Impact of chitosan and chitosan nanoparticles on reducing heavy metals within the Nile tilapia, Oreochromis niloticus

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ABSTRACT

The current study was conducted to assess the ability of chitosan and nanoparticles of chitosan to alleviate the effect of heavy metals in the Nile tilapia Oreochromis niloticus. 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 (water capacity=55 liters). The overall duration of the experiment was 82 days. Results revealed that fish fed on a fish meal-based diet recorded the highest retained heavy metals followed by those fed gluten meals. Moreover, supplementation of diets with chitosan and chitosan nanoparticles lowered the concentration of the retained heavy metals in the fish's whole body, especially chitosan nanoparticles. Consequently, it is recommended to use CS and CSNP, especially chitosan nanoparticles as feed additives for fish cultured in agricultural and sewage wastewaters, loaded with high concentrations of heavy metals to ameliorate the fish quality.

INTRODUCTION

The last years witnessed a remarkable spread of aquaculture in Egypt (Kaleem & Sabi, 2021). An eminent increase in aquaculture surpassed the capture of fisheries during the past couple of decades. It is assumed to serve as a principle source of aquatic animal requirements (Ahmed et al., 2020). In Egypt, agricultural drainage wastewater represents the main water source for aquaculture since the irrigation law prohibits the use of the Nile water in fish farming (Soliman, 2017). Interestingly, Khallaf et al. (1998) reported that, the drainage water is contaminated with domestic industrial effluents, sewage and agrochemicals that proved its harmful impact on the quality of farmed fish while negatively affecting the heath of human being an end user. Additionally, the drainage water may contain elevated concentrations of pesticides, runoff-derived fertilizers and heavy metals (Authman et al., 2013).

In the environment, heavy metals are enduring and non-biodegradable contaminants, causing various diseases to animals, fish and human (Bayomy et al., 2015). An accumulation of heavy metals was reported in the tissues and organs of fish entering the food chain and...
reaching the highest rate with respect to end-users (Akan et al., 2009). This, in return, changes the biological and structural functions of biomolecules (Newman, 1998).

Heavy metals are categorized into essential and non-essential. Essential heavy metals, such as Cu, Zn, Fe, Cr and Mn are essential for the living organisms, playing a role in the biological functions since they are required in low concentrations for the body welfare (Santos et al., 2014). The deficiency and the excess of these heavy metals are both detrimental (Santos et al., 2014). On the other hand, non-essential heavy metals, such as Cd and Pb are toxic metals released in water from fine suspended solids. Compared to the essential heavy metals, these metals do not have biological function in living organisms (Gati et al., 2016).

Heavy metal ion removal from aqueous solutions has grown in relevance in recent years, both for pollution management and raw material recovery. Adsorption-based technologies have proven to be one of the most viable alternatives proposed for the treatment of industrial wastewater contaminated by a wide variety of pollutants, both inorganic (Fu & Wang, 2011) and organic (Vocciante et al., 2019), due to their ease of operation, low processing and instrumentation costs and availability of different types of low-cost and environmentally friendly adsorbents. Metal oxides, polymers, activated carbon, carbon nanotubes, wastes of agriculture, and engineered and natural clays (Chen et al., 2011) have all been effectively used for the adsorption of heavy metals from aqueous solutions. This becomes more intriguing when low-cost adsorbent materials derived from industrial waste (Pietrelli et al., 2019) can be used. This would provide a double benefit to the environment while adhering to operational principles, such as the Circular Economy and "near-zero discharge" of harmful waste (Pietrelli et al., 2011) mandated by the most recent European legislation.

Chitosan (poly—(14)-2-amino-2-deoxy-D-glucose), a nitrogenous polysaccharide produced from chitin by partly deacetylating its acetoamide groups in strong alkaline solutions at 100°C, is a highly promising and economical substance in this area. (El-Naggar et al., 2019). Following cellulose, it is the second most prevalent polymer (Aranaz et al., 2009). Chitosan is non-toxic, biodegradable, biocompatible and highly soluble polymer (Shard et al., 2014). Containing both amino and hydroxyl groups, chitosan is highly potential for metal ion adsorption, which can function as chelation sites for metal ions. Amongst the most intriguing benefits of chitosan is its adaptability, since the material can be readily and physically changed to produce various polymer shapes, such as beads (Chiou & Li, 2003), membranes (Pietrelli & Xingrong, 2004) and/ or sponges (Ko, et al., 2010) for different applications. In addition, chitosan can be easily modified chemically to increase its applications (Guibal, 2004). Numerous critical evaluations on the diverse applications of chitosan as an ecologically benign biomaterial have recently been published, spanning from the medical industry to environmental protection and food technology (Gamage & Shahidi, 2007; Zhao et al., 2018; El-Naggar et al., 2020, 2022; Salaah et al., 2021).

A significant growth has been recorded in the field of nanotechnology (El-Naggar et al., 2019). Nanoparticles can resolve several matters related to the health production of human and animal and are apt to treat diseases they suffer as well (El-Naggar et al., 2020).
They are preferred due to their small size, which increases the available surface area to interact with biological support, enables efficient uptake by body cells and deep penetration into target sites, and increases the bioavailability of essential compounds. (Alishahi et al. 2011). The efficacy of nano chitosan have been proved, serving as immune enhancers in O. niloticus (El-Naggar et al., 2021) and a feed additive which improves the growth and meat quality of O. niloticus (Wang & Li, 2011; El-Naggar et al., 2022), and acting as a heavy metals chelating agent for water treatment (Abd-Elhakeem et al., 2016).

The Nile tilapia (Oreochromis niloticus) is the main farmed fish in East Africa and globally, it is the third most important cultured fish group after salmonids and carps (El-Sayed, 2006). The Nile tilapia is preferred as a source of protein for most of the Egyptians, especially those who have low income due to its suitable price (Kaleem & Sabi, 2021). The success of O. niloticus in aquaculture is assigned to its ability to survive at low oxygen tensions, ability to feed on wide range of foods (El-Sayed and Teshima, 1992; El-Sayed, 2019) and the strong immune system that enhances its potential to bear abiotic and biotic types of stress (Abdel-Tawwab et al., 2008; Salaah, 2021).

Unfortunately, there is no previous data in literature to support as a reference for the effectiveness of chitosan and nano chitosan for heavy metals removal from O. niloticus. Hence, to fill this gap, the current study was carried out to assess the effect of chitosan and nanoparticles of chitosan to detoxify heavy metals in the Nile tilapia.

**MATERIALS AND METHODS**

1. **Experimental fish and culture technique**

   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 located in Kafr El-Sheikh Governorate, Egypt. On the 21st of 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. Fish were grown out under laboratory recirculating aquaculture system (RAS) with a flow rate of 0.4 L min⁻¹. Fish were acclimatized to the experimental conditions for a week prior to the feeding trial. Fish were fed a floating diet of 30% crude protein at 2% of the fish body weight twice daily (10a.m. and 4p.m.). During the experiment, the fish were fed till apparent satiation. Fish weight was measured every 15 days, and the number of dead fish was recorded daily. The overall experiment lasted 82 days.

2. **Experimental design and diets**

   The experimental design was completely randomized with a 2 x 3 factorial design, with two protein sources, fish meal (FM) and gluten meal (GM), and three forms of chitosan (zero-chitosan, chitosan and chitosan nanoparticles). 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 (CS) or nanoparticles of chitosan (CSNP) (0.5%) to design
six isonitrogenous (30% crude protein) and isocaloric (4500 kcal/kg) for *O. niloticus* (NRC, 2011). The dose was managed bestowing to the technique of Wang & Li (2011).

The proximate composition of the experimental diets was analyzed in the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture (Table 1). The experimental diets were prepared by blending the ingredients into a homogeneous mixture and then passing it through a local minced meat machine, dried overnight at room temperature and stored in plastic bags at 4°C till furtherly used. The extraction of chitosan from *P. clarkii* wastes and the preparation of chitosan nanoparticles as well as their characterization were profoundly discussed in the study of El-Naggar et al. (2019).

3. **Water quality parameters**

Water temperature was registered using Senso Direct Oxi 200, and the pH value was determined using Milwaukee-pH600 digital pH meter twice a week. The alkalinity was estimated once a week by titration with sulfuric acid till the pH reached 4.5 (Boyd & Tucker, 1992). The total ammonia nitrogen (NH$_3$-N) and nitrite (NO$_2^-$) values were evaluated according to Boyd and Tucker (1992), using water analysis photometer (MultiDirect Lovibond) once a week. Following the instruction manual of the MultiDirect Lovibond, the ammonia concentration, including the ionized form (NH$_4^+$) and un-ionized form (NH$_3$) was calculated by the multiplication of the resultant value of NH$_3$-N by a conversion factor, 1.22 and 1.29, for NH$_3$ and NH$_4^+$, respectively.

4. **Heavy metals analysis**

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, fish were anesthetized using clove oil (40mg/L), and then six fish from each tank were randomly selected to serve as a final sample. The fish were then killed, oven-dried at 60-70°C, ground into homogenous powder and stored at -20°C until further analysis.

The determination of the heavy metals concentrations in fish carcass involves two main steps; namely, digestion and analysis. The digestion of the fish carcass, as well as heavy metals analysis were measured according to the Standard Method of the American Public Health Association (APHA-3111B, 2017) at the Central Laboratory of Faculty of Science, Ain Shams University. The digestion was performed using CEM Microwave Sample Preparation System (MDS-2000, USA).

- **Procedure of digestion:**

  One gram of the ground samples was placed in vessels and concentrated nitric acid (HNO$_3$) was added. The vessels were left overnight to allow sufficient reaction. Afterwards, the vessels were placed into a turntable connected to the system and a heating program and left to run till digestion completion. Eventually, the samples were left to cool for 5min, and the turntable was removed from the system.

  Heavy metals concentrations of the fish carcass were measured using Flame Atomic Absorption Spectrophotometer (Savant AA, GBC Scientific Equipment). The equipment is provided with acetylene as a source of fuel and air as an oxidant.
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- **Procedure of analyses:**

  The digested samples were added to 25 ml distilled water and placed in the spectrophotometer. The concentration of each heavy metal was measured at a certain wavelength and slit width. Six heavy metals were analyzed (Cu, Zn, Fe, Cd, Pb, Cr and Ni), and the resultant concentrations were expressed in mg/kg.

  The retained heavy metals were then calculated according to the following equation of Hernandez and Roman (2016), with slight modifications:

  \[(FBW \times HM_{\text{final}}) - (IBW \times HM_{\text{initial}})\]

  Where, FBW: fish final body weight; HM_{\text{final}}: heavy metal final concentration; IBW: fish initial body weight, and HM_{\text{initial}}: heavy metal initial concentration.

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

<table>
<thead>
<tr>
<th>Ingredient (g/100g)</th>
<th>FM</th>
<th>FMCS</th>
<th>FMCSNP</th>
<th>GM</th>
<th>GMCS</th>
<th>GMCSNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gluten</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Soybean</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Corn</td>
<td>8.43</td>
<td>7.93</td>
<td>7.93</td>
<td>8.43</td>
<td>7.93</td>
<td>7.93</td>
</tr>
<tr>
<td>Oil</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Bran</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin and mineral premix*</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Carboxy methyl cellulose</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Chitosan</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>Chitosan nanoparticles</td>
<td>-</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Proximate composition (% dry matter)

| Moisture | 7.8 | 7.9 | 7.7 | 7.8 | 8 | 8.6 |
| Crude protein | 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 (59 % 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 AW321 (acid-washed diatomaceous earth silica), 1,000 mg Agri-Vet Co., Cairo, Egypt.

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5. **Statistical analysis**

The experiment was conducted on triplicates which were analyzed using two-way analysis of variance (ANOVA) utilizing computer program Jeffreys’s Amazing Statistics Program (JASP 0.14) to detect the impact of the dietary protein source, chitosan (CS) forms and their interactions on the heavy metals retention in the whole fish. Tukey’s test (Abdi & Williams, 2010) was used to determine the differences among the experimental treatments.
RESULTS AND DISCUSSION

Water quality has a great impact on fish culture; it affects fish’s health status, behavior and growth. In the present experiment, the temperature varied from 21.53 to 26.90°C, with a mean of 24.51°C. Generally, water temperature has a remarkable impact on the initiation and the course of a number of fish diseases, with a considerable effect on the growth rate. The optimum temperature for rearing the Nile tilapia is about 25 to 27°C (DeWalle et al., 2011) (Table 2).

Throughout the experiment, the pH was directed towards the alkaline side, where it ranged between 7.97 to 8.45 with an average of 8.28. The optimal pH range for the Nile tilapia is from 6 to 9 (DeWalle et al., 2011) (Table 2). Destruction and mortality of fish species are associated with the alkaline pH values above 9.2 and acidity below 4.8 (FAO, 1993). The gills are the most susceptible organs to a great damage due to extremely high or low pH values, where hemorrhages may occur in the gills and on the lower part of the body. Moreover, excessive mucus secretion, often containing blood, can be observed in post mortem examination of the gills and skin (FAO, 1993).

During the current study, the total alkalinity fluctuated from 299.2 to 396 mg/L with a mean of 334.53 mg/L, and this confirms that the aquaria water is on the alkaline side (Table 2). The total alkalinity of water is the concentration of titratable bases, mainly carbonates (CO$_3^{2-}$) and bicarbonates (HCO$_3^-$), which is expressed as CaCO$_3$ equivalents. In order to achieve an optimum fish growth performance, water used for aquaculture must have a total alkalinity ≥ 20 mg L$^{-1}$ CaCO$_3$ (in fresh water) (Andrade et al., 2007). Notably, water with low total alkalinity is more susceptible to acidification than water with high total alkalinity.

Excreted ammonia exists in nontoxic ionized form NH$_4^+$ and un-ionized NH$_3$ form that is highly toxic for fish (Chervinski, 1982). There is a strong relationship between the toxicity of ammonia and the dissolved oxygen, CO$_2$ and pH. It is noteworthy mentioning that, the relationship between the toxicity of ammonia, the dissolved oxygen and CO$_2$ is inversely proportional, whereas it is directly proportional with the pH (Chervinski, 1982).

During the present trial, the ionized ammonia (NH$_4^+$) varied from 0 to 0.38 mg/L with a mean of 0.18 mg/L, while the unionized ammonia (NH$_3$) ranged from 0 to 0.40 mg/L, with an average of 0.17 mg/L. The average total ammonia nitrogen (NH$_3$-N) varied from 0 to 0.31 mg/L with a mean of 0.14 mg/L (Table 2). The toxicity of the total ammonia nitrogen is highly apparent if the level is higher than 1.0 mg/ L (DeLong et al., 2009).

During the nitrification process, ammonia is oxidized into nitrite (NO$_2$) then converted into nitrate (NO$_3$) through nitrifying bacteria grown on suspended organic matter. Nitrite has a toxic effect on fish, including tilapia, since it causes growth retardation and disturbs the physiological functions of the fish as well (Sudharsan et al., 2000). On the other hand, nitrate is relatively non-toxic to tilapia; on the contrary, prolonged exposure to inflated levels of nitrate may reduce the immune response and cause mortality (Plumb, 1997).
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Throughout the experiment, the level of nitrite (NO₂) ranged from 0.04 to 1.35mg/L, with a mean of 0.42 mg/L. The optimum tolerable range of nitrite (NO₂) for the culture of O. niloticus is 0.08 to 1mg L⁻¹ (Otoo et al., 2019) (Table 2). Nitrite concentration above 5mg/L is extremely toxic for tilapia (DeLong et al., 2009).

The present study showed that the parameters of the water quality under study were more or less convenient for rearing of O. niloticus under RAS system (Table 2). This result is coincided with that of Cruz and Ridha (2011).

Table 2. The water quality of the RAS system during the experimental study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>Optimum levels</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>21.53-26.90</td>
<td>25-27</td>
<td>DeWalle et al. (2011)</td>
</tr>
<tr>
<td>pH</td>
<td>7.97-8.45</td>
<td>6-9</td>
<td>DeWalle et al. (2011)</td>
</tr>
<tr>
<td>Total alkalinity (mg/L)</td>
<td>299.2-396</td>
<td>≥ 20</td>
<td>Andrade et al. (2007)</td>
</tr>
<tr>
<td>Total ammonia nitrogen (mg/L)</td>
<td>0-0.31</td>
<td>&lt; 1</td>
<td>DeLong et al. (2009)</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>0.04-1.35</td>
<td>0.08-1</td>
<td>Otoo et al. (2019)</td>
</tr>
</tbody>
</table>

In the aquatic ecosystem, heavy metals are regarded as the paramount pollutants, since they are present throughout the ecosystem and are detectable in trace amounts (Authman et al., 2015). They are detrimental for fish health, either above the threshold level, as in case of non-essential heavy metals, or below and above the permissible concentration, for essential heavy metals (Sfakianakis et al., 2015). Most of these metals are accumulated in tissues, causing fish poisoning, inducing pathological changes, affecting the reproduction and suppressing the immune system. Consequently, fish are used as bio-indicators monitoring heavy metals pollution (Authman et al., 2015).

The effect of CS and CSNP on the heavy metal retention in O. niloticus (whole fish) fed fish meal and gluten meal-based diets is illustrated in Table (3). Results showed that the chitosan forms affected significantly Cd (Fig. 1) and Ni retention (Fig.2). The highest significant retained heavy metal concentration was recorded in fish fed fish meal-based diet, followed by those fed gluten meal-based diet. Moreover, the dietary fortification with CS and CSNP lowered the retention of the Cd and Ni, especially CSNP supplementation.

On the other hand, no significant difference was recorded upon using CS and CSNP on the Cu, Zn, Fe, Pb and Cr retention in O. niloticus (Figs. 3-7). However, they differed numerically; fish fed fish meal-based diet recorded the highest retained heavy metals, followed by those fed gluten meal-based diet. Furthermore, the retention of the aforementioned heavy metals was numerically declined upon fortifying diets with CS and CSNP. Moreover, the lowest retained heavy metal concentration was recorded in fish fed diets supplemented with CSNP, especially gluten meal supplemented diets. Our study anticipates
that both dietary chitosan and nano chitosan have the capacity to reduce metals’ accumulation in the body and protect the vital organs function against metal intoxication.

Table 3. Impact of CS and CSNP on heavy metal retention in *O niloticus* (whole fish) fed fish meal and gluten meal-based diets

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>FM-based diets</th>
<th>GM-based diets</th>
<th>(P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>FMCS</td>
<td>FMCSNP</td>
</tr>
<tr>
<td>Cd</td>
<td>0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ni</td>
<td>0.022&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.021&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.017&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cu</td>
<td>0.144</td>
<td>0.082</td>
<td>0.066</td>
</tr>
<tr>
<td>Zn</td>
<td>0.224</td>
<td>0.120</td>
<td>0.107</td>
</tr>
<tr>
<td>Fe</td>
<td>0.395</td>
<td>-0.183</td>
<td>-0.413</td>
</tr>
<tr>
<td>Pb</td>
<td>0.067</td>
<td>0.058</td>
<td>0.036</td>
</tr>
<tr>
<td>Cr</td>
<td>0.012</td>
<td>0.007</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Each value denotes means (n=3). Different superscripts in each row designate significant difference (*P* < 0.05) by Tukey test. MSE: Mean standard error.

CS and CSNP are regarded as impressive metal ligands, giving rise to stable complexes with multiple metal ions *(Gamage & Shahidi, 2007)*. CS possesses an elevated adsorption capacity for various metal ions, such as Ni<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, Mg<sup>2+</sup> and Cu<sup>2+</sup> in acidic condition. Accordingly, it has been used for the recovery of metal ions in several industries *(Kurita, 1998)*. The detoxification process in aquatic animals have been well documented by two main mechanisms, comprising intracellular ligands: cytosolic metal binding compounds, such as metallothionein proteins and biomineralization; the relative relevance of both detoxification mechanisms varied according to species *(Marigomez et al., 2002)*. Chitosan has the tendency to form coordinate bond with the heavy metal forming a complex through the donation of its lone pair to the vacant orbital of the heavy metal *(Hussein et al., 2012)*.
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Fig. 2. Efficacy of CS and CSNP on Ni retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets

Fig. 3. Effect of CS and CSNP on Cu retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets

Fig. 4. Efficacy of CS and CSNP on Zn retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets
**Fig. 5.** Impact of CS and CSNP on Fe retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets

**Fig. 6.** Effect of CS and CSNP on Pb retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets

**Fig. 7.** Effect of CS and CSNP on Cr retention in *O. niloticus* (whole body) fed fish meal and gluten meal-based diets
Additionally, dietary chitosan and nano chitosan were found to enhance the antioxidant defense system in fish (El-Naggar et al., 2021; Salaah et al., 2021). One of the main antioxidant protective molecules in cells are thiols. Thiol groups are essential for metal detoxification in the liver, which may involve in the removal of metals from the body through the urine and gut (Eliaz et al., 2007).

Pietrelli et al. (2020) postulated that, chitosan is quite good in removing metal ions by adsorption. They used batch experiments to investigate the adsorption of chromium (III) by chitosan as a function of contact duration, pH, ion competition, and starting chromium (III) concentration. The adsorption rate was quite fast ($t_{1/2}$ 18min) and was modified by the presence of other metal ions. They discovered that chitosan has an outstanding loading capacity for chromium (III); however, metal ion adsorption was substantially impacted by pH. Approximately, 76% of the collected chromium was then readily eliminated by washing the utilized chitosan with 0.1 M EDTA solution. This research showed that chitosan has the potential to be a low-cost and effective agent for wastewater treatment and in-situ environmental restoration.

Furthermore, Setiyorini et al. (2022) studied the nano chitosan as a therapeutic agent for metal exposure in rats; chitosan showed a pharmacokinetics characterization as a cationic drug. Low molecular weight nano chitosan performed well in absorbing harmful metal in the blood circulation and gradually lowering its concentrations in the body. The reduction in metal content in the body was followed by a progressively improved body weight over the six-week nano chitosan supplementation. The molecular weight of chitosan is regarded to be crucial. Lower molecular weight chitosan has higher solubility and a considerable detoxification potential. Because of its amine (-NH$_2$) and hydroxyl (-OH) groups, chitosan is an excellent metal bio-sorbent. It combines with several heavy metals to generate complexes, and its amine groups serve as heavy metal coordination sites (Ngah & Fatinathan, 2010; Chauhan et al., 2012). On the other hand, Thilagar and Samuthirapandian (2020) documented low Pb accumulation in the fish receiving dietary chitosan supplements.

Ismaiel et al. (2015) compared plant protein-based diets to fishmeal-based diets and suggested that the latter might accumulate harmful substances, causing organs histopathological alterations. Moreover, it was noted that, the ash content was higher in FM-based diet than GM-based diet (Table 1). Thus, it is more efficient to supplement fishmeal-based diet with CSNP that has higher effective chelation capacity for heavy metal compared to CS. Small size, large surface and high stability may be the reasons for CSNP high ability of chelation (Seyedmohammadi et al., 2016; Zareie et al., 2019). The higher density of adsorption sites of CSNP increases the probability of coordinate bond formation between the CSNP functional group and heavy metals compared to CS (Yu et al., 2013).

**CONCLUSION**

In conclusion, it was found that the retained heavy metals in fish fed GM-based diets were lower than those fed FM-based diets. Moreover, the supplementation of CS and CSNP to fish meal and gluten meal-based diets lowered the concentrations of the retained heavy metals in the fish whole body, particularly CSNP. So, it is preferable to use the gluten meal in
the fish diet, as it has lower levels of heavy metals, and it is cheaper than the fish meal. Moreover, it is recommended to use CS and CSNP as feed additives for fish cultured in agricultural and sewage wastewaters, which are always loaded with high levels of heavy metals. Furthermore, CS and CSNP can be used in fish finisher diets to maintain the accumulated heavy metals at permissible levels for the sake of the consumer’s safe and health.

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Role of chitosan and chitosan nanoparticles in removing heavy metals from the Nile tilapia


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المخصص العربي

تقيم دور الكيتوزان والجسيمات النانوامريكا الكيتوزان على التقليل أو تأثير المعدان الثقيلة من المبطي النيلي، *Oreochromis niloticus*

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أجريت هذه الدراسة لتقديم دراسة لقيم دور الكيتوزان والجسيمات النانوامريكا للكيتوزان، المستخلصه من نمايات اقتصادية المياوية، للتحقيق من تأثير بعض المعدان الثقيلة في المبطي النيلي. لقد سمحت هذه التجربة باستخدام نظام تحليل المعاملات المتعدد (٢ × ٣) مع نظامين غذائيين مختلفين؛ نظام غذائي قائم على مسحوق السمك، ونظام غذائي قائم على مسحوق الجلوتين. ومع ثلاثة أشكال من الكيتوزان (صفر كيتوزان كجمعة ضامنة، ثم كيتوزان وحده، وجيب الجلوتين). وقد تم استخدام عدد ٢٧٠ من أصبيع الأسماك المبطي النيلي وحيد الجنس بمتوسط وزن٢٠٨ جم، وتم توزيعها عشوائياً على ٦ معالجات مختلفة يوقف ١٥ سمكة لكل حوض، وكررت كل معالجة ٣ مرات. واستمرت التجربة لمدة ٢٨ يوماً، وأعطت النتائج أن عتبة المحرونة على مسحوق السمك سجلت أعلى نسبة محسطة في المعالجات التالية: ٢٨ يوماً، وأوضحت النتائج أن العملية للمعدان الثقيلة في أسماك الأسماك كانت بلياً لمسحوق الجلوتين. بالإضافة إلى ذلك فقد وجد أن اضافة الكيتوزان والجسيمات النانوازيرية للكيتوزان قد نجحنا في تقليل النسب المحسطة من تلك المعالج في جسم الأسماك، خاصة الجسيمات النانوامريكا للكيتوزان. وبناءً على هذه النتائج، توصي الدراسة الحالية باستخدام مسحوق الجلوتين التنباتي بدلاً من مسحوق الأسماك وذلك لأحتاءه على مستويات مخفضة من المعالج الثقيلة، وأيضًا بإضافة الكيتوزان والجسيمات النانوازيرية للكيتوزان ككممل غذائي لمسحوق الجلوتين النباتي مترأ في مياه الصرف الزراعي و الصحي المحمولة بتركيزات عالية من المعالج الثقيلة و ذلك لتحسين جودة الأسماك وحفاظ على صحة الإنسان.