Effects of Chitosan and Chitosan Nanoparticles on Water Quality, Growth Performance, Survival Rate and Meat Quality of the African Catfish, Clarias Gariepinus

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Abstract:
The culture of African catfish (Clarias gariepinus) is hamstrung by high feed cost, and efforts to reduce it have been geared toward exploiting fishmeal alternatives which are hitherto competed for by both human and livestock. There is a growing need to search for waste products which can save this situation. A 91-day feeding trial was conducted in nine tarpaulin tanks with twenty C. gariepinus fingerlings each of average weight 2.79±0.05 g to check the effect of chitosan and its nanoparticles on growth performance and feed utilization. The basal diet (BD) which served as control was formulated to contain 40% crude protein. Two test diets were formulated to contain BD replaced with 5g kg⁻¹ diet of chitosan (BD+CH) and chitosan nanoparticles (BD+CHN) respectively. These were replicated thrice. Chitosan supplementation was found to significantly (P<0.05) improved daily weight gain, survival and meat quality P<0.05) of C. gariepinus fingerlings while chitosan nanoparticle supplementation significantly improved water quality, daily weight gain, feed utilization, survival as well as body composition. Chitosan nanoparticles from shells of arthropods and shellfish which lay waste globally has the potentials to revolutionize aquaculture. Fish nutritionists as well as farm managers should key into this platform technology.

Keywords:
Chitosan, Nanoparticles, Nutrition, Feed Conversion, Growth Performance, Body Composition

1. Introduction
The story of Aquaculture in Nigeria is basically that of African catfish. The African catfish species (*Clarias gariepinus or lazer*) are the most resistant and widely accepted and highly valued fish that are cultivated in Nigeria. According to Adewumi [1], it is the major species reared in Nigeria because its body has no scales, has omnivorous feeding habit and the flesh is very tasty and free from bones. Since 1970s, a significant change took place in aquaculture as better control over the production process enabled a number of new technologies and production practices to develop - Green growth. These changes dramatically improved the competitiveness of aquaculture products both as sources of basic food and as cash crops. The competitiveness of aquaculture has further been increased by the product development and marketing that was possible with a more predictable supply. The combined effect of productivity and market growth has made aquaculture the world’s fastest growing animal-based food sector of the last decades [2, 3]. However, the gap between supply and demand of fish and other fisheries products is wide.

Nevertheless, aquaculture is not without challenges. Production of high quality feed is one of the persistent bottlenecks holding back great rapid expansion of aquaculture in Nigeria. According to Fagbenro [4] and Isyaki [5] feed cost constitutes about 40 and 60 percent of the operational cost in aquaculture. This has hindered many investors from investing in aquaculture. Many approaches have been employed in an attempt to reduce feed cost in aquaculture [6] - [8]. However, most of the ingredients suggested for incorporation in fish diets are competed for by humans and livestock. Therefore, there is need to search for waste products to substitute fishmeal which is the costliest ingredient in fish diet.

Crab shells, snail shells and other shells of aquatic organisms which lay waste after the consumption of the edible part all over the world contain chitin which is deacetylated to varying degrees to yield chitosan, a nontoxic polyglucosamine widespread in nature [9]. Production and utilization of chitosan constitutes an economically attractive means of crustaceans shell waste disposal sought worldwide [10]. Its application in aquaculture arouses interests because of low side effects, growth performance enhancement, and immune functions improvement. Growth performance improvement by chitosan has recently been reported in rainbow trout [11], grouper [12] and sea bass [13]. However, none has been reported on *C. gariepinus* which is the major fish species cultured in Nigeria.

Moreso, the production of more effective fish feed is one of the numerous benefit of nanotechnology application in aquaculture [14]. Chitosan nanoparticles have attracted much attention because of their unique properties and interesting application [9]. Improved growth performance, survival and meat quality status of Nile tilapia (*Oreochromis niloticus*) by nanoparticles of chitosan has been experimented by Wang [15]. However, none has been reported on *C. gariepinus*. This research therefore seeks to study the effect of chitosan and chitosan nanoparticle on growth performance, feed utilization, survival and body composition of *C. gariepinus*.

2. Materials and Methods

2.1. Description of the Experimental Site

The 91 days’ experiment was carried out in the hatchery unit of the Department of Fisheries and Aquatic Environmental Management, University of Uyo, Uyo. (Latitude 5° 230”N and Longitude: 7° 553”E) Akwa Ibom State, Nigeria between April and June
2017. Nine tarpaulin tanks measuring 1 m³ were used. The containers were arranged in rows and column of 5x3 and provided with outlets which allowed the removal of water. The source of water was borehole and the tanks were filled using hose. Each tank has a water holding capacity of 3000 liters but water was maintained at the 1000 liters levels throughout the study.

2.2. Preparation of Chitosan/Chitosan Nanoparticle

Preparation of chitosan and nanoparticle was carried out at the Biochemistry Laboratory of the University of Uyo, Uyo, Nigeria according to Bolat[16]. The selected crab shells were cleaned, dried and ground into small shell pieces with the starting weight of 1000 g. Shells were then demineralized at room temperature by putting in 1.57 M of concentrated HCl for 5 hours under stirring and then dried at 60 °C with 233.93 g of shell remaining. Decolourization of the shell was carried out using 0.32% sodium hypochlorite (1:10W/V) concentration for 3 minutes. Deproteinization was later classically processed at 70 °C for 24 h using 1 M NaOH. (A. B. Enterprises Mumbai, Maharashtra) with 114.98 g of ground shell remaining. According to the homogeneous method, alkali chitin was prepared after dispersion of chitin in concentrated NaOH (30 g NaOH/45 g H₂O/ 3 g Chitin) at 25 °C for 5 h, followed by dissolution in crushed ice around 0°C. This method results in a soluble chitosan with an average degree of acetylation of 48%–55%. Chitosan nanoparticles were produced using ionic gelation method; incorporating a polyanion, TPP (triphosphophate) into the crude chitosan solution under constant stirring. The content was freeze-dried to obtain pure chitosan nanoparticles.

2.3. Experimental Diet Preparation

Experimental diets were formulated using feed formulation software for windows (Winfeed 2.8) which formulates feed by linear programming technique. All diets were formulated on dry matter basis. Blood meal, soybean and fishmeal (65%, anchovy) served as protein sources (Table 1). All ingredients were ground to less than 40 μm and stored in a freezer with nitrogen gas to prevent spoilage. Dry ice was used during grinding of all nutrients to avoid decomposition. The vitamin premix was prepared prior to diet preparation to ensure the freshness of ingredients. Diets formulated in percentage basis were converted to weight basis at 5 kg bag size. These were weighed individually using an electronic sensitive weighing balance (Model JY10S-01, China). Chitosan and chitosan nanoparticles (5 g kg⁻¹) were then added and combined thoroughly to obtain a homogenous mix. Cassava starch (Fisher Scientific, Pittsburgh, PA, USA) was used as a binder and placed in solution with 50% cool water (50 mL water/50 g starch).

Table 1. Formulation and proximate composition of the experimental diets (on dry matter basis)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>BD (control)</th>
<th>BD+CH</th>
<th>BD+CHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood meal¹</td>
<td>220.5</td>
<td>220.5</td>
<td>220.5</td>
</tr>
<tr>
<td>Yellow maize meal²</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Soybean meal³</td>
<td>215.0</td>
<td>215.0</td>
<td>215.0</td>
</tr>
<tr>
<td>Fishmeal⁴</td>
<td>245.0</td>
<td>240.0</td>
<td>240.0</td>
</tr>
<tr>
<td>Wheat bran⁵</td>
<td>105.5</td>
<td>105.5</td>
<td>105.5</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Chitosan Nanoparticle</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin + premixes</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Palm oil</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Cassava starch</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Common salt (NaCl)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Proximate analysis, g kg⁻¹**

<table>
<thead>
<tr>
<th></th>
<th>g kg⁻¹</th>
<th>g kg⁻¹</th>
<th>g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>957.6</td>
<td>960.2</td>
<td>956.9</td>
</tr>
<tr>
<td>Ash</td>
<td>74.2</td>
<td>74.5</td>
<td>75.8</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>44.6</td>
<td>45.0</td>
<td>45.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>400.00</td>
<td>350.00</td>
<td>350.0</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>501.8</td>
<td>568.6</td>
<td>565.6</td>
</tr>
<tr>
<td>Ether extract</td>
<td>71.8</td>
<td>71.6</td>
<td>72.1</td>
</tr>
<tr>
<td>Digestible Energy (MJ kg⁻¹ DM)†</td>
<td>17.9</td>
<td>18.1</td>
<td>18.1</td>
</tr>
</tbody>
</table>

BD = Basal Diet; BD+CH = Basal Diet + Chitosan; BD+CHN = Basal Diet + Chitosan nanoparticles.

† calculated digestible energy, DE (MJ kg⁻¹ DM) = [(CP x 4) + (EE x 9) + (TC x 4)] x 0.042

Hot water was then poured slowly into the solution while stirring until a gelatinous mixture was obtained. Oil and hot water (approximately one third of the total weight of the prepared diet) were added to the dry ingredients. The prepared starch was then slowly added until all were mixed thoroughly. The dough was pelleted using a Hobart A-200T mixing and pelleting machine. Long strings of pellets were created with a 2 mm die. The already prepared vitamin + mineral premix was then sprayed gently on the pellets and freeze-dried overnight at 40°C to a final moisture of approximately 8-10%. Samples of the pelleted feeds were taken to the laboratory for proximate analysis. The dried feeds were packed in polythene bags, labelled and stored in the refrigerator at 20°C prior to use.

### 2.4. Biochemical Analysis

Formulated diets and fish carcasses were analysed for proximate composition using standard methods by Eurofins Scientific, Des Moines, IA, USA [17]. Moisture content was estimated by drying the samples to constant weight at 95 °C in drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 hours. Crude fiber was determined in a Tecator Fibertec System M1020 Hot Extractor Unit as described by Tecator application note 01/7. Total carbohydrates were determined by the anthrone-sulfuric acid reagent method as initially described by Morris [18] and based on the methodology employed by [19].
2.5. Experimental Design

The setup took the form of completely randomized design (CRD). There were three treatments with three replicate. Treatment 1 was the Basal diet (BD) which served as control. Fish in treatment 2 were fed BD supplemented with chitosan particle of 5g kg\(^{-1}\) diet according to Wang [30] and designated (BD+CH) while fish in treatment 3 were fed BD supplemented with chitosan nanoparticle of 5g kg\(^{-1}\) diet and designated (BD+CHN).

2.6. Source of Fingerlings

Fingerlings of *C. gariepinus* were procured from Prime Park Farm located at No. 13 Aka Itiam Street Uyo in Uyo Local Government Area, Akwa Ibom State. Two weeks old fingerlings were transported in a 20 litres rubber container with water and an opening on the topmost part for oxygen to penetrate. These were brought to the experimental site with no transport mortality incurred. They were then acclimated for two weeks in the hatchery unit of the fish farm. During this period the fish were fed *ad libitum* (as we chose) with diet of 45 percent crude protein.

2.7. Tank Preparation, Stocking and Feeding

Nine (9) tarpaulin tanks were used for the experiment. The tanks were washed and dried. Water was supplied from the bore-hole through tap by the hose; water level used in stocking this fish was 2 feet from the bottom. Twenty fingerlings of *C. gariepinus* of mean weight 2.79±0.05 g were randomly stocked in each of the nine tanks with the same water levels. Fish were fed thrice a day at 8.00, 14.00 and 20.00 hours at 5% fresh body weight for 91 days. Sampling was done fortnightly using hand net. The feeding rate was adjusted accordingly during each sampling date. Physico-chemical parameters were recorded weekly, where there was fluctuation, 10% of the cultured water was replaced with fresh water from the reservoir.

2.8. Measurement of Physico-Chemical Parameters

Dissolved oxygen concentration (DO), morning temperature and pH were measured in situ in each pond using a calibrated JENWAY 3405 electrochemical analyser (Barloword Scientific Ltd, Essex, UK), with independent probes for each variable. Portions of the water samples were filtered through a Glass microfiber GF/C 2-micron filter paper and a Technicon II Autoanalyzer was used to determine total ammonia using spectrophotometric methods described by Goltermann[20] for total ammonia. The analyses were carried out at Soil Science Laboratory, University of Uyo-Nigeria following the standard analytical procedures detailed in APHA [21].

2.9. Sampling and Data Collection

Data on fish growth were recorded weekly. The weight of fish was taken using electronic sensitive weighing balance (Model JY10S-01, China). The experimental tanks were inspected daily to remove dead fish, if any. Fish daily weight gain, feed conversion ratio, specific growth rate and survival were determined as follows:

2.9.1. Daily Weight Gain

Daily weight gain (g) is calculated as the difference between the initial and final mean weight values of the fish divided to the number of days the experiment was conducted.
Where:

\[
\text{DWG} = \frac{\text{FE} - \text{IW}}{\text{N}}
\]

FW= final weight of fish
IW= Initial weight of fish
N= number of days the fish were culture

**2.9.2. Specific Growth Rate (SGR)**

Specific Growth Rate (SGR) was calculated according to Hepher[22], using the formula:

\[
\text{SGR} = \left( \frac{\ln \text{FBW} - \ln \text{IBW}}{\text{No. of days}} \right) 
\]

Where;

FBW = Final Body Weight at each harvest,
IBW = Initial Body Weight
In= Natural logarithm

**2.9.3. Feed Conversion Ratio (FCR)**

This was calculated by dividing the total amount of feed given (feed intake) by the mean weight gain (MWG). The calculation was based on total dry weight of feed [22] using the formula:

\[
\text{FCR} = \frac{\text{Dry Weight Feed Fed (g)}}{\text{Weight gain (g)}} 
\]

**2.9.4. Survival Rate (SR)**

The survival rate (SR) was calculated as total fish harvested/total fish stocked expressed in percentage.

\[
\text{Survival (\%)} = \frac{\text{Total fish number harvested}}{\text{Total fish number stocked}} \times 100 
\]

**2.9.5. Protein Efficiency Ratio (PER)**

This was calculated by dividing the mean weight gain (MWG) by the total protein intake [31]. This is done using the formula: \( \text{PER} = \frac{\text{Weight gain}}{\text{Protein intake}} \).

**2.10. Statistical Analysis**

Data obtained were subjected to analysis of variance (ANOVA) test and the means from the various treatments were compared for significant differences (P<0.05), by means of [23]. Results with \( P \leq 0.05 \) were considered significant [24]. Where there was significant difference, post hoc test was conducted using Duncan Multiple Range Test (DMRT).

**3. Results and Discussion**

**3.1. Effect of Chitosan and Chitosan Nanoparticles on Water quality**
The physico-chemical parameters of the cultured water are presented in Table 2. There was no significant difference (p<0.05) among the different treatments in the DO and morning temperature. However, pH was significantly higher in BD+CH and BD+CHN than in BD while ammonia was significantly lower in BD+CHN than BD and BD+CH. The present findings showed that supplementation of chitosan significantly improved the quality of the cultured water by increasing the pH concentration of the cultured water while chitosan nanoparticles supplementation significantly improved the quality of the cultured water by reducing the ammonia concentration and increasing the pH concentration of the cultured water. These findings agree with that of Wang [15] who reported improved water quality as a result of chitosan and chitosan nanoparticles supplementation in the diet of Oreochromis nilotica.

### Table 2. Physico-chemical parameters of tank water during the 91 days culture period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BD</th>
<th>BD+CH</th>
<th>BD+CHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen</td>
<td>5.75±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.94±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.17±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Morning temperature</td>
<td>28.05±1.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.65±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.88±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>6.70±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.13±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.17±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ammonia-nitrogen</td>
<td>0.05±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean±SD from three replicates. Mean with the same superscript letter are not significantly different (P<0.05). BD = Basal Diet; BD+CH = Basal Diet + Chitosan; BD+CHN = Basal Diet + Chitosan nanoparticles.

### 3.2. Effect of Chitosan and Chitosan nanoparticles on Growth Performance of African Catfish (C. gariepinus) Fingerlings

The weekly weight of C. gariepinus fingerlings over thirteen weeks and growth response are shown in Fig. 1 and Table 3 respectively. Generally, the best response was observed when fingerlings were fed diet BD+CHN while the worst response was observed in fingerlings fed the basal diet (BD). On the whole, there was no significant difference (P>0.05) among the treatments in terms of final weight and SGR. Daily weight gain was however significantly higher (P<0.05) in diet BD+CHN which was higher than BD. This result is not in agreement with the works of Gopalakannan[25] who reported that addition of 1% chitosan to the diet of Carp (Cyprinus carpio) resulted in a significantly improved growth, Kuriba[26] who reported that chitosan supplemented diet gave the best growth performance of the Indian major carp (Labeo rohita), Zaki[13] who recorded improvement of growth performance in Sea Bass (Dicentrarchus labrax) and Suriya[27] who reported that chitosan supplemented diet gave significantly higher total weight, average weight and number of molting than the control treatment in Freshwater Prawn (Macrobrachium lanchesteri). Wang[15] who had the same result in O. nilotica and Zhou[28] who found that nano-Se supplementation improved the growth performance of crucial carp (Carassius aurantus gibelio). The difference may be due to short culture period as indicated by the sharp improvement in daily weight gain. It is believed that if the fish are culture to marketable size then improved final weight SGR would also be recorded.
Figure 1. Growth Trend of C. gariepinus during the 91 days culture.

Table 3. Growth performance and feed utilization of C. gariepinus fed different experimental diets for 91 days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental diets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BD</td>
</tr>
<tr>
<td><strong>Growth performance</strong></td>
<td></td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>52.28±0.14a</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>556.73±92.4a</td>
</tr>
<tr>
<td>Daily weight gain (g)</td>
<td>5.54±2.52a</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>2.60±0.69a</td>
</tr>
<tr>
<td><strong>Feed utilization</strong></td>
<td></td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.14±0.62b</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.76±0.22a</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>86.66±10.27a</td>
</tr>
</tbody>
</table>

Values are mean±SD from three replicates. Mean with the same superscript letter are not significantly different (P<0.05). BD = Basal Diet; BD+CH = Basal Diet + Chitosan; BD+CHN = Basal Diet + Chitosan nanoparticles.

Ringwood [29] reported that young carp and sturgeon exhibited a faster rate of growth (30% and 24% respectively) when they were fed nanoparticles of iron. In this study 29% faster growth rate has been realized as a result of chitosan nanoparticles supplementation. This agrees with value for carp. Supplementation of chitosan and nanoparticles resulted in significantly higher daily weight gain. Various species of fish fed with the diet supplemented with 1 to 5% beta-chitosan had the highest weight gain, which further attest to the growth promoting effect of chitosan [30] - [35]; hence, the chitosan may play a crucial role in enhancing the digestion and absorption of nutrients at lower levels. Noteworthy, Shiau [36] argued that chitin but not chitosan supplementation enhances growth of grass shrimp, *Penaeus monodon*.

3.3. Effect of Chitosan and Chitosan Nanoparticles on Feed Utilization of African Catfish (C. gariepinus) Fingerlings
All other diets except BD had similar feed conversion ratio. Diet BD+CHN had significantly lower (P<0.05) FCR than other diets. The basal diet had similar feed conversion ratio with the chitosan supplemented diet. Diet BD+CHN had significantly (P<0.05) higher protein efficiency ratio value than all other diets which had similar protein efficiency ratio. In this study chitosan supplementation did not improved the feed utilization parameters. Both feed conversion ratio and protein efficiency ratio were not improved. This is in disagreement with the findings of Masume[37] who reported that feed conversion ratio significantly decreased in Caspian kutum (Rutilus frisii kutum) fingerlings fed diet containing 1 g kg$^{-1}$ of chitosan compared to the other groups (P < 0.05) this disparity may be species driven. In this study chitosan nanoparticle supplementation significantly improved the feed utilization parameters. Both feed conversion ratio and protein efficiency ratio were significantly (p<0.05) improved which agrees with the findings of Wang [15].

3.4. Effect of Chitosan and Chitosan Nanoparticles on Survival Rate of African Catfish (C. gariepinus) Fingerlings

Survival rate was significantly (P<0.00) higher in treatments BD+CH and BD+CHN than in the control diet. Generally, survival rate was improved in this study by chitosan incorporation. This is in agreement with the findings of Kiruba[26] who reported reduced mortality as a result of chitosan supplementation in diet of Indian Major carp Labeo rohita. Zaki[13] also suggested that chitosan incorporated into diets of sea bass fish certainly reduced mortality and also improved the growth performance of fish. Survival rate was improved in this study by chitosan nanoparticle incorporation.

3.5. Effect of Chitosan and Chitosan Nanoparticles On Meat Quality of African Catfish (C. Gariepinus) Fingerlings

Proximate composition of fish carcass is shown in Table 4. Initial values had a significantly higher crude protein than other diet while the basal diet had the lowest (P<0.05). However, there was no significant difference (P<0.05) between diet BD+CH and diet BD+CHN.

<table>
<thead>
<tr>
<th>Nutrient, g kg$^{-1}$</th>
<th>Initial</th>
<th>BD</th>
<th>BD+CH</th>
<th>BD+CHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>618.7±1.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>524.8±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>590.0±0.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>594.4±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ether extract</td>
<td>205.9±0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>111.2±1.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.3±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145.8±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total ash</td>
<td>71.6±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>126.4±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.1±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.1±2.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>00.1±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>03.0±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>01.3±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>00.4±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caloric value (Kcal kg$^{-1}$)</td>
<td>447.30±1.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>349.66±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>387.17±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>418.48±1.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>29.8±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.8±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.3±0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>125.4±0.43&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture</td>
<td>340.6±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>678.9±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>703.0±0.35&lt;sup&gt;d&lt;/sup&gt;</td>
<td>691.9±0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean±SD from three replicates. Mean with the same superscript letter are not significantly different (P<0.05). Nitrogen Free Extract = 1000 - (moisture + Ash +Crude fiber +Crude protein +lipid). BD = Basal Diet; BD+CH = Basal Diet + Chitosan; BD+CHN = Basal Diet + Chitosan nanoparticles.

Similar trend was observed in the composition of ether extract. Total ash was significantly higher in fish fed diet BD, lower in initial and similar in fish maintained in diet BD+CH and BD+CHN. Crude fibre was similar in fish fed the basal diet, diet supplemented with chitosan and chitosan nanoparticles and significantly higher than
the initial value. Caloric values were significantly different (p<0.05) in all the treatments. The initial values were significantly higher than fish fed diet BD+CH which in turn was higher than fish fed BD+CHN which is also significantly higher than fish maintained on the basal diet. Significantly difference (p<0.05) also existed in all the treatments in terms of nitrogen free extract. Fish fed diet BD+CHN had the highest NFE followed by fish maintained on BD+CH, follows by the basal diet feeders while the least value was obtained from the initial values. Fish samples were significantly different (p<0.05) in terms of moisture. Fish fed diet BD+CH had the highest moisture content followed by those fed diet BD+CHN, while the least moisture content was observed in the initial values. The improvement in chemical body composition, crude protein, ether extract, caloric value, nitrogen free extract and moisture different compared to control groups agrees with the findings of Zaki[13] who reported that the chemical body composition, crude protein, dry matter, crude fat and ash were significant (P ≤ 0.01) different in Sea Bass (Dicentrarchus labrax) fingerlings fed chitosan supplemented diet compared to control groups.

Proximate composition: crude protein, ether extract, caloric value, nitrogen free extract and moisture improved as compared to control groups and in some cases as compared with the chitosan supplemented diet. These agree with the findings of Wang [15] who reported that dietary chitosan nanoparticles supplementation improved the growth performance and meat quality status of tilapia. The 5 g kg⁻¹ diet inclusion level was taken after that used for tilapia by these authors since there is no information for C. gariepinus. Ecotoxicity data so far suggest that manufacture nanomaterial have low acute toxicity to aquatic species, and so immediate threats to aquaculture systems and fisheries may be very small [38].

4. Conclusions

On an average, the sea food industry produces 80,000 tons of waste per year. The sheer amount of waste makes degradation a slow process causing accumulation of waste over a period of time. A very simple and effective solution to this environmental hazard is the recycling of shell waste to commercially viable products like chitin. Chitosan is the N-acetyl derivative of chitin obtained by N-deacetylation. Generally, incorporation of chitosan at a level of 1-5 g in fish diet enhanced the performance and reduced the fish mortality under stress. Application of nanotechnology to aquaculture has resulted in improved water quality and better fish yield. In this new findings, supplementation of chitosan in the diet of African catfish (C. gariepinus) at 5 g kg⁻¹ inclusion level significantly improved weight gain, survival rate and body composition of the experimental fish. Incorporation of chitosan nanoparticles at the same inclusion level significantly improved water quality, daily weight gain and feed utilization, survival and meat quality of the African catfish (C. gariepinus). Large-scale production of chitosan nanoparticles should be encouraged by all forms of Governmental and non-governmental agencies as this has the potentials of revolutionizing aquaculture.

Conflicts of Interest

There is no conflict of interest regarding the publication of this article.

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