBW). The fry (1500 fish) was carefully produced at the Wet Laboratory of Freshwater, National Institute of Oceanography and Fisheries (NIOF). The average weight of these fish was 0.7- 1.0g $fish^{-1}$. They were stocked in a $2m^3$ fiberglass tank with aerators and acclimatized for 15 days with fed control diet (35% crude protein, 19kJ kg^{-1} gross energy) at a rate of 5% of their body weight, receiving the feed twice a day at 9:00 and 13:00h. Observations were made about the fish's appetites, and the health of the fry was checked. There were no diseases found. After acclimatization, 945 uniformly sized fry fish were

randomly distributed among the seven treatments, and stocked in in circular fiber glass tanks (a 100-liter water capacity) at a rate of 45 fish $tank^{-1}$ with aerators. Stocking of the fry were done early in the morning. Initial mean weight (0.86± 0.04 g) of the fry were recorded at the beginning of the experiment. The fish were hand-fed at 5% of the total biomass twice daily at 9:00 and 14:00h for 70 days. Every 15 days, feed adjustments were made for each tank.

Experimental design

A completely randomized design (CRD) was implemented using seven treatments with three replicates. The diets were processed by blending the dry ingredients in a homogenous mixture. A laboratory pellet mill was used to produce 2mm pellets, and then was mixed with the chitosan nanoparticles (CsNPs) as follows: Treatment 1 (C, control diet): had no CsNPs added 0%, Treatment 2 ($CsNPs_{0.05}$): basal diet mixed with 0.05g kg^{-1} diet CsNPs, Treatment 3 ($CsNPs_{0.1}$): basal diet mixed with 0.1g kg^{-1} diet CsNPs, Treatment 4 ($CsNPs_{0.2}$): basal diet mixed with 0.2g kg^{-1} diet CsNPs, Treatment 5 ($CsNPs_{0.4}$): basal diet mixed with 0.4g kg^{-1} diet CsNPs, Treatment 6 ($CsNPs_{0.8}$): basal diet mixed with 0.8g kg^{-1} diet CsNPs, Treatment 7 ($CsNPs_{1.6}$): basal diet mixed with 1.6g kg^{-1} diet CsNPs. The pellets were air dried for 6 hours (approximately 10% moisture content). Before usage, all diets were wrapped in cellophane bags and cooled to 4°C.

Preparation of chitosan nanoparticles (CNPs)

Chitosan powder is derived from chitin, the natural chitinous shell of crustaceans (shrimps and crabs). The purchased particles had molecular weights ranging from 3800 to 20,000 daltons and a de-acetylation degree (DD) of approximately 75%. CsNPs were produced using chitosan's ionic gelation method with tripolyphosphate (TPP) anion. The synthesis was carried out at the Animal Health Research Institute (AHRI) in Dokki, Giza, Egypt, according to the guidekines of **Zhang *et al.* (2012)**. Briefly, chitosan was dissolved in 1% acetic acid and agitated for 1 hour to clarify the solution. Sodium tripolyphosphate (0.5mg/ mL) was dissolved in deionized water. 1.0ml of TPP was added dropwise to 100ml of chitosan solution under magnetic stirring at room temperature. The mixture was stirred for 20 minutes before sonication. The suspension was then centrifuged at 10,000rpm for a further 20 minutes. The resulting supernatants were discarded, and the precipitate was suspended in distilled water and freeze-dried before further use or analysis. The freeze-dried CsNPs were suspended in deionized distilled water for characterization or kept at 4°C for directly used.

Proximate examination of trial diets

Seven isonitrogenous (35% crude protein) and isocaloric (19kJ kg^{-1}, gross energy) **(NRC, 2011)** were formulated (Table 1) using meat meal, soybean meal, corn gluten meal, yellow corn meal, wheat bran, soybean oil, NaCl, Vit. C, vitamins and minerals

mixture, and carboxymethyl cellulose. Using a blender, all of the feed ingredients were ground and mixed with water. CsNPs were prepared by using the ionic gelation method according to **Masarudin *et al.* (2015)**, and were subsequently mixed with ingredient blends containing vitamin-mineral premixes. Following that, the feeds were passed through an aluminum wire sieve to produce pellets (0.8mm in diameter). The pelletized feed was oven dried at 70°C, packed in plastic small plastic bags, and preserved at 4˚C to maintain microbial viability.

The seven experimental diets were analyzed for crude protein, dry matter, ash, crude fiber, and crude lipids according to **AOAC (2016)**. Dry matter and moisture were determined via oven drying at 105°C to a constant weight, and ash by burning in a muffle furnace (Memmert UFB500 and Carbolite furnace Memmert CWF 11/13, Germany) at 600°C. Crude protein was analyzed using the micro-Kjeldhal method at N% 6.25 (on a Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden). Crude lipid was determined using **Bligh and Dyer (1959)** with a mixture of 2:1 chloroform and methanol in Soxhlet apparatus (Gerhardt Soxtherm) for 8h. Fiber contents were determined by alkali and acid digestion of lipid residue. Nitrogen free extract (NFE) was calculated as 100 - (% crude lipid + % crude fiber + % crude protein + % crude ash) and gross energy (kJ kg-1) were calculated using the physiological values, CP × 23.9 + lipid × 39.8+ carbohydrates × 17.6, according to **Brett (1973)**. The results are shown in Table (I). During the feeding trial (70 days), the fish were hand-fed at 5% of the total biomass, twice daily at 9:00 and 14:00h. Feed adjustments were made for each tank every 15 days after sampling following a 24-h starvation period and weighing.

Assessment of water quality

All tanks were supplied with clean water from a well. Every day, 15% of the water in each tank was replaced. To ensure that there was sufficient oxygen available for the fish in the tanks, air pumps (RSElectical®) (Rs-1000: 220-240V, 9 liters/ minute output, 8W) and air stones (Aquaneat®) were used to supplement dissolved oxygen. All seven experimental fish were cultured under a 12/ 12h light/ dark cycle. Water and air temperature were determined daily at 9 am. Dissolved oxygen (DO) was checked at 7 am and pH were recorded using a DO meter and a pH meter. According to **APHA (2017)**, ammonia, nitrate, and nitrite were assessed using a DREL, 2000 spectrophotometer (Hash CO., USA).

Table 1. Formulation and proximate composition of the experimental diets (g/ kg)

	C 0.0	CsNPs 0.05	CsNPs 0.1	CsNPs 0.2	CsNPs 0.4	CsNPs 0.8	CsNPs 1.6
Meat meal	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Soybean meal	268.0	268.0	268.0	268.0	268.0	268.0	268.0
Corn gluten meal	160.0	160.0	160.0	160.0	160.0	160.0	160.0
Yellow corn meal	179.40	179.35	179.30	179.20	179.00	178.60	177.80
Wheat bran	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Soybean oil	60	60	60	60	60	60	60
Chitosan nano particles	0.00	0.05	0.10	0.20	0.40	0.80	1.60
NaCl	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vit. C	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Anti- oxidant[1]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Premix mixture [2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Carboxymethyl cellulose(CMC)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Proximate composition							
Dry matter	89.71	89.71	89.71	89.71	89.72	89.72	89.74
Crude protein	35.20	35.19	35.18	35.16	35.12	35.08	35.04
Crude lipid	6.66	6.66	6.65	6.63	6.59	6.55	6.47
Crude fiber	5.56	5.60	5.70	5.89	6.26	6.90	7.57
Carbohydrate[3]	46.83	46.79	46.71	46.56	46.26	45.67	44.48
Ash	5.75	5.76	5.76	5.76	5.77	5.80	6.44
GE(KJ/kg) [4]	19.31	19.30	19.28	19.22	19.16	19.03	18.88

1. Anti-oxidant (Hadox dry) Hameco Agro – Netherlands.

2. Vitamin and mineral mixture: Each 1kg of mixture contains: Retinyl acetate: 3,000IU, cholecalciferol: 2,400IU, all-rac-α-tocopheryl acetate: 60IU, menadione sodium bisulfite: 1.2mg, ascorbic acid monophosphate (49 % ascorbic acid): 120mg, cyanocobalamine: 0.024mg, d-biotin: 0.168mg, choline chloride: 1,200mg, folic acid: 1.2mg, niacin: 12mg, d-calcium pantothenate: 26mg, pyridoxine. HCl: 6mg, riboflavin: 7.2mg, thiamin. HCl: 1.2mg, sodium chloride (NaCl: 39% Na, 61% Cl): 3,077mg, ferrous sulfate (FeSO4·7H2O, 20% Fe): 65mg, manganese sulfate (MnSO4, 36 % Mn): 89mg, zinc sulfate (ZnSO4·7H2O, 40 % Zn): 150mg, copper sulfate (CuSO4.5H2O, 25 % Cu): 28mg, potassium iodide (KI, 24 % K, 76 % I): 11mg.

3. Nitrogen-free extract = 100 − (CP + crude lipid + crude fiebr+ ash).

4. Gross energy (kJ g-1) were calculated using the physiological values, CP × 23.9 + lipid × 39.8+ carbohydrates × 17.6 **(Brett, 1973)**.

The estimation of growth performance and feed efficiency

Throughout the 70-day trial, dead fish were recorded daily. Fish samples were collected from treatments by sampling 50% of the total fish in each tank and determining the average weight every two weeks. At the end of the experiment, the fish were collected and analyzed to determine weight gain, specific growth rate, average daily growth, feed conversion rate, protein efficiency ratio, and survival using the following formula:

A. Weight gain% (g /fish) = (Final weight – Initial weight /(Initial weight)× 100.

B. Specific growth rate (SGR, % / days) = (ln Fish weight at end of experiment –ln Fish weight at the beginning of experiment) /Number of days during experiment)× 100.

C. Average daily growth (ADG, g/ fish/ day) = (Fish weight at end of experiment – Fish weight at the beginning of experiment) / Number of days during experiment).

D. Feed Conversion Rate (FCR) = Weight of feed /Weight gain.

E. Protein efficiency ratio (PER) = Weight gain / Amount of protein consumed.

F. Survival (S, %) = (Number of surviving fish /Number of initial fish)× 100.

G. Feed conversion efficiency (FCE) = (Weight gain /Weight of feed) × 100.

H. Energy efficiency ratio (EER) = Weight gain /Energy of feed.

Histomorphological examination

Samples of five fish proximal intestines and liver were collected from each tank. They were fixed at 10% buffered formalin for 48h and then processed following the histological methods. Briefly, the samples were dehydrated in ascending grades of ethanol (70- 100%), cleaned in xylene, according to **Suvarna *et al.* (2018)**; paraffin slices were produced and stained with hematoxylin and eosin (H&E) dyes. 4μm thick sections were obtained by microtome, dehydrated in alcohols and stained. Hematoxylin–eosin was used to stain tissue sections, and a light microscope with a digital camera was used to examine and photograph the tissue (Olympus, Tokyo, Japan). The villi length was measured using a computerized image analysis system (Image J software; Bethesda, MD, USA) **(Schneider *et al.*, 2012)**. The results were presented as a mean standard error (±SE). We performed the statistical analysis using the statistical software package SPSS 22 (SPSS® Inc., Chicago, IL, USA).

Statistical analysis

The data were subjected to the one-way analysis of variance ANOVA using SPSS 18 (Chicago, IL, USA). Duncan's multiple range test **(Duncan, 1955)** was used to compare differences between treatment means when significant F values were observed at $P \leq 0.05$ level. All percentage data were arc-sin transformed before analysis **(Zar, 2010)**. The relationship between dietary chitosan nanoparticles CsNPs levels and water quality criteria, including total ammonia, NH_3 (mg l^{-1}), nitrite, NO_2 (mg l^{-1}), and nitrate; NO_3 (mg l^{-1}) was tested using simple and polynomial correlation analysis.

RESULTS

Water quality parameters

Table (2) illustrates the differences in water quality parameters among the experimental groups throughout the trial period. There is a slight upward trend from the control group to $CsNPs_{1.6}$ in dissolved oxygen (DO). Significant differences were observed between the control and other treatments in total ammonia ($NH_{3)}$, nitrite, (NO_2) and nitrate, (NO_3). DO concentrations (5.36- 5.53mg l^{-1}), pH (8.71– 8.38), and temperature (29.47− 29.17 °C) were within the ranges for fish culture conditions. The total ammonia level increased in the control treatment and then decreased upon increasing CsNPs concentration up to 1.6g kg^{-1} diet. Change in nitrite and nitrate were similar to that of total ammonia.

Table 2. Water quality characterization of the Nile tilapia reared for 70-days experimental treatments

	C	CsNPs 0.05	CsNPs 0.1	CsNPs 0.2	CsNPs 0.4	CsNPs 0.8	CsNPs 1.6
Temperature (°C)	29.47±0.35	29.32±0.10	29.42±0.10	29.52±0.05	29.62±0.02	29.42±0.42	29.17±0.07
Dissolved oxygen (mg L^{-1})	5.36±0.01	5.39±0.04	5.45±0.14	5.42±0.03	5.49±0.09	5.46±0.07	5.53±0.09
pH	8.71±0.14	8.52±0.01	8.50±0.05	8.34±0.02	8.36±0.00	8.40±0.01	8.38±0.02
Total ammonia, NH_3 (mg L^{-1})	0.057±0.00^{a}	0.058±0.00^{a}	0.052±0.00^{b}	0.032±0.00^{c}	0.017±0.00^{d}	0.016±0.00^{d}	0.016±0.00^{d}
Nitrite, NO_2 (mg L^{-1})	0.128±0.00^{a}	0.127±0.00^{a}	0.116±0.00^{b}	0.071±0.00^{c}	0.038±0.00^{d}	0.036±0.00^{d}	0.035±0.00^{d}
Nitrate, NO_3 (mg L^{-1})	0.31±0.00^{a}	0.31±0.00^{a}	0.29±0.00^{a}	0.18±0.00^{bc}	0.12±0.00^{c}	0.08±0.00^{c}	0.08±0.00^{c}

Values represented as mean ± SD (n = 7). Values in the same raw with different superscript letters significantly differ at $P < 0.05$.

The correlation (R^2) between the different dietary levels of CsNPs (Fig. 1) and water total ammonia, nitrite, and nitrate content were observed in treatments. The correlation (R^2) was 0.81 for total ammonia; R^2 was 0.82 for nitrite, and R^2 was 0.81 for nitrate, respectively.

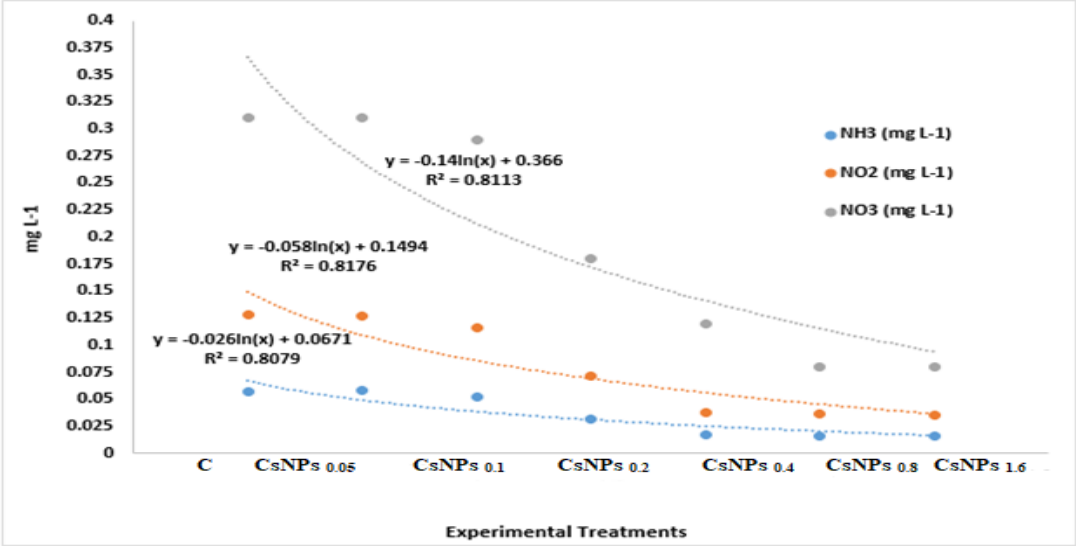

Fig. 1. The correlation between the different dietary levels of CsNPs and water total ammonia, nitrite, and nitrate content

Growth parameters

There were significant differences among the experimental treatments in terms of final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE), energy efficiency ratio (EER) and survival (S%) *(P*<0.05) after 70-days of culture (Table 3). With the increase of CsNPs ratios from CsNPs $_{0.05}$ to CsNPs $_{0.2}$, FW, WG, SGR, EER and S% were significantly *(P*< 0.05) increased compared with the control. However, protein efficiency ratio (PER) was not significantly different (*P*> 0.05) between control and other treatments. However, no significant differences were found in the feed intake among all the trial groups *(P*> 0.05) (Table 3).

Table 3. Growth performance and survival (%) of the Nile tilapia reared for 70-day experimental treatments

	C	**CsNPs $_{0.05}$**	**CsNPs $_{0.1}$**	**CsNPs $_{0.2}$**	**CsNPs $_{0.4}$**	**CsNPs $_{0.8}$**	**CsNPs $_{1.6}$**
Initial weight (g / fish)	0.86±0.03	0.86±0.06	0.86±0.04	0.86±0.03	0.86±0.05	0.86±0.07	0.86±0.03
Final weight (g / fish)	13.79±0.37^{c}	14.18±0.10^{b}	14.81±0.04^{ab}	15.28±0.18^{a}	14.32±0.16^{b}	14.04±0.14^{b}	13.99±0.12^{bc}
Weight gain (g / fish)	12.97±0.03^{b}	13.32±0.10^{ab}	13.95±0.04^{ab}	14.42±0.13^{a}	13.46±0.11^{ab}	13.18±0.13^{b}	13.13±0.06^{b}
SGR (%/days)	3.97±0.02^{c}	4.00±0.01^{ab}	4.07±0.00^{ab}	4.11±0.05^{a}	4.02±0.03^{ab}	3.99±0.01^{b}	3.98±0.05^{b}
Feed intake (g /	17.52±0.	17.22±0.08	17.68±0.0	18.14±0.0	17.00±0.0	17.79±0.0	18.71±0.0

fish)	02		6	5	2	3	4
FCR	1.35±0.02^{bc}	1.29±0.03^{ab}	1.27±0.02^{bc}	1.26±0.04^{c}	1.29±0.02^{bc}	1.35±0.01^{ab}	1.42±0.03^{a}
FCE (%)	74.03±1.50^{c}	77.35±1.25^{b}	78.90±1.67^{ab}	79.49±1.61^{a}	79.18±2.05^{a}	74.09±1.43^{c}	70.18±1.53^{d}
Protein intake (g / fish)	6.17±0.22	6.06±0.31	6.22±0.23	6.38±0.29	5.97±0.21	6.24±0.19	6.56±0.24
PER (%)	2.10±0.04	2.20±0.01	2.24±0.01	2.26±0.04	2.25±0.02	2.11±0.03	2.00±0.05
Energy intake (Kj / fish)	338.31±8.32^{b}	332.35±12.44^{bc}	340.87±11.57^{b}	348.65±9.61^{a}	325.72±9.83^{c}	338.54±12.71^{b}	353.25±10.55^{a}
EER (%)	38.33±3.61^{bc}	40.08±4.04^{ab}	40.92±3.45^{a}	41.36±6.57^{a}	41.32±5.91^{a}	38.93±5.22^{b}	37.17±5.69^{c}
Survival (%)	89.62±1.28^{c}	91.85±1.28^{bc}	92.59±1.28^{b}	97.04±1.28^{a}	94.07±1.28^{b}	92.59±1.28^{b}	91.85±1.28^{bc}

Values are represented as mean ± SD (n = 7). Values in the same raw with different superscript letters significantly differ at $P<0.05$.

Histological examination

The experimental diets supplemented with CsNPs which were used in the current work demonstrated an important effect on the histomorphological structures of the intestine and liver tissues of the Nile tilapia larva (Figs. 2, 3).

According to histological micrographs, intestine of the Nile tilapia larvae is composed of tunica muscularis (muscular layers), serosa, sub-mucosa, and mucosa which is forming the intestinal walls (Fig.2). The photomicrograph in the control group C showed a slight degeneration in muscularis external layer, while the other treated groups demonstrated normal histomorphological architecture of intestinal walls (Fig. 2). The intestine of group fed on 0.05g CsNPs kg^{-1} diet verified edema and flat villi architecture (Fig. 2B). The second treated group which was fed on 0.1g CsNPs kg^{-1} diet illustrated sloughing of enterocytes (Fig. 2C). The group fed on 0.2g CsNPs kg^{-1} diet demonstrated normal healthy intestinal walls and branched villi with intact enterocytes comprising the intestinal villi (Fig. 2D). Moreover, the group fed on 0.4g CsNPs kg^{-1} diet showed a normal architecture of intestine displaying goblet cells (Fig. 2F). Whereas, the two other groups which were fed on 0.8 and 1.6gm CsNPs kg^{-1} diet showed a slight deterioration in the intestinal tissue as the enterocytes were not intact, and the lamina propria showed slight degeneration (Fig. 2F, G).

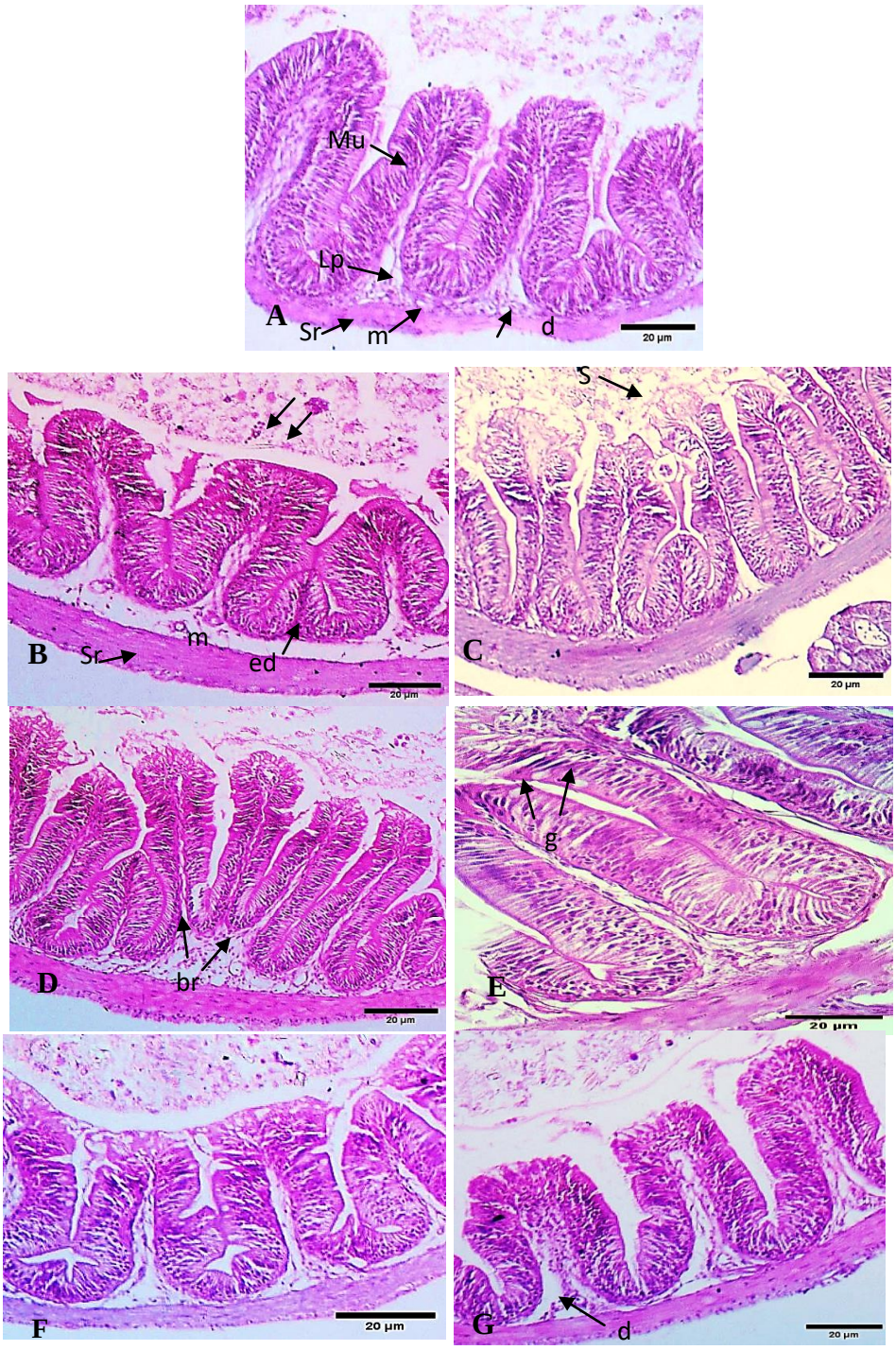

Fig. 2. Photomicrograph showing the intestine of the Nile tilapia larva fed on different concentrations of nano chitosan: **(A)** Control group showing normal serosa (Sr), (d) Degeneration of muscularis (m) lamina propria (Lp) and mucosa layer (Mu), **(B)** Group fed on CsNPs 0.05 showing flat end villi (arrow) and edema (ed), **(C)** Group fed on CsNPs 0.1 showing sloughing of enterocytes (S), **(D)** Group fed on CsNPs 0.2 showing normal branched villi (br), **(E)** Group fed on CsNPs 0.4 showing normal villi with obvious goblet cell (g), **(F)** Group fed on CsNPs 0.8 showing unintact enterocytes, and **(G)** Group fed on CsNPs1.6 showing degeneration in lamina propria

The intestinal villi demonstrated the maximum height and width in the group fed on 0.2gm CsNPs kg^{-1} diet, followed by the group fed on 0.4gm CsNPs kg^{-1} diet, as these two groups were significantly different from other experimental groups and the control one (Table 4)

Table 4. Villi length and width of the Nile tilapia fed with different CsNPs supplemented diets

Group mean	C	CsNPs $_{0.05}$	CsNPs $_{0.1}$	CsNPs $_{0.2}$	CsNPs $_{0.4}$	CsNPs $_{0.8}$	CsNPs $_{1.6}$
Villi height (µm)	57.99 ± 0.03^{b} ^{c}	52.87 ± 0.06 ^{c}	61.04 ± 0.01 ^{b}	87.53 ± 0.06 ^{a}	60.80 ± 0.10 ^{b}	46.27 ± 0.01 ^{d}	59.46 ± 0.47^{b} ^{c}
Villi width (µm)	28.23 ± 0.05^{b}	33.46 ± 0.05 ^{a}	18.86 ± 0.05 ^{d}	34.33 ± 0.15 ^{a}	19.80 ± 0.10 ^{d}	22.43 ± 0.06 ^{c}	32.70 ± 0.10^{a}

Values represented as mean ± SD (n = 7). Values in the same raw with different superscript letters significantly differ at $P<0.05$.

The histological analysis of liver tissue revealed some structural variation in the histomorphological structure of the group fed on the control diet in comparison to supplemented groups. The photomicrographs of liver in the Nile tilapia larvae fed with CsNPs revealed that the control group had congested blood sinusoids and fatty vacuolization of hepatocytes (Fig. 3A). The liver of the Nile tilapia larvae fed on 0.05gm CsNPs kg^{-1} diet had congested blood sinusoids, lymphocytic infiltration, and nucleus pyknosis (Fig. 3B). The liver sections in larvae fed on 0.1gm CsNPs kg^{-1} diet group exhibited congested blood sinusoids, hepatocyte degeneration, and necrosis (Fig. 3C). In contrast, the 0.2gm CsNPs kg^{-1} diet fed group showed almost normal hepatocytes with a slight lymphocyte infiltration (Fig. 3D). The liver of larvae fed on 0.4gm CsNPs kg^{-1} diet exhibited hepatocyte degeneration and necrosis (Fig. 3E); nonetheless, fish fed on 0.8gm CsNPs kg^{-1} diet showed congested blood sinusoids and hepatocyte degeneration (Fig. 3F), moreover fish fed on 1.6gm CsNPs kg^{-1} diet showed vacuolization of hepatocytes (Fig. 3G).

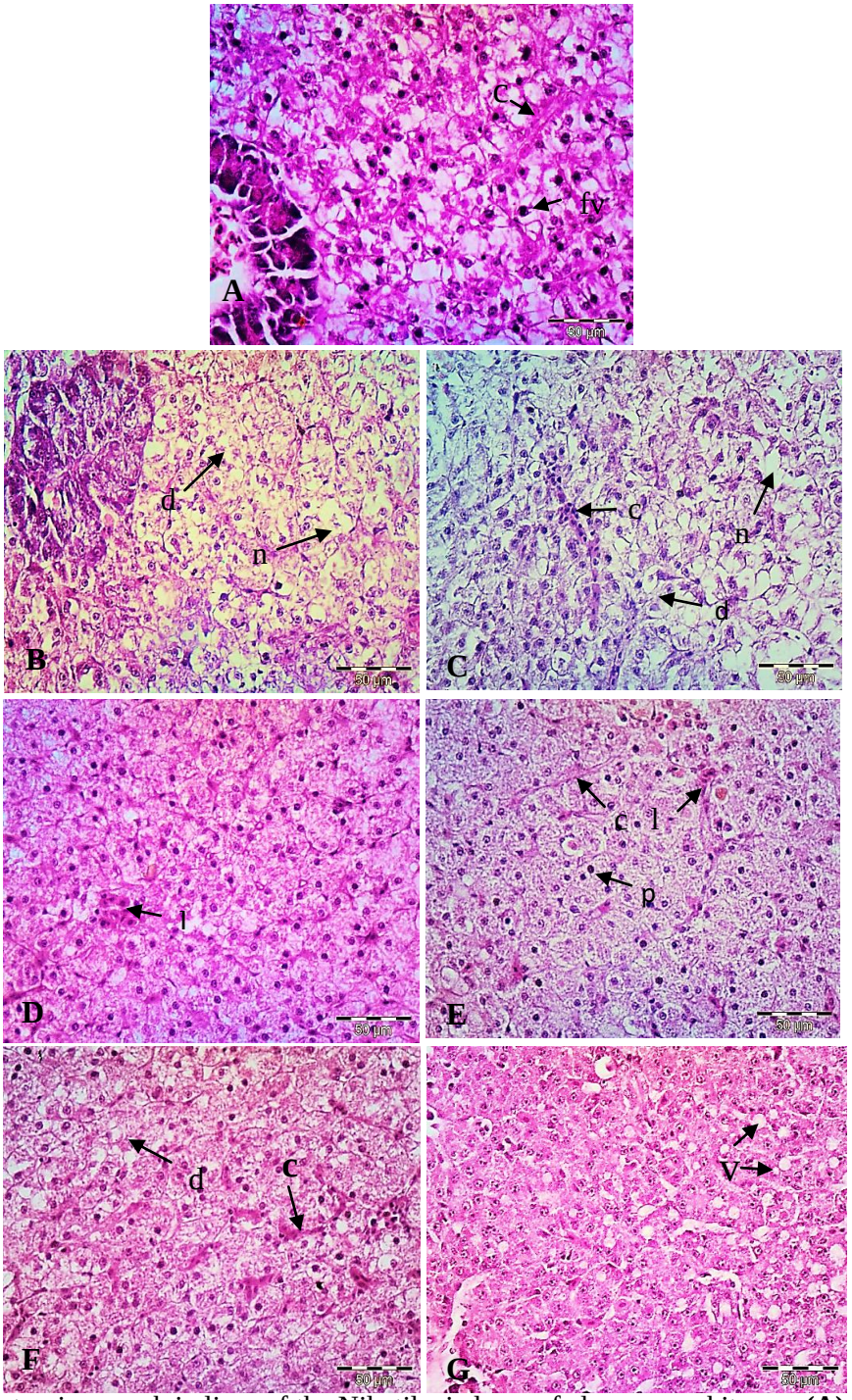

Fig. 3. Photomicrograph in liver of the Nile tilapia larvae fed on nano chitosan: **(A)** Liver of control group showing congested blood sinusoid (c) and fatty vacuolization of hepatocytes (fv), **(B)** Group fed on CsNPs0.05 showing degeneration of the hepatocyte (d) and necrosis(n), **(C)** group fed on CsNPs0.1 showing congested blood sinusoid (c), degeneration of the hepatocyte (d) and necrosis (n), **(D)** Group fed on CsNPs 0.2 normal hepatocyte with slight lymphocyte infiltration NCh, **(E)** group fed on CsNPs 0.4 showing congested blood sinusoid (c), lymphocytic infiltration (l) and picknosis **(F)** group fed on CsNPs 0.8 showing group congested blood sinusoid (c) and degeneration of the hepatocyte (d), and **(G)** Group fed on CsNPs 1.6 show vacuolization of hepatocytes

DISCUSSION

Water quality parameters

Different physicochemical parameters were recorded throughout the experimental period (Table 2). There were no significant differences ($P > 0.05$) observed in water temperature, DO and pH, among different CsNPs treatments. Water temperature is a significant physical parameter that directly influences the chemical, physical, and biological nature of the water body. **Nelson (2008)** stated that warm-water fish do well in a range of 17– 32°C depending on the species, but their optimum growth occurs at 26.7–30.0°C. The pH of water has a profound effect on the productivity of the water body. The pH values in the present study ranged from 8.34 to 8.71. A pH of 6.5 to 8.5 is ideal for pond fish culture and biological production, whereas a pH higher than 9.5 is undesirable since fish become stressed in water **(Ekubo & Abowei, 2011; Bhatnagar & Devi, 2013)**. Throughout the experiment, the pH was within the acceptable range and optimal for fish production. DO levels in the present investigation ranged from 5.36 to 5.53mg l^{-1}. Accordingly, **Boyd and Tucker (2012)** stated that 5.0 to 7.0mg l^{-1} of DO content in ponds water is good for fish production, and water with a DO level below 3mg l^{-1} is unproductive.

The primary source of total ammonia in tank water is fish excretion. Table (2) illustrates the results of water quality characteristics measured in experimental tanks during the treatment period. The results showed that total ammonia levels decreased from 0.057mg l-1 in the control diet to 0.016mg l-1 at CsNPs1.6 group. **Boyd and Tucker (2012)** demonstrated that the safe limit of total ammonia is 1.0mg l-1. The evidence presented here shows that the water quality parameters were appropriate for the Nile tilapia growth and survival. In other words, the addition of CsNPs into practical fish diets had no deleterious effect on water quality during fish culture. However, reductions were recorded in the values of nitrite and nitrate. A highly positive correlation (R^2 = 0.81, 82, and 81) was found between the different dietary levels of CsNPs and total ammonia, nitrite, and nitrate water content (Fig. 1). **Fajardo *et al*. (2022)** demonstrated that nanotechnology has also been used to treat water pollution, which is one of the main problems in aquaculture. The photo-catalysis and adsorption efficiencies of nanomaterials offers effective and inexpensive approaches to water purification. In the same context, graphene nano-sheets and graphene oxide, linked to removal of several types of pollutants from water, have attracted tremendous attention in last few years **(Motamedi *et al*., 2014; Liu *et al*., 2016)**.

Growth performance, feed efficiency, and survival rate

In aquaculture, several requirements should be observed before introducing any fish food supplement to avoid any negative impacts on fish and to boost physiological and biological activities of the cultured fish **(Fath El-Bab *et al*., 2020)**. For this reason,

researchers in the aquaculture field have shown an interest in diets supplemented with feed additives, the most prominent of which are chitosan (CS) and chitosan nanoparticles (CsNPs). In the recent years, considerable attention has been paid to develop biodegradable nanoparticles as effective components for delivering many bioactive feed compounds, such as antioxidants, natural pigments, fatty acids, antimicrobial agents, phenolic compounds, and vitamins **(Akbari-Alavijeh *et al.*, 2019; Khani Oushani *et al.*, 2020)**. Due to its ultrafine size, appropriate stability, very low toxicity, and exclusive functional groups for a wide range of entrapments, chitosan has gained a lot of interest in being used as a delivery vehicle for bioactive molecules, such as hydrophobic drugs, vitamins, proteins, nutrients, and phenolic compounds into biological systems **(Assadpour *et al*., 2016; Katouzian, & Jafari, 2016)**. The study showed that adding 0.1% and 0.2g kg^{-1} CsNPs to the fry diet significantly increased *O. niloticus* growth ($P< 0.05$) when compared to the control group. There was also a decrease in body weight when fish were treated with higher dosages of dietary chitosan than 0.2g kg^{-1} CsNPs feed. Influences of dietary chitosan supplementation on growth and feed utilization have been evaluated with several aquaculture species with varied results. **Dawood *et al.* (2020)** showed that CsNPs significantly increased the FBW, WG, and SGR parameters compared to the control ($P< 0.05$). The value of feed conversion ratio (FCR) was reduced in the group of *Liza ramada* receiving 0.5, 1, and 2g kg^{-1} of chitosan nanoparticles, with the lowest values being in fish fed 2g kg^{-1} ($P< 0.05$). **Najafabad *et al.* (2016)** investigated the effects of dietary chitosan on growth performance, in the Caspian kutum *(Rutilus frisii kutum, Kamenskii,* 1901) fingerlings (1.7± 0.15g) were fed diets containing chitosan at different levels (0, 0.25, 0.5, 1, and 2g kg^{-1} diet) for a period of 60 days. Results showed that the feed conversion ratio significantly decreased in fish fed diet containing 1g kg^{-1} of chitosan compared to the other groups ($P< 0.05$), but there were no significant differences between treatments in terms of specific growth rate and condition factor ($P> 0.05$). **Gopalakannan and Arul (2006)** showed that dietary chitosan supplementation enhanced the growth of the common carp (*Cyprinus carpio*). On the contrary, **Shiau and Yu (1999)** observed depressed growth in tilapia (*O. niloticus* × *O. aureus*) after feeding chitin and chitosan at the 2, 5 and 10% level. They also speculated that the depressed growth in tilapia may be due to the interposition of chitosan and chitin in the absorption of nutrients.

The FCR in the control, $CsNPs_{0.05}$, $CsNPs_{0.1}$, $CsNPs_{0.2}$, $CsNPs_{0.4}$, $CsNPs_{0.8}$ and $CsNPs_{1.6}$ groups were 1.35, 1.29, 1.27, 1.26, 1.29, 1.35 and 1.42, respectively (Table 3). The worst FCR values was recorded in the fish treated with no dietary CsNPs. On the other hand, the FCR was significantly increased with increasing the doses of dietary CsNPs up to 0.2g kg^{-1} ChNPs diet group (Table 3). FCE, PER, EER and S% were significantly ($P< 0.05$) increased in fish fed diet containing 0.2g kg^{-1} CsNPs and 0.1 CsNPs groups, respectavily. **Zaki *et al.* (2015)** showed that prebiotic dietary chitosan has beneficial effects on treated fish in improving the quality of feeds, aquaculture in a safe

way and growth performance, as well as reducing the mortality of fishes **(El-Sayed & Barakat, 2016)**. **Zhang (2019)** attributed the enhanced growth rate and feed utilization to chitosan's role in activating digestive enzymes, which are responsible for the hydrolysis of nutrients (e.g., carbohydrates, lipids, and proteins) in the gastrointestinal tract (GIT) and the digested nutrients can be easily absorbed by the intestinal villi and be available for the cells. In addition to the developmental stage and amount of dietary chitosan supplied, chitosan effects exerted on fish growth performance also seem to depend on the species **(Abdel-Ghany & Salem, 2020)**. Though as a consequence, micronutrients in the form of nanoparticles, incorporated in aquaculture feeds can penetrate in cells more efficiently, and therefore, raise the absorption rate **(Zhou *et al.*, 2009; Ogunkalu, 2019)**. Additionally, this helps the body to digest and absorb nutrients **(Jiang *et al.*, 2012)**. This has been demonstrated in sturgeon and young carp, which showed faster growth rates when fed with iron nanoparticles **(ETC, 2003)**. While, **Deng *et al*. (2003)** reported that CsNPs can stay in the intestinal tract for a longer time due to its small particle size. **Chen *et al.* (2014)** found that apparent digestibility coefficient of dry matter and the apparent digestibility coefficient of protein after 75 days of feeding on diets supplemented with 10– 20g chitosan kg^{-1} significantly decreased the growth performance of gibel carp (*Carassius gibelio*) (initial body weight, 4.80± 0.01g). However, the supply of 0– 0.2g chitosan kg^{-1} diet caused a dose dependent increase of the average daily weight and SGR in post-larvae sea bass (*Dicentrarchus labrax*) **(El-Sayed & Barakat, 2016)**. Recently, **Wang *et al*. (2023)** observed that the addition of 0.5 and 0.1% CsNPs into the feed has a significant promoting effect on the growth of sea cucumber. This effect may be due to the strong stability of CsNPs, its small particle size, easiness to enter cells, and significant enhancement of the activities of ALP and lysozyme (Lyz) immune enzymes. ALP is a phosphomonoesterase that decomposes phosphomonoesters, provides phosphate groups for the body, regulates membrane transport, participates in the metabolism of DNA, RNA, lipids and proteins, and has an important impact on the body's immunity **(Chen *et al.*, 1998)**. While, lysozyme is an alkaline enzyme that decomposes the insoluble mucopolysaccharides into soluble glycopeptides by destroying the cell wall of bacteria, which leads to the rupture of cell wall and the release of cell wall contents to cause the dissolution of bacteria **(Liu *et al.*, 2017; Ma *et al.*, 2020)**, hydrolyze mucopolysaccharide from pathogenic bacteria, which has antibacterial, anti-inflammatory and antiviral effects.

In the present study, regarding the survival (S%) (Table 3), there were significant differences among the experimental treatments. Significantly ($P< 0.05$) higher value (S% = 97.04%) was obtained in $CsNPs_{0.2}$ receiving tilapia feed than control treatment (S%= 89.62 %). Dietary CsNP was investigated by **Saleh *et al*. (2022)** for the amelioration of the systemic inflammatory responses of an induced fish model. One hundred and forty-four rainbow trout were assigned to one pathogen-free and non-supplemented group (negative control), and three challenged groups: Non-supplemented (positive control),

CsNPs-preventive, and CsNPs-therapeutic. They have shown low cytotoxicity and high anti-microbial efficacy, mucoadhesion, and hemocompatibility in several bionanotechnological applications in aquaculture **(Ahmed *et al*., 2019; Wu, *et al*., 2020)**. CSNPs exhibit antimicrobial effects *in vitro* against some major pathogenic bacteria and fungi of fish **(Abdel-Razek, 2019; Ahmed, *et al*., 2020**).

Histological examination

The current study revealed that the groups fed with CsNPs from 0.2 to 0.4g kg-1 diets had essentially expanded villi length, width, and normal tissue. In these groups, the architecture and intaglio were nearly typical; these progressed morphometric characteristics clarify the development of intestinal structure in the Nile tilapia fry that received this supplemented feed. This result is consistent with the findings of **Elsabagh *et al.* (2018)** and **Elabd *et al.* (2023)**. Our results illustrated that the villi height increased significantly *(P*< 0.05) with supplemented chitosan nanoparticles in the diet-fed groups of 0.1 and 0.2g CsNPs kg^{-1} diet, however group fed with 0.8 to 1.6g kg^{-1} CsNPs diet reduced significantly *(P*< 0.05) the villi height (µm). **Vechklang *et al.* (2011)** hypothesized that intestinal morphology influences the physiology and metabolism of nutrients absorption. **Blomberg *et al*. (1993)** showed that the gut surface area, as assessed by macroscopic morphological parameters such as villus height and epithelial thickness, influences ingestion and absorption, and hence the net utilization of dietary nutrients. The same authors suggested that increasing villus height and decreasing epithelial thickness facilitated the absorption process. Histological sections of organs are essential for determining their health status **(Abdel-Latif *et al*., 2020)**. **Salam *et al.* (2021)** examined influence of dietary chitosan on the length of intestinal villi of juvenile *Barbonymus gonionotus*. They showed that the three doses of chitosan-treated feed (1, 2, g kg^{-1} feed) remarkably increased the surface area of the intestine of juvenile *B. gonionotus* compared to that of the control fish (0g kg^{-1} feed). This study revealed that dietary chitosan significantly (*P*< 0.05) enhanced the length (µm) of the intestinal villi of juvenile *B. gonionotus*. Notably, the same authors showed that the highest length of intestinal villi of *B. gonionotus* was recorded in treatment fish that were fed on chitosan of 1g kg^{-1} compared to that in the control and other treatments (*P*< 0.05). In the same context, **Najafabad *et al.* (2016)** investigated the effects of dietary chitosan (at levels of 0, 0.25, 0.5, 1.0, and 2.0g kg^{-1} diet) on intestinal histology in the Caspian kutum (*Rutilus frisii kutum, Kamenskii*, 1901) and found that administration of chitosan for 60 days enhanced villus length in the fish intestine They reported that the intestinal villus length increased in fish fed diet containing 1g kg^{-1} of chitosan compared to control group (*P*< 0.05). This result was in agreement to our current findings which might be activated by feeding of chitosan. The gastrointestinal tract morphology of tilapia found that dietary immunostimulants such as Ergosan enhanced villus height **(Merrifield *et al*., 2011)**. Moreover, **Kumari *et al*. (2013)** reported the benefits for the productive performance and

the healthiness of the intestinal villi from chitosan-encapsulated trypsin more than from bare trypsin of rohu (*Labeo rohita*). **Chen *et al.* (2014**) showed that the number of goblet cell, intraepithelial lymphocyte of mid-intestine and microvilli height of distal intestine of the gibel carp (*Carassius auratus gibelio*) increased at 4.0g kg^{-1} dietary chitosan.

The current results demonstrated that goblet cells are clearly observed in sections of tilapia fry fed CsNP diets, which is consistent with **Dawood *et al*. (2020)**, who stated that goblet cells were enhanced in grey mullet fish fed chitosan supplemented feed. Our findings were also consistent with **Ibrahim *et al*. (2021)**, who observed that adding chitosan vitamin C nano-composite to tilapia diets increased villi height and the number of goblet cells in the fish intestine improved the intestinal absorption by providing a larger surface area for nutrient absorption and immune protection against infections.

Shiu *et al*. (2015) reported that the histological status of the liver is a good indicator of the actual nutritional status of the fish. Some histopathological changes such as congestion, necrosis of hepatocytes, hepatocyte vacuolization, and leukocyte infiltration were observed in liver sections of fish fry. Our investigation found that adding CsNsP to tilapia fry diets reduced hepatocyte vacuolization, indicating that cell vacuolization was primarily a fatty type. This is consistent with the findings of **Stanek *et al*. (2023)** who demonstrated that, adding chitosan to *Cyprinus carpio* fry diets can improve abnormal lipid accumulation in the liver. However, hepatocyte vacuolization was identified when low concentration supplementation of CsNPs in groups receiving 0.05 and 0.1g kg^{-1} are in agreement with those documented in hybrid tilapia juveniles when chitosan inclusion levels were 0.4 and 0.5g kg^{-1}, respectively **(Méndez-Martínez *et al*., 2023)**. A positive effect of chitosan on liver morphology which matches with the current results were detected in the study of **Salam *et al*. (2021)** who found that, dietary chitosan (1, 2, and 3g kg^{-1} feed) improved the morphology of the liver of *B. gonionotus* compared to that with the control (0g kg^{-1} feed). **Thilagar and Samuthirapandian (2020)** reported that dietary chitosan- treated feed improved the morphology of the intestine, liver, and kidney of *O. mossambicus*. Moreover, there were no significant changes in morphology in fish fed chitosan alone, which indicated the nontoxic nature of dietary chitosan for fish **(Gopalakannan & Arul, 2006)**. **El-Naggar *et al.* (2022)** evaluated the effect of supplementing fish and gluten meals with chitosan and chitosan nanoparticles (0.05 %) on the Nile tilapia liver histology. They found that the liver of fish fed fish meal diet appeared as a continuous mass of hepatocytes that radiated from central vein, forming a cord-like pattern though the wall of the central vein and sinusoids was lined with kupffer cells that appeared as simple squamous cells with flattened nuclei and surrounded with a thin rim of cytoplasm. The same authors found that the hepatocytes were polygonal in shape that possessed acidophilic cytoplasm. They had a large, rounded, central and basophilic nucleus with a single central darkly stained nucleolus.

Hepatocytes with regularly shaped sinusoids were noted in our experimental groups, while hepatocytes in the control group were characterized with congested blood sinusoids and fatty vacuolization of hepatocytes. Our results coincide with others reported about the positive effects on *O. niloticus* livers upon using chitosan in the diet **(Salaah *et al.*, 2022)**. On the other hand, chitosan and chitosan nanoparticles were identified as significant in the hepatocytes protection against oxidative stress **(Ozcelik *et al*., 2014)**.

CONCLUSION

Our study indicates that 0.2g kg^{-1} of CsNPs is the ideal supplementation level in early feeding of the Nile tilapia fry (*Oreochromis niloticus*) to improve growth performance, diet utilization efficiency, feed conversion ratio, and histomorphological health of the liver and gut. This study has significant implications for using CsNPs as feed supplements in aquaculture and preservation of water quality for rearing pond from deterioration.

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الملخص العربي

حظيت جزيئات الشيتوزان النانوية بالكثير من الاهتمام في السنوات الأخيرة نظرًا لخصائصها المفيدة كمكمل صحي لتزويد المواد النشطة بيولوجيًا في صناعة الأغذية والتغذية. كان الغرض من هذه الدراسة هو دراسة تأثير جزيئات الشيتوزان النانوية كمحفزات غذائية عند التغذية المبكرة لزريعة البلطي النيلي (*Oreochromis niloticus*) على أداء النمو وكفاءة التغذية والصحة النسجية للكبد والأمعاء. تمت إضافة مستويات متدرجة من CsNPs (0.05، 0.1، 0.2، 0.4، 0.8 و1.6 جم لكل كجم) إلى النظام الغذائي الأساسي، وتم تقسيم كل نظام غذائي تجريبي إلى ثلاث مجموعات من الأسماك بوزن تقريبي (0.86 ± 0.04 جم)) لمدة 70 يومًا. خلصت الدراسة التجريبية إلى أن النظام الغذائي 0.1 CsNPs و0.2 جم كجم أدى إلى ارتفاع ملحوظ في الوزن النهائي(FW) ، وزيادة الوزن(WG) ، ومعدل النمو النوعي(SGR) ، ونسبة كفاءة الطاقة(EER) ، والبقاء على قيد الحياة (S%)مقارنة بالمجموعة الضابطة .(P<0.05) ومع ذلك، زادت نسبة كفاءة البروتين (PER) ولكنها لم تختلف بشكل كبير (P>0.05) بين السيطرة والمعاملات الأخرى. أظهرت أمعاء زريعة البلطي النيلي أن ارتفاع الزغب زاد في المجموعات التي غذيت على 0.1 جرام CsNPs كجم-1 عليقة و0.2 جرام CsNPs كجم-1 عليقة و0.4 جرام CsNPs كجم-1 عليقة، في حين تم تسجيل أعلى طول للزغب. في المجموعة 0.2 جرام من CsNPsكجم -1 من النظام الغذائي. أظهرت المجموعة التي تغذت على (0.2 جم من CsNPs كجم-1 علف) جدران معوية صحية وزوائد متفرعة مع خلايا معوية سليمة تشتمل على الزغابات المعوية. أظهر التركيب النسيجي المرضي لكبد زريعة البلطي النيلي التي تم تغذيتها بـ CsNPs أن المجموعة الضابطة كانت تعاني من احتقان و التفريغ الدهني لخلايا الكبد. في حين عرضت مجموعة النظام الغذائي 0.2 جرام CsNPs كجم -1 خلايا كبدية طبيعية مع تسلل طفيف للخلايا الليمفاوية. أوضحت نتائج الدراسة الحالية التأثير الإيجابي للمكملات الغذائية بمقدار 0.2 جرام كغم-1 من جزيئات الشيتوزان النانوية والتي تعتبر مركب واعد في تحسين أداء النمو وكفاءة التغذية وزيادة طول الزغابات لتسهيل عملية الامتصاص في الأمعاء. البلطي النيلي.(*Oreochromis niloticus*)