Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 427 – 448 (2022)
www.ejabf.journals.ekb.eg

Effects of Dietary Supplementation of Chitosan and Chitosan Nanoparticles on Growth Performance, Fatty Acids Profile and Liver Histology of the Nile Tilapia (*Oreochromis niloticus*) Fed on Fish Meal-Free Diets

Marwa M. El-Naggar [1,*], Hamza El-Shabaka [1], Fawzia Abd El-Rahman [1], Wael Abou- Elmagd [2], Magdy Khalil [1], Ashraf Suloma [3]

[1]Departments of Zoology and [2]Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt.
[3]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.
[*]Corresponding Author: m.alnggar@hotmail.com

ARTICLE INFO

Article History:
Received: July 2, 2022
Accepted: July 19, 2022
Online: July 25, 2022

Keywords:
Nile Tilapia,
Chitosan,
Chitosan nanoparticle,
Gluten meal,
Growth performance,
Omega-3

ABSTRACT

The current study was conducted to evaluate the effect of supplementing fish and gluten meals with chitosan and chitosan nanoparticles on the Nile tilapia growth performance, feed utilization, fatty acids profile and liver histology. The experimental design was completely randomized with a 2 x 3 factorial design; two protein sources; fish and gluten meals, and three forms of chitosan (zero-chitosan, chitosan and chitosan nanoparticles). A total number of 270 *O. niloticus* fingerlings were randomly distributed among 18 tanks (55 liters water capacity). The experiment lasted for 82 days. Results indicated that the growth performance parameters and protein efficiency ratio of fish fed gluten meal-based diets were improved by the addition of chitosan nanoparticles. Additionally, it was found that supplementation of gluten meal-based diet with chitosan nanoparticles increased the total omega-3 level, while decreased the omega-6/omega-3 ratio. Moreover, supplementation of gluten meal-based diets with chitosan nanoparticles improved the histological architecture of the liver and reduced fish mortality. Consequently, it is recommended to use chitosan nanoparticles as feed additives to gluten meal-based diets at a level of 0.5%.

INTRODUCTION

During the past couple of decades, a remarkable enormous progression in aquaculture has outpaced capture fisheries. Additionally, in the upcoming years, it is predicted to serve as a principle source of aquatic animal requirements **(Ahmed *et al*., 2020; FAO, 2022)**. Within the last few decades, the aquaculture industry has developed and is well sustained. In spite of its success, aquaculture industry has serious limitations; among low resistance to diseases, lack of fry and high cost of fish meal have been recognized **(Khalil, 2014)**.

Fish meal (FM) has been radically used in the world aquaculture feed industry due to its basic nutrients, namely: vitamins, indispensable amino acids, essential fatty acids, major minerals, several growth factors and essential fatty acids **(Zhou *et al*., 2005)**. With the

aquaculture extension, an improved alternative stock of protein sources is required to cover the escalating cost, high demand and the fish meal unbalanced supply **(Phumee *et al*., 2011:El-Husseiny *et al*., 2018)**.

One of the commercially attainable plant protein-based products is corn gluten meal (GM), which is the wet milling of corn product **(Wu *et al*., 1995)**. GM is regarded as a good alternative plant protein source for FM **(Metwalli, 2013)**. It contains about 60% crude protein (CP), 2% ash and 1% crude fiber and crude lipid, respectively **(Wu *et al*., 1995)**. In addition to its high protein content, GM has a sufficient essential amino acids profile, though it lacks arginine and lysine **(Amerio *et al*., 1998)**. It is favored due to its high digestibility by *Oreochromis niloticus*, low fiber content in addition to the absence of any anti-nutritional factors **(Korprucu & Ozdemir, 2005)**. Several studies reported that gluten meal can substitute fish meal in many fishes **(Wu *et al*., 1995)**. **Metwalli (2013)** reported that inclusion of GM up to 75% as low cost plant protein instead of fishmeal can be utilized in *O. niloticus* diets without adverse effect on growth performance, blood characteristics and feed efficiency.

Chitosan [β-(1-4)-N-acetyl-D-glucosamine] (CS) has proved to be one of the smartest and non-toxic natural cationic biopolymers. It belongs to polysaccharides with structural traits compared to glycosaminoglycans **(Muzzarelli *et al*., 1988)**. CS is the second most abundant polymer after cellulose **(Aranaz *et al*., 2009)**. It has remarkable properties including non-toxicity, biodegrability, biocompatibility and improved solubility, in addition to, immunorestorative properties **(Shard *et al*., 2014)**. CS is obtained from the deacetylation of chitin that is considered as an important component found in the exoskeletons of aquatic crustaceans, such as: crayfish, crabs, shrimps and terrestrial organisms as well as cell walls of some microorganisms **(Xu *et al*., 2008)**.

Chitosan nanoparticles (CSNP) have attracted attention due to their distinctive properties and interesting applications **(Radhika-Rajasree & Gayathri, 2014; El-Naggar *et al*., 2022a,b)**. CSNP have effective antibacterial activity, enhancing growth performance and survival of fish **(Kaur *et al*., 2014)**. Supplementation of CSNP to *O. niloticus* diet significantly influenced the liver status, digestive enzymes, meat quality, immune response, whole body composition, hematology, intestinal bacterial count and growth rate **(Wang & Li, 2011; Abd El-Naby et al., 2019a)**.

In East Africa, the Nile Tilapia (*Oreochromis niloticus*) is the highly favored cultured fish, while globally it has been estimated the third outstanding fish group in the world, after carps and salmonids. It is widely cultured in about 100 countries in the tropical and subtropical regions **(El-Sayed, 2006; GAFRD, 2022)**. The success of *O. niloticus* in aquaculture is attributed to its high survival rate in poorly oxygenated environments, high resistance to diseases and its capability to depend on a broad variety of foods **(Prabu *et al*., 2019)**. It worth noting that among other cultured fish, the tolerance of *O. niloticus* to feed on higher concentrations of carbohydrates and

dietary fiber has made it distinctive **(El-Sayed & Teshima, 1992)**. However, a good and sustained feed is radical to obtain prosperous *O. niloticus* culture to guarantee a rapid growth at low cost and high outcome.

There is no previous literature about the effect of chitosan forms on the utilization of protein sources. Therefore, the present study aimed to evaluate and compare the potential of CS and CSNP to enhance the properties of gluten meal for FM-based diet total replacement.

MATERIALS AND METHODS

1.1. Experimental fish and culture technique

Following the procedures of the study of **El-Naggar *et al*. (2021)**, the present study was carried out at the Fish Nutrition Laboratory, Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt. Mixed sex *O. niloticus* fingerlings, with an average initial body weight of 15.3 ± 0.08 g, were collected from a local hatchery in Kafr El-Sheikh Governorate, Egypt. At 21st November 2018, under controlled thermal condition, a total of 270 *O. niloticus* fingerlings were randomly distributed into 6 different treatments with a triplicate of 15 fish each. Acclimatization of fish was performed for a week before the feeding trial. Fish were fed a floating diet of 30% CP at 2% of the fish body weight twice daily (10 a.m. and 4 p.m.) . During the experiment, the fish were fed till apparent satiation, twice daily (10 a.m. and 4 p.m.). Fish weight was measured every 15 days. The experiment lasted 82 days, and the number of dead fish was recorded daily.

1.2. Experimental design and diets

The experimental design was completely randomized with a 2 x 3 factorial design; with two protein sources; fish meal and gluten meal, and with three forms of chitosan (zero-chitosan, chitosan and chitosan nanoparticles) **(El-Naggar *et al*., 2021)**. Fish meal basal diet was formulated and 100% of fish meal was replaced with GM in gluten meal-basal diet. Each of the basal diets were supplemented with chitosan or chitosan nanoparticles (0.5%) to design six isonitrogenous (30% CP) and isocaloric (4500 kcal/kg) for *O. niloticus* **(NRC, 2011)**. The dose was administered according to **Wang & Li (2011)** and **El-Naggar *et al*. (2021)**.

The proximate composition of the experimental diets is shown in Table 1. Preparation of the experimental diets required blending the ingredients till a homogenous mixture was obtained; then the mixture was passed through a local minced meat machine, dried overnight at room temperature and stored in plastic bags at 4 °C till used. The extraction of chitosan and from *P. clarkii* wastes and preparation of chitosan nanoparticles as well as their optimization and characterization has been already discussed previously in **El-Naggar *et al*. (2019, 2020)**.

Chitosan and chitosan nanoparticles were optimized by 9 hrs deacetylation and applying 1:1 TPP to chitosan, respectively **(El-Naggar *et al*., 2019, 2020)**

Table 1. Formulation and proximate composition of fish meal and gluten meal-based diets supplemented with CS or CSNP

	FM-based diet			GM-based diet		
Ingredient (g/100g)	**FM**	**FMCS**	**FMCSNP**	**GM**	**GMCS**	**GMCSNP**
Fish meal (70% CP)	14	14	14	-	-	-
Gluten	-	-	-	14	14	14
Soybean (42% CP)	34	34	34	34	34	34
Corn	8.43	7.93	7.93	8.43	7.93	7.93
Oil	6	6	6	6	6	6
Bran[e]	35	35	35	35	35	35
Vitamin and mineral premix[*]	2	2	2	2	2	2
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Carboxy methyl cellulose	0.50	0.50	0.50	0.50	0.50	0.50
Butylated hydroxytoluene	0.02	0.02	0.02	0.02	0.02	0.02
Chitosan	-	0.50	-	-	0.50	-
Chitosan nanoparticles	-	-	0.50	-	-	0.50
Total	100	100	100	100	100	100
Proximate composition (% dry matter)						
Moisture	7.8	7.9	7.7	7.8	8	8.6
Crude protein (CP)	30.7	30.5	30.8	29.1	29.5	29.5
Crude lipid	8.62	8.46	8.57	8.22	8.23	8.17
Ash	6.8	6.9	6.8	5	5.3	5.3
Crude fiber	6.73	6.99	6.94	7.02	6.91	6.89
Nitrogen free extract (NFE)	39.35	39.25	39.19	42.86	42.06	41.54
Gross energy kcal/kg	4469	4449	4472	4501	4486	4458

[*]Provides per kg of diet: retinyl acetate, 3,000 IU; cholecalciferol, 2,400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49 % ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1,200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3,077 mg; ferrous sulfate (FeSO4·7H2O, 20 % Fe), 65 mg; manganese sulfate (MnSO4, 36 % Mn), 89 mg; zinc sulfate (ZnSO4·7H2O, 40 % Zn), 150 mg; copper sulfate (CuSO4.5H2O, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1,000 mg Agri-Vet Co., Cairo, Egypt.
Nitrogen free extract was determined by the difference: NFE = 100 − (% crude protein + % crude fat + % crude fiber + % total ash + % moisture) **(AOAC, 1995)**.
Dietary gross energy was calculated using the conversion factors of 5.6, 9.4 and 4.2 kcal/kg for protein, lipids and NFE, respectively, **(Brett & Groves, 1979)**.

1.3. Experimental unit

Fish were grown out under laboratory recirculating aquaculture system (RAS) whose rate of flow was 0.4 L min^{-1} (Fig. 1). A total of 18 plastic tanks whose capacity was 55 liters were provided with a side flow (OD) opening and connected to a standpipe (SP) provided with a valve that regulates the quantity of water flowing out through the side drain. The water passed from the side over flow to a sump then was mechanically filtered (M.f) to get rid of the food remains and feces. Water was drained from the mechanical filter to biological filter 1 (B.f1) then by gravity to biological filter 2 (B.f2) through which the ammonia was disposed. Water in the B.f2 passed through a pump to a collecting tank (CT) and filtered water was drained back to the RAS tanks. Aeration was continuously provided to the tanks using an air pump and porous stones to ensure the maintenance of oxygen supply greater than 5 mg L^{-1}.

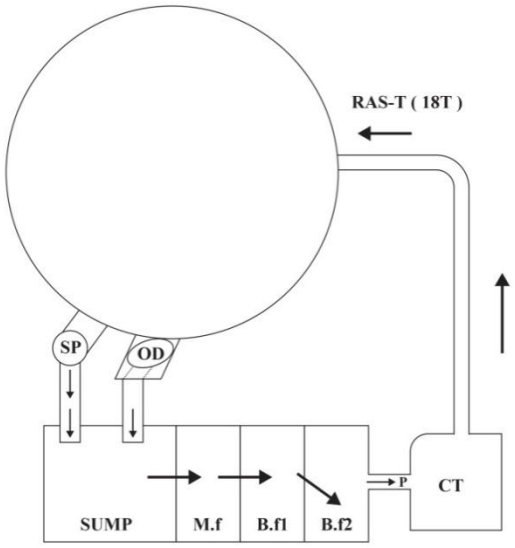

Fig.1. A schematic diagram of the Recirculating Aquaculture System.
Abbreviations: B.f, biological filter; CT, collecting tank; M.f, mechanical filter; P, pipe; OD, side flow; RAS, recirculation aquaculture system; SP, stand pipe.

1.4. Growth performance parameters

- **Specific growth rate (SGR)**

The specific growth rate was calculated using the following equation according to **Hopkins (1992)**

$$SGR\ (\%) = \frac{In\ W2 - In\ W1}{T}\ X\ 100$$

Where, ln is the natural logarithm.
W_1 is the initial body weight (g).
W_2 is the final body weight (g).
T is the experimental period in days.

- **Survival rate (%)**

The survival rate was calculated according to **Hung** et al**. (1993)** as follows:

$$Survival\ rate\ (\%) = \frac{No.of\ survival\ fish}{Initial\ no.of\ fish}\ X\ 100$$

1.5. Feed utilization parameters

- **Feed intake (FI)**

The feed intake per fish was calculated according to **Da *et al*. (2016)** as follows:

Feed intake per fish (FI) (g/fish) = Total feed intake (g)/Total no. of fish

- **Feed conversion ratio (FCR)**

The feed conversion ratio is a parameter that is used to determine the value of feed for providing the necessary food amount required for one unit of growth. A lower value indicates an improved outcome. Feed conversion ratio was calculated according to **Hung *et al*. (1993)** by the following equation:

$$FCR = Feed\ intake\ (g)/Body\ weight\ gain\ (g)$$

- **Protein efficiency ratio (PER)**

The protein efficiency ratio is an indicator for the efficiency of protein consumption utilization. It was calculated by the following equation according to **Hung *et al*. (1993)** as follows:

$$PER = Weight\ gain\ (g)/Protein\ intake\ (g)$$

- **Protein productive value (PPV)**

The protein productive value is an indicator for the retained protein from protein intake. It was calculated according to **Nose (1971)** as follows:

$$PPV\ (\%) = \frac{Protein\ gain\ (g)}{Total\ protein\ intake\ (g)}\ X\ 100$$

Where, protein gain = final fish protein content – initial fish protein content.

1.6. Determination of the chemical composition of *O. niloticus* carcass and the experimental diets

At the start of the experimental study, a batched sample of thirty fish was assembled to act as an initial one. At the end of the experiment, six fish from each tank were randomly selected to serve as a final sample. 40 mg/L clove oil was used to anaesthetize the fish samples. The fish were then sacrificed, oven-dried at 60-70 °C, ground into homogenous powder and stored at -20 ºC until further analysis. The fish carcass samples were analyzed for moisture, CP, crude lipid, ash contents and fatty acids profile. Moreover, the amino acids analysis was performed in the experimental diets (Table 2).

The moisture content of the fish carcass was determined, according to the method of Association of Official Analytical Chemist **(AOAC, 1995)**. The CP content of fish carcass samples were measured in the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, by Dumas method using Tru Spec Nitrogen determinator **(AOAC, 2016a)**. Crude lipid and ash contents of fish carcass were determined, according to the method of Association of Official Analytical Chemist **(AOAC, 1995)**.

The fatty acids analysis of the fish carcass was performed according to methyl esters boron trifluoride method of **AOAC (2016b),** using a spectrophotometer (Schimadsu UV-1800). Fatty acid analysis was carried out in the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture.

Amino acids analyses of the experimental diets were carried out according to the method of (AOAC, 2012), namely: performic oxidation method. Amino acids determination was measured

in the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, using amino acid analyzer (Biochrom 30).

Table 2. Amino acids composition of fish meal and gluten meal-based diets supplemented with CS or CSNP (g kg^{-1} dry diet)

Amino acids	FM-based diets			GM-based diets			Amino acid requirement of *O. niloticus*[a]
	FM	FMCS	FMCSNP	GM	GMCS	GMCSNP	
Non-essential amino acids							
Alanine	15.6	15.7	18.0	19.5	20.1	21.4	-
Aspartic acid	30.7	30.3	28.6	25.3	25.4	24.4	-
Glutamic acid	49.7	49.3	48.7	52.9	53.1	54.4	-
Glycine	14.7	14.6	14.5	11.2	11.5	11.0	-
Proline	16.3	16.6	15.6	19.1	19.8	20.6	-
Serine	14.3	14.2	14.1	14.3	14.3	15.0	-
Tyrosine	11.5	11.0	10.8	12.1	12.6	13.0	-
Essential amino acids							
Arginine	20.0	19.8	20.3	16.8	17.8	16.7	11.8
Histidine	8.7	8.7	8.5	7.2	7.4	7.2	10
Isoleucine	12.7	12.6	12.7	11.7	11.9	11.8	8.7
Leucine	22.2	21.9	22.1	28.1	28.5	31.1	19
Lysine	19.8	19.5	19.2	12.7	12.9	11.9	14.3
Methionine+cystine	11.2	12	11	10.4	11.5	11	9.0
Phenylalanine	14.8	14.5	13.9	15.3	15.6	16.2	10.5
Threonine	12.7	12.5	12.4	10.7	10.9	10.9	10.5
Valine	14.4	15.4	19.4	17.5	14.9	14.8	15

[a] **NRC (2011).**

1.7. Liver histology

From each replicate, five specimens of *O. niloticus* were randomly selected and carefully dissected to examine the general viscera. Tiny parts of liver were dissected out and fixed in aqueous Bouin's fluid for 24 hrs. After fixation and washing, dehydration was carried out into ascending grades of ethyl alcohol. The materials were then cleared in terpineol and placed in a clear molten parablast. Sections of 5-6 µm in thickness were cut and stained with Harris' haematoxylin and counterstained with eosin **(Humason, 1972)**. Light microscope was used to examine the sections and photomicrographs were taken as required.

1.8. Statistical analysis

The experiment was conducted on triplicate that were analyzed using two-way analysis of variance (ANOVA) using computer program Statistical Package for Social Sciences **(SPSS, 2013)** to detect the impact of the dietary protein source, chitosan (CS) forms and their interactions. The data of the fatty acids profile was analyzed using one-way ANOVA. To determine the differences among the experimental groups, Duncan's multiple range test incase significant differences existed **(Duncan, 1955)**.

RESULTS

Growth performance parameters

The growth performance parameters of *O. niloticus* fed different experimental diets are presented in Table (3). The interaction between the protein sources and chitosan forms showed that fish meal (FM) diet had the highest ($P < 0.05$) final body weight (FBW), body weight gain (BWG) and specific growth rate (SGR) (31.5, 15.83 g/fish and 1.00%, respectively).

Fish growth was negatively affected neither by FM-based diet supplemented with chitosan (CS) or with chitosan nanoparticles (CSNP) nor by FM substitution with gluten meal (GM). On the other hand, fortifying GM-based diet with CSNP enhanced the fish growth, as no significant difference was noticed among the latter and FMCS or FMCSNP diets. The survival rate differed significantly ($P < 0.05$) among the experimental treatments. Fish fed FMCS and FMCSNP diets showed the highest survival rate (100%), which is considered as a direct effect of the CS forms. Similar observations were noticed in fish fed GMCS and GMCSNP diets.

Table 3. Growth performance parameters of *O. niloticus* fed fish meal and gluten meal-based diets supplemented with CS or CSNP (mean± SEM) (n=3)

Parameters	FM-based diets			GM-based diets					Protein source X CS forms effect (*P*-value)
	FM	FMCS	FMCSNP	GM	GMCS	GMCSNP	Protein source effect (*P*-value)	CS forms effect (*P*-value)	
FBW (g/fish)	31.5 ±0.95^{a}	28.40 ±1.49^{ab}	28.62 ±0.16^{ab}	26.16 ±0.16^{b}	26.13 ±1.13^{b}	27.80 ±1.69^{ab}	0.021	0.417	0.195
SGR (%)	1.00 ±0.00^{a}	0.85 ±0.05^{ab}	0.89 ±0.00^{ab}	0.73 ±0.05^{b}	0.74 ±0.05^{b}	0.81 ±0.10^{b}	0.009	0.454	0.225
Survival rate (%)	90 ±3.33^{ab}	100 ±0.00^{a}	100 ±0.00^{a}	87 ±6.67^{b}	97 ±3.33^{ab}	90 ±3.33^{ab}	0.108	0.079	0.53

Different superscripts in each row designate significant difference ($P < 0.05$) by Duncan's test
Abbreviations: FBW, Final body weight; SEM, standard error of mean; SGR, Specific growth rate

Feed utilization parameters

The feed utilization parameters of *O. niloticus* fed different experimental diets are shown in Table (4). It is noteworthy mentioning that FM-based diets showed better values regarding FCR and PER than GM-based diets. The best FCR and PER values were noted in fish fed FM diet (1.72 and 1.89, respectively), whereas the worst FCR and PER values were observed in fish fed GMCS diet (2.47 and 1.37, respectively). Upon supplementation of GM diet with CSNP, the FCR and PER values improved (2.19 and 1.55, respectively) in comparable with other GM-based diets.

Table 4. Feed utilization parameters of *O. niloticus* fed fish meal and gluten meal-based diets supplemented with CS or CSNP (mean± SEM) (n=3)

Parameters	FM-based diets			GM-based diets					Protein source X CS forms effect (*P*-value)
	FM	FMCS	FMCSNP	GM	GMCS	GMCSNP	Protein source effect (*P*-value)	CS forms effect (*P*-value)	
FI (g/fish)	27.22 ±1.40	22.52 ±1.24	24.73 ±1.38	25.17 ±0.36	26.27 ±1.48	26.34 ±2.11	0.379	0.486	0.202
FCR	1.72 ±0.02^{c}	1.75 ±0.08^{c}	1.86 ±0.12^{bc}	2.40 ±0.14^{a}	2.47 ±0.08^{a}	2.19 ±0.13^{ab}	0.001	0.719	0.211
PER	1.89 ±0.02^{a}	1.87 ±0.09^{a}	1.75 ±0.12^{ab}	1.43 ±0.08^{c}	1.37 ±0.05^{c}	1.55 ±0.09^{bc}	0.001	0.868	0.218
PPV (%)	30.03 ±1.16	30.17 ±2.16	33.95 ±4.33	31.98 ±0.32	26.92 ±1.32	33.73 ±2.66	0.803	0.162	0.575

Each value denotes means± SEM, SEM: standard error of mean (n=3).
Different superscripts in each row designate significant difference ($P < 0.05$) by Duncan's test
Abbreviations: FCR, Feed conversion ratio, PER, Protein efficiency ratio, PPV, Protein productive value; SEM, Standard error of mean

Chemical composition of *O. niloticus* carcass

The chemical composition of *O. niloticus* fed different experimental diets is presented in Table (5). Fish fed FM-based diets recorded higher moisture content than those fed GM-based diets. CSNP supplementation to FM or GM diet significantly decreased the moisture content. The interaction results showed that fish fed diets supplemented with CSNP showed higher protein content in comparison with those fed diets fortified with CS. The protein source and chitosan forms affected significantly ($P < 0.05$) the crude lipid content. Fish fed GM-based diets showed higher lipid content than FM-based diets. Dietary fortification with CSNP resulted in decrease in fish lipid content.

Table 5. The chemical composition of *O. niloticus* carcass fed fish meal and gluten meal-based diets supplemented with CS or CSNP (as wet basis %) (mean± SEM) (n=3)

Parameters	FM-based diets			GM-based diets					Protein source X CS forms effect (*P*-value)
	FM	FMCS	FMCSNP	GM	GMCS	GMCSNP	Protein source effect (*P*-value)	CS forms effect (*P*-value)	
Moisture	75.11 ±1.50^{ab}	76.25 ±0.25^{a}	72.74 ±0.75^{bc}	72.03 ±0.85^{b} ^{c}	72.31 ±0.05^{bc}	71.36 ±1.20^{c}	0.011	0.129	0.433
Crude protein (CP)	14.29 ±0.19^{b}	14.26 ±0.27^{b}	15.78 ±0.55^{ab}	16.57 ±0.32^{a}	15.55 ±0.27^{ab}	16.62 ±1.01^{a}	0.013	0.114	0.412
Crude lipid	4.40 ±0.28^{c}	4.48 ±0.13^{c}	4.32 ±0.24^{c}	5.22 ±0.21^{b}	6.05 ±0.14^{a}	4.37 ±0.01^{c}	0.002	0.008	0.019
Ash	4.79 ±0.51	5.58 ±0.25	5.05 ±0.15	4.52 ±0.13	5.33 ±0.15	5.26 ±0.53	0.708	0.124	0.731

Different superscripts in each row designate significant difference ($P < 0.05$) by Duncan's test
SEM, standard error of mean

Fatty acids profile of *O. niloticus* carcass

In the present study, the chitosan forms affected significantly the total omega-3 (Σ n-3) level in the fish whole body. Within FM-based diets, the supplementation of CS and CSNP increased significantly ($P < 0.05$) the Σ n-3 level (2.83 and 2.23%, respectively) compared to fish fed FM diet (1.52%). On the other hand, dietary supplementation of CSNP to GM-based diet increased

significantly the Σ n-3 level in fish (2.27%) compared to GM and GMCS diets (1.02 and 0.91%, respectively). Among fish fed GM-based diets, the lowest significant ($P < 0.05$) total omega-6 (Σ n-6) and n-6/n-3 values were recorded in GMCSNP treatment (Table 6).

Table 6. Fatty acids profile of *O. niloticus* whole body (% of total fatty acids) fed fish meal and gluten meal-based diets supplemented with CS or CSNP (mean± SEM) (n=3)

Fatty acids	FM-based diets			GM-based diets		
	FM	**FMCS**	**FMCSNP**	**GM**	**GMCS**	**GMCSNP**
C10:0	ND[1]	ND	ND	ND	ND	0.21±0.1^{a}
C12:0	ND	ND	ND	ND	ND	0.17±0.06^{a}
C14:0	1.06±0.05^{c}	1.34±0.11^{b}	1.33±0.00^{b}	1.06±0.04^{c}	1.075±0.035^{c}	1.66±0.04^{a}
C15:0	0.29±0.02^{b}	0.42±0.02^{ab}	0.36±0.01^{ab}	0.34±0.01^{ab}	0.30±0.01^{b}	0.48±0.11^{a}
C16:0	15.45±1.35	15.54±0.86	15.14±0.21	14.7±0.03	14.48±0.35	15.69±0.06
C16:1n-7	1.84±0.17^{c}	2.52±0.18^{a}	2.46±0.05^{ab}	2.00±0.09^{bc}	2.09±0.02^{abc}	2.4±0.16^{ab}
C16:3n-4	0.12±0.01^{ab}	0.15±0.02^{ab}	0.13±0.00^{ab}	0.13±0.02^{ab}	0.11±0.00^{b}	0.21±0.05^{a}
C17:0	0.70±0.03^{d}	0.80±0.00^{b}	0.78±0.01^{bc}	0.76±0.01^{bc}	0.72±0.02^{cd}	0.90±0.01^{a}
C18:0	5.68±0.48	5.40±0.17	5.08±0.18	4.96±0.09	4.81±0.10	5.89±0.82
C18:1n-9	25.10±1.20	22.64±1.14	22.30±0.37	25.08±0.49	23.98±0.75	25.02±1.25
C18:1n-7	1.77±0.15^{c}	2.19±0.01^{a}	2.10±0.00^{ab}	1.82±0.14^{bc}	1.80±0.02^{bc}	2.20±0.04^{a}
C18:2n-7	ND	0.13±0.00	0.11±0.00	0.12±0.01	0.13±0.01	0.12±0.00
C18:2n-6	37.37±3.17	37.99±2.19	39.68±0.12	38.74±0.31	39.39±0.33	34.04±2.32
C18:2n-4	0.37±0.03	0.36±0.04	0.35±0.04	0.34±0.02	0.33±0.05	0.29±0.02
C18:3n-6	1.20±0.10^{a}	1.14±0.18^{a}	1.31±0.12^{a}	1.33±0.17^{a}	1.66±0.18^{a}	1.08±0.22^{a}
C18:3n-3	0.59±0.02	0.78±0.12	0.66±0.02	0.67±0.10	0.61±0.02	0.72±0.14
C20:0	0.29±0.04	0.27±0.00	0.28±0.02	0.26±0.00	0.24±0.00	0.25±0.02
C20:1n-9	ND	ND	ND	ND	0.77±0.02	1.03±0.23
C20:2n-6	1.63±0.18^{ab}	1.41±0.06^{ab}	1.42±0.02^{ab}	1.68±0.11^{a}	1.66±0.01^{a}	1.32±0.01^{b}
C20:3n-6	0.69±0.08^{b}	0.71±0.04^{b}	0.71±0.05^{b}	0.80±0.01^{ab}	0.91±0.03^{a}	0.63±0.06^{b}
C20:4n-6	1.29±0.02^{b}	1.47±0.18^{ab}	1.55±0.09^{ab}	1.43±0.13^{ab}	1.81±0.21^{a}	1.30±0.07^{b}
C20:5n-3	0.13±0.01^{a}	ND	ND	ND	ND	0.55±0.31^{a}
C22:0	0.19±0.04	0.12±0.01	ND	0.20±0.01	4.64±4.51	0.08±0.08
C22:1n-9	0.33±0.03	ND	ND	0.25±0.04	0.20±0.04	0.20±0.07
C22:4n-6	0.53±0.12	0.68±0.13	0.63±0.02	0.62±0.07	0.81±0.11	0.52±0.09
C22:5n-6	0.77±0.1^{ab}	0.72±0.14^{ab}	0.66±0.02^{b}	0.96±0.22^{ab}	1.25±0.14^{a}	0.75±0.21^{ab}
C22:5n-3	0.16±0.04^{b}	0.60±0.14^{a}	0.45±0.02^{a}	ND	ND	ND
C22:6n-3	0.64±0.10^{bc}	1.51±0.00^{a}	1.12±0.13^{ab}	0.35±0.06^{c}	0.30±0.04^{c}	1.03±0.39^{ab}
Σ SFA[2]	23.66±2.01	23.90±1.18	22.97±0.41	22.29±0.11	26.27±4.04	24.96±0.59
Σ MUFA[3]	29.04±1.55^{ab}	27.35±1.31^{ab}	26.87±0.32^{b}	29.15±0.21^{ab}	28.85±0.73^{ab}	30.85±1.35^{a}
Σ PUFA[4]	45.38±3.76	47.54±2.72	48.67±0.52	47.07±0.07	48.89±1.14	42.34±1.88
Σ HUFA[5]	5.85±0.44	7.12±0.42	6.55±0.27	5.85±0.27	6.75±0.53	6.08±0.38
Σ n-3[6]	1.52±0.04^{c}	2.89±0.26^{a}	2.23±0.12^{b}	1.02±0.16^{cd}	0.91±0.06^{d}	2.27±0.22^{b}
Σ n-6[7]	43.48±3.77^{ab}	44.14±2.94^{ab}	45.97±0.36^{ab}	45.58±0.19^{ab}	47.51±1.01^{a}	39.66±1.64^{b}
n-6/n-3	28.61±3.32^{b}	15.47±2.433^{b}	20.62±0.99^{b}	45.85±7.38^{a}	52.36±2.34^{a}	17.57±0.98^{b}

SEM, standard error of mean

Different superscripts in each row designate significant difference ($P < 0.05$) by Duncan's test

[1]Not detected

[2]Total saturated fatty acids, including: C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0

[3]Total mono-unsaturated fatty acids, including: C16:1n-7, C18:1n-9, C18:1n-7, C20:1n-9 and C22:1n-9

[4]Total polyunsaturated fatty acids, sum of all fatty acids with chain length ≥ 18 carbon atoms and ≥ 2 double bonds

[5]Total highly unsaturated fatty acids, sum of all fatty acids with chain length ≥ 20 carbon atoms and ≥ 2 double bonds.[6]Total omega 3, including: C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3

[7]Total omega 6, including: C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6 and C22:5n-6

Histological structure of the liver

The liver of fish fed FM diet appeared as a continuous mass of hepatocytes that radiated from central vein, forming a cord-like pattern. 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 (Figure 2A). 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 (Figure 2A).

The histological architecture of liver of FMCS and FMCSNP treatment resembled FM treatment (Figs. 2B and 2C). Minimal histopathological changes were found in the liver of fish fed GM diet, such as: congestion of blood sinusoids and necrosis (Figure 2D). Supplementation of GM-based diets with CS and CSNP improved the histological architecture of the liver, which became exactly like the FM treatment (Figures 2E and 2F).

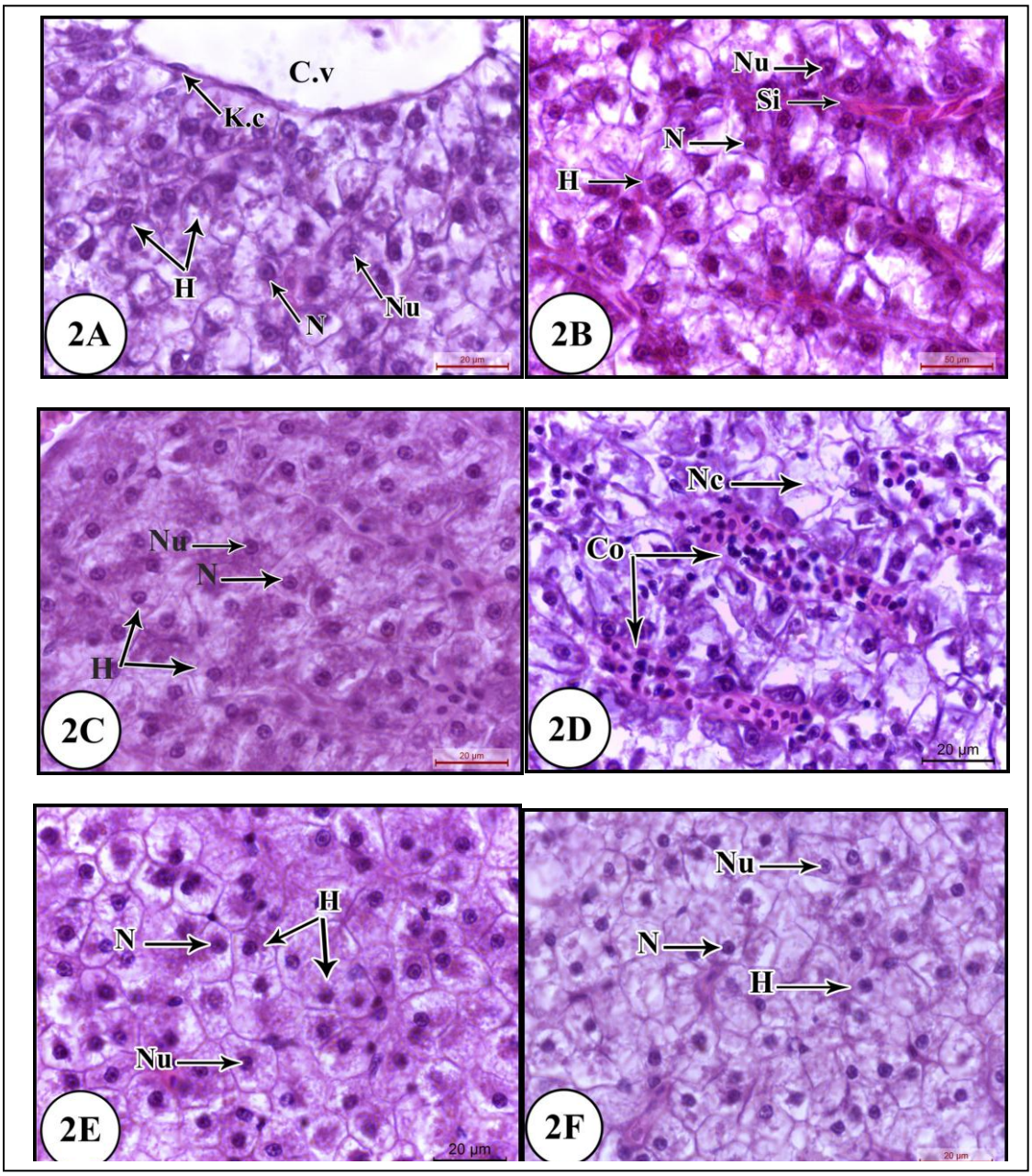

Fig. 2. A photomicrograph of S. of liver of *O. niloticus* fed (A) FM, (B) FMCS, (C) FMCSNP, (D) GM, (E) GMCS and (F) GMCSNP diets.

Abbreviations: Co, congestion; C.v, central vein; H, hepatocyte; K.c, kupffer cell; N, nucleus; , Nc, necrosis; Nu, nucleolus, Si, sinusoid.

DISCUSSION

The dietary protein source had a recorded effective impact on fish growth. Fish meal (FM) is a favored protein source due to its well-balanced amino acids profile, palatability and growth potential **(Al-Thobaiti *et al*., 2018)**, which is deficient in plant protein sources **(Watanabe, 2002; Mukhtar *et al*., 2017)**. Growth deterioration detected in many fish species, as for instance, in *O. niloticus*, resulted from the total substitution of fish meal with gluten meal (GM), is attributed to the deficiency of lysine **(Metwalli, 2013; Mukhtar *et al*., 2017)**. Lysine is remarkably a significant fish feed component that leads to high growth performance and fish population wellness **(Zhou *et al*., 2007:El-Husseiny *et al*., 2017)**. Moreover, lysine was proved to enhance the protein deposition in the fish body **(Hamid *et al*., 2016)**. On the other hand, GM was recorded as an enteritis inducer which negatively affects the gut health in *Scophthalmus maximus* **(Bai *et al*., 2019)**.

In the current study, the recorded decline in the growth performance of fish fed FM diets supplemented with chitosan agrees with the findings of **Shiau and Yu (1999)** in *O. niloticus* X *O. aureus* and that of **Geng *et al*. (2011)** in *Rachycentron canadum*, especially the diets with high FM inclusion. The previous authors added that fish growth performance decreased as CS supplementation increased. Reversely, the present result disagrees with that of **Gopalakannan and Arul (2006)**, who used CS concentration of 1% and recorded acceleration in the growth rate of *Cyprinus carpio* compared to the control.

Notably, chitosan can bind to both fatty acids and amino acids **(Wydro *et al*., 2007; Gawad & Ibrahim, 2012)**. Meanwhile, not all CS is absorbed in the gastrointestinal tract; some of which excrete in the feces **(Maezaki *et al*., 1993)**. The recorded decline in the growth of fish fed FMCS diet indicates that the excreted CS might negatively affected the amino acids blood profile; causing imbalanced amino acids which subsequently decreased the efficacy of protein synthesis.

On the other hand, an improvement in the growth performance parameters was attributed to the dietary fortification of GM-based diets supplemented with CSNP, having GM as an enteritis inducer in fish **(Bai *et al*., 2019)**. In addition, CSNP might have improved the microbial community structure of the gut. This improvement might be attributed to the fact that CSNP might inhibit potential pathogens, enhance the population of beneficial microorganisms, and/or enhance the microbial enzyme activities in fish gut that consequently improve feed digestibility and nutrient absorption/assimilation **(Abd El-Naby *et al*., 2019b)**. It is worthy to mention that, compared to CS, CSNP of 0.5% was more efficient due to the higher bioavailability; it remains for a long time in the blood stream, promotes the digestion and absorption of nutrients and enhances the growth of *O. niloticus* **(Abd El-Naby *et al*., 2019b; Wang & Li, 2011)**. Eminently, the small size and large surface area of CSNP may be another reason that played a role in enhancing the growth of fish fed GMCSNP diet.

Furthermore, the enhancement of the survival rate of fish fed diets supplemented with CS and CSNP indicates that, being natural products, neither CS nor CSNP had toxic effect on the

fish under study. Both CS and CSNP are considered immunostimulants; triggering the immune and the antioxidant systems. This means that both have the ability to reduce or destruct any pathogen **(Abdel-Tawwab *et al*., 2019)**.

Protein source seems to be the direct factor that affected the ratios of both feed conversion (FCR) and protein efficiency (PER), where fish fed FM-based diets recorded better FCR and PER values compared to GM-based diets. This observation coincides with that of **Metwalli (2013)**, who found that the substitution of FM with GM up to 75% affected neither the FCR nor the PER, recording best values in FM diet. It was noticed that, the complete substitution of FM with GM had a negative effect on both FCR and PER. Moreover, the FM diet possesses a well-balanced amino acids profile (Table 2). The lack of an indispensable amino acid, as lysine in GM led to poor utilization of the dietary protein, and consequently, reduced fish growth and decreased feed efficiency **(Halver & Hardy, 2002)**. Additionally, the present results showed that the dietary protein was better utilized in FM-based diets compared to GM-based ones. **Metwalli (2013)** postulated that, the complete substitution of FM with GM decreased the PER. This might be due to the deficiency of essential amino acids in the diet. **Wassef *et al*. (2003)** and **El-Ebiary (2005)** added that, the deficiency of lysine and methionine in GM diet was due to the reduced digestion coefficient of the protein. The improved values of both FCR and PER upon fortification of GM-based diets with CSNP are similar to those recorded in the study of **Abd El-Naby *et al*. (2019b)** who detected the best values for the two variables upon adding 0.5% CSNP to the feed of *O. niloticus*.

The high lipid content in fish carcass may cause more accumulation in the moisture content of fish fed GM-based. **Jobling *et al*. (1998)** deduced that the relation between both factors is inversely proportional. On the other hand, moisture increase in fish fed FM-based diets may be related to the increase in ash content with respect to salt consumption that induced water retention **(Rakova *et al*., 2017)**. It was found that CSNP supplementation to either FM or GM-based diets decreased the moisture content of fish carcass, indicating higher nutritive content of fish compared to other treatments. Due to their large surface area and small particle size, a decrease in moisture content recorded in fish fed diets supplemented with CSNP may have caused the observed high protein content. It is worth noting that, the superiority of CSNP over CS in binding to amino acids was determined. Moreover, their small size increases their bioavailability, thus leads to higher absorption along the gut **(Wang & Li, 2011)**. In other words, compared to CS, the CSNP causes less excretion in the feces, less loss of amino acids and better amino acids profile in blood and leads to higher protein content in the tilapia carcass. **Gopalakannan and Arul (2006)** reported that the increase in the CP in case of CSNP can be attributed to specific metabolic pathways in fish compared to ordinary CS and could have an essential role in promoting the absorption and assimilation of nutrients in case of lower levels in *O. niloticus*.

Fish fed GM-based diets recorded higher crude lipid content compared to those fed FM-based diets. The deficiency in the amino acids in GM-based diet, especially in term of lysine, may cause more metabolisms of dietary amino acids generating energy **(Cheng & Hardy, 2003)**.

In this context, fish may convert non-utilized amino acids to lipid **(Jurss & Bastrop, 1995)**. Compared to those supplemented with lysine, plant-based diets that lacked lysine showed higher lipid deposition **(Cheng *et al*., 2003)**.

It seems that supplementing diets with CSNP enhanced protein synthesis and promoted energy utilization because higher protein content was recorded in parallel with lower lipid accumulation in fish carcass. The decrease in crude lipid content upon dietary supplementation with CSNP concurs with the finding of **Wang and Lee (2011)**. Conversely, **Abd El-Naby *et al*. (2019b)** observed an increase of crude lipid upon fortification of diets with CSNP.

It is well-known that *O. niloticus* is rich with omega-6 while omega-3 is reversely deficient **(Young, 2009)**. High n-6 to n-3 ratio, which causes an increase in arachidonic acid (C18:2n-6), can cause various diseases, such as cardiovascular diseases, inflammation and cancers **(Young, 2009)**. Optimal and safe ratio between n-6 and n-3 is notedly1:1 **(Candela *et al*., 2011)**. One of the very interesting recorded results in the present study is that the chitosan forms affected significantly the total omega-3 (Σ n-3) level in the fish whole body. Within FM-based diets, the supplementation of CS and CSNP elevated the Σ n-3 level significantly compared to fish fed FM diet. **Chiu *et al*. (2017)** reported that Cs enhanced the Σ n-3 level, and decreased the triglycerides level. The decrease of the total n-6 and n-6/n-3 ratio was obvious upon the fortification of GM-based diet with CSNP that could be induced either through binding of the cationic groups of CSNP and the functional groups of the fatty acids or as a result of the β-oxidation and catabolism of the fatty acids to produce energy **(Candela *et al*., 2011)**.

Histology of aquaculture species plays a significant role in understanding the pathological alteration related to nutritional sources **(Jimoh *et al*., 2020)**. The liver, for instance, is a vital organ that shares in many essential metabolic processes, such as regulation of protein and carbohydrate metabolism, as well as detoxification and storage of harmful metabolites **(El-Hady & Abdel-Hameid, 2009)**. Some minimal histopathological changes were found in the liver of fish fed GM diet among which the congestion of blood sinusoids and necrosis were recognized. These observations correspond with those of **Ismail *et al*. (2015)** examining *O.* niloticus and those of **Lopez *et al*. (2015)** in their study conducted on *Totoaba macdonaldi*. The aforementioned authors added that those alterations might be due to the deficiency in some essential amino acids and unbalanced nutrients in the plant-based diets. Moreover, **Mukhtar *et al*. (2017)** deduced that, corn gluten is deficient in essential amino acids; one of which is lysine that regulates the carnitine synthesis in liver. Carnitine plays an important role in transferring long chain fatty acids to the mitochondria to stimulate beta-oxidation as an energy source. Hence, the deficiency of lysine would cause an accumulation of fats histologically presented as numerous vacuolations in the hepatocytes. In this respect, **Mahbub *et al*. (2018)** confirmed the aforementioned interpretation, assessing that the deficiency of lysine showed histopathological changes in the liver, including compact hepatocytes and vacuolations. Nevertheless, it is assumed that, the decrease in the lysine level may be contributed to other unknown factors that may cause this mild histopathology. The improvement of the liver architecture resulted from the dietary addition of CS and CSNP is simultaneous with the decline of the ALT and AST levels

(El-Naggar *et al*., 2021). **Abdel-Naby *et al*. (2019b)** reported that both variables (ALT and AST) are indicators of hepatotoxicity. Ozcelik et al. On the other hand, CS and CSNP were identified significant in the hepatocytes protection against oxidative stress **(Ozcelik *et al*., 2014)**. The previous authors added that, the amelioration of ALT and AST might be related to the antioxidant activity of CS and CSNP; their efficacy to get rid of free radicals and reactive oxygen species (ROS) was determined.

CONCLUSSION

The supplementation of GM-based diets with CSNP improved growth performance, FCR and omega-3 levels in fish carcass and liver histology, whereas they reduced fish mortality with respect to GM diet. CSNP could be considered as a functional ingredient for producing functional food (fish with high omega-3). The present results revealed that adding CSNP as an additive to plant-based aqua feed is recommended to reduce production cost, and minimize aquatic environmental impacts as well.

FUNDING

This article is part of results of Project ID: 9450781006 – ASRT JESOR 2015, entitled 'Novel extraction and industry from wastes and exoskeleton of the introduced freshwater crawfish', so we thank for funding this work.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Rania Ahmed, Associate Professor of Fish Nutrition, Fish Nutrition Lab (FNL), Department of Animal production, Faculty of Agriculture, Cairo University for reading and revising the article, we appreciate her great effort.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author. The data are not publicly available to preserve privacy of the data.

REFERENCES

Abd El-Naby, A.S.; Al-Sagheer, A.A.; Negm, S.S. and Mohammed, A.E. (2019 a). Dietary combination of chitosan nanoparticle and thymol affects feed utilization, digestive enzymes, antioxidant status, and intestinal morphology of *Oreochromis niloticus*. Aquacult., http:// doi.org/10.1016/j. aquaculture.2019.734577.

Abd El-Naby, F.S.; Naiel, M.A.E.; Al-Sagheer, A.A. and Negm, S.S. (2019 b). Dietary chitosan nanoparticles enhance the growth, production performance, and immunity in *Oreochromis niloticus*. Aquacult., https://doi.org/10.1016/j. aquaculture.2018.11.014.

Abdel-Tawwab, M.; Abdel Razek, N. and Abdel-Rahman, A.M. (2019). Immunostimulatory effect of dietary chitosan nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.). Fish shellfish Imunol., **88**: 254-258.

Ahmed, I.; Reshi, Q.M. and Fazio, F. (2020). The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquacult. Int., **28**: 869-899.

Al-Thobaiti, A.; Al-Ghanim, K.; Ahmed, Z.; Suliman, E.M. and Mahboob, S. (2018). Impact of replacing fish meal by a mixture of different plant protein sources on the growth performance in Nile Tilapia (*Oreochromis niloticus* L.) diets. Braz. J. Biol., **78(3)**: 525-534.

Amerio, M.; Vignali, C.; Castelli, L.; Fiorentini, L. and Tibaldi, E. (1998). Vegetable protein sources, protein evaluation indexes and ideal protein of sea bream (*Sparus aurata*). Rivista taliana di Acquacult., **33**:135-45.

Annet, C. S. (1985). A model to facilitate optimal aquaculture production by quantitavely relating fish growth to feed and other environmental resources. Ph.D. thesis, Michigan State University, East Lansing, Michigan, USA.

Aranaz, I.; Mengibar, M.; Harris, R.; Panos, I.; Miralles, B.; Acosta, G. G. and Heras, A. (2009). Funcional characterization of chitin and chitosan. Current Chem. Biol., **3**: 203-230.

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis of the Association Official Analytical Chemists, vol. 1. 16th edition. **Cunnif, P**. (ed.). vol. 1. AOAC, Arlington, USA. 1298 pp.

Association of Official Analytical Chemists (AOAC) (2012). Official Methods of Analysis. 19th edition, vol. 1, no. 985.01. AOAC, INC. Suite, Virginia 22201. USA. Chapter 3, 6 pp.

Association of Official Analytical Chemists (AOAC) (2016 a). Dumas method no. 968.06. In: ″Official Methods of Analysis″. 20th edition. **Latimer, G.W.Jr.** (ed.). AOAC, INC. Suite, Rockville, Maryland 20850-3250, USA. Chapter 4.

Association of Official Analytical Chemists (AOAC) (2016 b). Official Methods of Analysis of the Association of Official Analytical Chemists. 17th edition. 969.3 and 991.39 fatty acids in Oils and Fats Preparation of Methyl Esters Boron Tri Fluoride - AOAC-IUPAC Method Codex-Adopted-AOAC Method. Chapter 41, pp.19-20.

Bai, N.; Gu, M.; Liu, M.; Jia, Q.; Pan, S. and Zhang, Z. (2019). Corn gluten meal induces enteritis and decreases intestinal immunity and antioxidant capacity in turbot (*Scophthalmus maximus*) at high supplementation levels. PLoS ONE 14(3): e0213867. https://doi.org/10.1371/ journal.pone.0213867.

Brett, J.R. and Groves, T.D.D. (1979). Physiological energetics. In: ″Fish Physiology″. **Hoar, S.; Randall, D.J. and Brett, J.R.** (eds.). Acad. Press, NY, pp. 279-352.

Candela, C.G.; López, L.M.B and Kohen, V.L. (2011). Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Nutr. Hospit., **26(6)**: 323-329.

Cheng, Z.J. and Hardy, R.W. (2003). Dietary lysine in plant protein diets improves Rainbow trout performance, water quality. Glob. Aquacult. Advoc.–Feed Nutr., Section, 56-57.

Cheng, Z.J.; Hardy, R.W. and Usry, J.L. (2003). Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. Aquacult., **215(1-4)**, 255-265.

Chiu, C.-Y.; Chang, T.-C.; Liu, S.-H. and Chiang, M.-T. (2017). The regulatory effects of fish oil and chitosan on hepatic lipogenic signals in high-fat diet-induced obese rats. J. Food Drug Anal., **25**, 919-930.

Da, C.R.; Lunduh, T.; Lindberg, J.E. and Berg, H. (2016). Growth performance, feed utilization and biological indices of Tra catfish ***(Pangasianodon hypophthalmus)*** cultured in net cages in pond fed diets based on locally available feed resources. Int. Aquat. Res., **8**: 309-321.

Duncan, D.B. (1955). Multiple range and multiple F-tests. Biomet., **11**, 1-42.

El-Ebiary, E.H. (2005). Use of soybean meal and/ or corn gluten meal as partial substitutes for fish meal in Nile tilapia (*Oreochromis niloticus*) fingerling diets. Egypt. J. Aquat. Res., **31(2)**: 432-442.

El-Hady, M. and Abdel-Hameid, N. A. (2009). Histo pathological alterations on the liver of northern plains killifish (*Fundulus kansae*, Garman 1895) induced by exposure to pesticides (terbufos and atrazine). Egypt. J. Exp. Biol. (Zool.), **5**: 273-278.

El-Husseiny, O.M.; El-Haroun, E.R.; Goda, A.; Woodward, B. and Suloma, A. (2017). Effects of lysine and tryptophan supplementations in plant protein-based diets on the performance of Nile tilapia (*Oreochromis niloticus*). J. Appl. Aquacult., **29(3-4)**: 266-276.

El-Husseiny, O.M.; Hassan, M.I.; El-Haroun, E.R. and Suloma, A. (2018). Utilization of poultry by-product meal supplemented with L-lysine as fish meal replacer in the diet of African catfish *Clarias gariepinus* (Burchell, 1822). J. Appl. Aquacult., **30(1)**: 63-75.

El-Naggar, M. M.; Abou-Elmagd, W. S. I.; Suloma, A. M.; El-Shabaka, H. A.; Khalil, M. T. and Abd El-Rahman, F. A. A. (2019). Optimization and physico-chemical characterization of chitosan and chitosan nanoparticles extracted from the crayfish *Procambarus clarkii* wastes. J. Shellfish Res., **38(2)**: 385-395.

El-Naggar, M. M.; Salaah, S. M.; Ashraf, S.; Khalil, M. T. and Emam, W. W. M. (2022b). Impact of chitosan and chitosan nanoparticles on reducing heavy metals within the Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Biol. Fish., **26(2)**: 859 – 874.

El-Naggar, M.M. (2020). Effects of chitosan nanoparticles on the growth rate and reproductive performance of Nile tilapia, *Oreochromis niloticus*. Ph.D. thesis. Faculty of Science, Ain Shams Univ., Cairo, Egypt.

El-Naggar, M.M.; Haneen, D.S.A.; Mehany, A.B.M. and, Khalil, M.T. (2020). New synthetic chitosan hybrids bearing some heterocyclic moieties with potential activity as anticancer and apoptosis inducers. Int. J. Biol. Macromol., **150**: 1323-1330.

El-Naggar, M.M.; Medhat, F. and Taha, A. (2022a). Applications of chitosan and chitosan nanoparticles in fish aquaculture. Egypt. J. Aquat. Biol. Fish., **26(1)**: 23-43.

El-Naggar, M.M.; Salah, S.; El-Shabaka, H. A.; Abd El-Rahman, F. A. A.; Khalil, M. T. and Suloma, A. (2021). Efficacy of dietary chitosan and chitosan nanoparticles supplementation on health status of Nile tilapia, *Oreochromis niloticus* (L.). Aquacult. Rep., 19: 100628.

El-Sayed, A.F.M. (2006). Tilapia Culture. A.F.M. El-Sayed (ed.). CABI Pub.: Wallingford, GB, 256 pp.

El-Sayed, A.F.M. and Teshima, S. (1992). Protein and energy requirement of Nile tilapia, *Oreochromis niloticus*, fry. Aquacult., **103**, 55-63.

Food and Agricultural Organization (FAO) (2022). The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, Italy, FAO, 266 pp. https://doi.org/10.4060/cc0461en

Gawad, A.A. and Ibrahim, M.A. (2012). Spectroscopic analyses of chitosan interactions with amino acids. J. Comput. Theor. Nanosci., **9(8)**: 1-5.

General Authority for Fisheries Resources Development (GAFRD) (2022). Yearly Book for fish Production in Egypt, Agriculture Ministry.

Geng, X.; Dong, X.-H.; Tan, B.-P.; Yang, Q.-H.; Chi, S.-Y.; Liu, H.-Y. and Liu, X.-Q. (2011). Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, , *Rachycentron canadum*. Fish Shellfish Immunol., **31**: 400-406.

Gopalakannan, A. and Arul, V. (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquacult., **255**:179-187.

Halver, J.E. and Hardy, R.W. (2002). Fish nutrition (3rd edition). Acad. Press, New York, 824 pp.

Hamid, S.N.I.N.; Abdullah, M.F.; Zarina, Z.; Yusof, S.J.H.M. and Abdullah, R. (2016). Formulation of fish feed with optimum protein-bound lysine for African catfish (*Clarias gariepinus*) fingerlings. Procedia Eng., **148**: 361-369.

Hopkins, K.D. (1992). Reporting fish growth, a review of the basics. J.World Aquacult. Soc., **23**, 173-179.

Humason, G.L. (1972). Animal tissue techniques. 3rd edition. W. H. Freeman Company, San Francisco, 400 pp.

Hung, S.S.O.; Lutes, P.B.; Shqueir, A.A. and Conte, F.S. (1993). Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*)**.** Aquacult., **115**: 297-303.

Ismaiel, Y.M.; Khedr, N.A. and Ahmed, T.E. (2015). Effect of fish meal and plant protein alternatives on the histological picture of different organs on Nile tilapia in Egypt. Benha Vet. Med. J., **28(2)**: 273-282.

Jimoh, W.A.; Ayeloja, A.A.; Yusuf, O.Y.; Abdulsalami, S.A. and Shittu, MO.O. (2020). Histopathological changes in liver and kidney of sharptooth catfish fed on cooked *Jatropha curcas* seedmeal based diets. Savannah Vet. J., **3**: 52-59.

Jobling, M.; Koskel, J. and Savolainen, R. (1998). Influence of dietary fat level and increased adiposity on growth and fat deposition in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult. Res., **29**: 601-607.

Jurss, K. and Bastrop, R. (1995). Chapter 7 Amino acid metabolism in fish. Biochem. Mol. Biol. Fishes. **4**: 195-189.

Kaur, P.; Choudhary, A. and Thakur, R. (2013). Synthesis of chitosan-silver nanocomposites and their antibacterial activity. Int J Sci Eng Res, **4(4)**:869.

Khalil, M. T. (2014). Achieving sustainability in the fishery sector. Road map for green economy in Egypt. Workshop on 25/6/2014, Acad. Sci.Technol, Cairo, Egypt.

Koprucu, K. and Ozdemir, C. (2005). Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). Aquacult., **250**: 308-316.

Lopez, L.M.; Ibarra, M.F.; Vargas, I.B.; Galaviz, M.A. and True, C.D. (2015). Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (*Totoaba macdonaldi*). Fish Physiol. Biochem., **41**: 921-936.

Maezaki, Y.; Tsuji, K.; Nakagawa, Y.; Kawai, Y.; Akimoto, M.; Tsugita, T.; Takekawa, W.; Terada, A.; Hara, H. and Mitsuoka, T. (1993). Hypocholesterolemic effect of chitosan in adult males. Biosci. Biotech. Biochem., **57**: 1439-1444.

Mahbub, J.; Iqbal, M.M.; Hossain, Md.S.; Mian, S.; Jabed, Md.N. and Munzurul, H.A.K.M. (2018). Effects of L-Lysine on growth and histological changes in liver and intestine of GIFT Strain *Oreochromis niloticus*. Bangladesh J. Fish., **30(1)**: 27-35.

Metwalli, A.A.A. (2013). Effects of partial and total substitution of fish meal with corn gluten meal on growth performance, nutrients utilization and some blood constituents of the Nile tilapia *Oreochromis niloticus*. Egypt. J. Aquat. Biol. Fish., **17(1)**: 91-100.

Mukhtar, B.; Malik, M.F.; Shah, S.H.; Azzam, F.; Liaqat, S. and Liaqat, I. (2017). Lysine Supplementation in Fish Feed. Int. J. Appl. Biol. Foren., **1(2)**: 26-31.

Muzzarelli, R.; Baldassarre, V.; Conti, F.; Ferrara, P.; Biagini, G.; Gazzanelli, G. and Vasi, V. (1988). Biological activity of chitosan: ultrastructural study. Biomat., **9(3)**: 247-252.

National Research Council (NRC) (2011). Feed composition tables. In: ″Nutrient Requirements of Fish and Shrimp″. **Whitacre, P.T.** (ed.). Nat. Acad. Press, Washington, DC, pp. 334-347.

Nose, T. (1971). Determination of nutritive value of food protein in fish. 111. Nutritive value of casein, whitefish meal and soybean meal in rainbow trout fingerlings. Bull. Freshwat. Fish. Res. Lab., **21**: 85-98.

Ozcelik, E.; Uslu, S.; Burukoglu, D. and Musmul, A. (2014 a). Chitosan and blueberry treatment induces arginase activity and inhibits nitric oxide production during acetaminophen-induced hepatotoxicity. Pharmacogn. Mag., **10(2)**: 217-224.

Phumee, P.; Wei, W.Y.; Ramachandran, S. and Hashim, R. (2011). Evaluation of soybean meal in the formulated diets for juvenile *Pungusiandon hypophthalms* (Sauvage, 1878). Aquacult. Nutr., **17**:511-517.

Prabu, E.; Rajagopalsamy, C.; Ahilan, B.; Jeevagan, I.J.M.A. and Renuhadevi, M. (2019). Tilapia – An excellent candidate species for world aquaculture: A Review. Ann. Res. Rev. Biol., http://dx.doi.org/10.9734/arrb/2019/v31i330052.

Radhika-Rajasree, S.R. and Gayathri, S. (2014). Biocompatible Nanocomposite from crab shell waste, preparation, characterization and their possible application. Int. J. Pharm Bio Sci., **5(2)**: B758-B766.

Rakova, N.; Kitada, K.; Lerchl, K.; Dahlmann, A.; Birukov, A.; Daub, S.; Kopp, C.; Pedchenko, T.; Zhang, Y.; Beck, L.; Johannes, B.; Marton, A.; Müller, D.N.; Rauh, M.; Luft, F.C. and Titze, J. (2017).Increased salt consumption induces body water conservation and decreases fluid intake. J. Clin. Inv., **127(5)**: 1932-1943.

Shard, P.; Bhatia, A. and Sharma, D. (2014). Optimization and physico-chemical parameters on synthesis of chitosan nanoparticles by ionic gelation technique. Int. J. Drug Deliv., **6**: 58-63.

Shiau, S.Y. and Yu, Y.P. (1999). Dietary supplementation of chitin and chitosan depresses growth in tilapia, ***Oreochromis niloticus* × *O. aureus***. Aquacult., **179**: 439- 446.

Statistical Package for Social Sciences (SPSS) (2013). Computer Software, Ver. 17. SPSS Company, London, UK.

Wang, Y. and Li, J. (2011). Effects of chitosan nanoparticles on survival, growth and meat quality of tilapia, *Oreochromis nilotica*. Nanotoxicol., **5(3)**, 425-431.

Wassef, E.A.; Sweilam, M.A. and Attalah, R.F. (2003). The use of fermented fish silage as a replacement for fish meal in Nile tilapia (*Oreochromis niloticus*) diets. Egypt. J. Nutr. Feeds, **6 (Special Issue)**: 357- 370.

Watanabe, T. (2002). Strategies for further development of aquatic feeds. Fish. Sci., **68**: 242-252.

Wu, Y.V.; Rosati, R.; Sessa, D.J. and Brown, P. (1995). Utilization of corn gluten feed by Nile tilapia. Prog. Fish Cult., **57**: 305-309.

Wydro, P.; Krajewska, B. and Hac-Wydro, K. (2007). Chitosan as a lipid binder: a Langmuir monolayer study of chitosan–lipid interactions. Biomacromol., **8**: 2611- 2617.

Xu, Y.; Gallert, C. and Winter, J. (2008). Chitin purification from shrimp wastes by microbial deproteination and decalcification. Appl. Microbiol. Biotechnol., **79**: 687-697.

Young, K. (2009). Omega-6 (n-6) and omega-3 (n-3) fatty acids in tilapia and human health: a review. **60(S5)**: 203-211.

Zhou, Q.C.; Mai, K.S.; Tan, B.P. and Liu, Y.J. (2005). Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquacult. Nutr., **11(3)**: 175-182.

Zhou, Q.C.; Wu, Z.H.; Chi, S.Y. and Yang, Q.H. (2007). Dietary lysine requirement of juvenile cobia (*Rachycentron canadum*). Aquacult., **273**: 634-640.