Original Research Article

Response of Juvenile *Clarias gariepinus* to Varying Dietary Levels of Ionotropic Gelated Chitosan Nanoparticles Synthesized from Wasted Crab Shells

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ABSTRACT

Background: Crab shells lie wasted globally after the extraction of the edible soft tissues. Chitosan nanoparticles (CNPs) derived from these shells have the potentials to revolutionized aquaculture.
Aims: Evaluated the effects of CNPs on water quality parameters, growth performance, feed utilization and survival rate of African catfish (*Clarias gariepinus*).
Study design: The experimental diets were incorporated with chitosan nanoparticles at the rate of 0, 2.5 5.0 and 7.5 g kg^{-1} representing Treatments CNP00, CNP2.5, CNP5.0 and CNP7.5 respectively and replicated thrice.
Place and Duration of Study: Department of Fisheries and Aquatic Environmental Management, University of Uyo, Uyo – Nigeria from August to November 2021.
Methodology: We synthesized CNPs by ionic gelation method. Dried flour of FM, SBM, Wheat bran and yellow maize meal were analyzed for proximate composition used in the formulation of experimental diets. These were milled at 2 mm pellets, CNPs were dissolved in 0.3 liters of water and sprayed on the pellets. These were stored at 10% moisture content, in black polythene bags. Diets were fed to two hundred and sixteen fingerlings of African catfish (*C. gariepinus*) with mean body weight 23.86±3.11. Growth performance, feed utilization and survival rate parameters were calculated and statistically compared.
Results: Ammonia was significantly ($p<0.05$) low in CNP7.5. The highest daily weight gain (6.98±0.81 g) was recorded in CNP7.5 while the lowest (1.02±0.01 g) was recorded in the control (CNP00). SGR, DWG, FCR, PER followed the same pattern. However, there was no difference in percentage survival rate.
Conclusion: Chitosan nanoparticles supplemented at the rate of 7.5 g k^{-1} diet can give a better water quality, growth performance and feed utilization of *C. gariepinus* juvenile. Incorporation of CNPs in diet of *C. gariepinus* at a concentration of 7.5 g kg-1 is recommended for more profitable and sustainable culture of this fish species.

Keywords: growth; feed; ion; ammonia; protein

1. INTRODUCTION

The aquaculture attributes of African catfish (*C. gariepinus* have been recognized since the 1940s while the basic technologies and protocols for the farming of the species were developed much later. The significant increases in annual production around 2000 onwards can partially be ascribed to the concerted research efforts on the nutritional requirements of the species during all life history stages, feed formulation, optimization of feeding and feed management practices and the development of high-density tank farming practices. However, feed costs constitute a major percentage of variable costs in culture of *C.*

gariepinus. This is as a result of price soaring of the ingredients used in aquafeed production and the competition by man in the use of such ingredients. From the foregoing, it is deemed appropriate to incorporate relevant technology to ensure that the high feeding cost can be adequately justified by the economic benefits that can be realized by improved weight gain, meat quality and survival rate caused by positive impacts of such technology on this species. One of such technologies is nanotechnology. It is a field that specializes on the use of smaller sized materials to allow for adjustment of molecules and substances at the nanoscale level, which can further enhance the mechanical properties of materials or grant access to less physically accessible areas of the body.

Nanotechnology has a wide range of application in aquaculture especially in the aquafeed industry where it is effective in micronutrient delivery example chitosan nanoparticles (CNPs). Chitosan [poly(1,4-β-*D*-glucopyranosamine)] is a polysaccharide with low immunogenicity, low toxicity, and antimicrobial potential being widely used in feed production for human and animals [1-3]. Research interest on the applications of CNPs for the delivery of micronutrients is on the early-stage and is developing at a rapid rate. Use of CNPs has been shown to significantly increased shelf life and delivery of vitamin C in rainbow trout after 20 days of feeding [4]. Jiménez-Fernández *et al*. [5] confirmed that CNPs had the ability to penetrate fish intestinal epithelium showing a significant increase of ascorbic acid on both models. Chitosan Nanoparticles can serve as an encapsulating agent for nutrients that can easily leach in in water [6,7]. With good micronutrient delivery and prevention of leakage of useful nutrient, CNPs have improved fish growth performance and feed utilization. In our previous work, supplementation of CNPs in diet of African catfish (*C. gariepinus*) was found to significantly improve daily weight gain, survival rate and meat quality [8]. However, we used a single concentration of CNPs (0.5 g kg^{-1}) in fish feed and there exist a gap in knowledge as to what concentration will be best for optimum growth performance of African catfish. This recent work was aimed at using varying concentrations of dietary CNPs in fish feed to ascertain which concentration will be best for optimum growth of *C. gariepinus.* The main objective of this research work is to assess the response of varying dietary chitosan nanoparticles on the water quality, growth performance and feed utilization of *C. gariepinus.*

2. MATERIAL AND METHODS

2.1 Experimental Site

The 90 days experiment was carried out in concrete fish ponds in the Fish Farm complex of the Department of Fisheries and Aquatic Environmental Management University of Uyo, Uyo-Nigeria located on Latitude**:** 5° 03' 4.57"and Longitude**:** 7° 56' 0.60" between August and November, 2021. Four concrete ponds partitioned into three replicate units were used. The sources of water were borehole and rainfall.

2.2 Synthesis of Chitosan Nanoparticles

Chitosan and CNPs were synthesized at the Biochemistry Laboratory of the University of Uyo, Uyo, Nigeria according to Bolat [9]. Crab shells were harvested in Itam Market, Uyo-Nigeria. After gathering enough shells, cleaning, drying, and grinding of shells into powder form was done. Chitosan preparation was done by dissolving chitosan polysaccharides in 1.57 M of concentrated hydrochloric acid (HCl) for 5 hours under stirring and subsequent repeated decantation and then dried at 60^{o}C with 233.93 g of shell remaining. Subsequently, the solution was decolorized (using 0.32% sodium hypochlorite, 1:10W/V concentration) for 3 minutes. Deproteinization of the solution was later carried out at 70°C for 24 h using 1 M NaOH (A. B. Enterprises Mumbai, Maharashtra) with 114.98 g of ground shell remaining. Alkali chitin was prepared after dispersion of chitin in concentrated NaOH (30 g NaOH/45 g H_2O/ 3g 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 (tripolyphosphate) into the crude chitosan solution under constant stirring. The content was freeze-dried to obtain pure CNPs.

2.3 Selection of Ingredients for Feed Formula

Fishmeal and soybean meal were the main protein sources, yellow maize meal and wheat bran meal were the source of carbohydrate and crude fibre, respectively. Chitosan NPs were added at the rates of 2.5 g, 5.0 g and 7.5 g, respectively per kg of feed for different treatments (Table 1).

Table 1**:** Proximate composition and digestible energy of the feed ingredients used in this experiment

Feedstuff	**Composition (g kg^{-1})**										**(Kcal kg^{1} DM)**
	DM	**CP**	**CF**	**EE**	**Ash**	**Ca**	**P**	**NFE**	**LS**	**MT**	**DE**
FM	90.00	72.00	1.70	12.00	20.00	5.95	3.00	20.00	4.20	2.00	353200
SBM	88.50	45.30	6.50	3.50	26.34	0.20	0.20	28.00	2.80	0.60	2230.00
WBM	87.60	16.90	11.30	7.16	35.02	0.13	1.89	34.90	1.09	0.50	2794.00
YMM	88.51	7.31	2.00	3.20	25.51	0.20	0.20	64.59	2.80	0.60	3060.00

FM=fishmeal; SBM=soybean meal; WBM=Wheat bran meal; YMM=yellow maize meal
DM=dry matter CP=crude protein; CF=crude fibre; EE=ether extract; P=phosphorus; Ca=calcium; NFE=nitrogen free extract; LS=lysine; MT=methionine; DE=digestible energy.

2.4 Experimental Diet Formulation

Experimental diet was formulated using feed formulation software which formulates feed by linear programming (LP). Diet was formulated on dry matter basis and the result presented on Table 2.

Table 2. Diet formula and proximate composition on dry matter basis

Ingredient g kg^{-1}	**CNP00**	**CNP2.5**	**CNP 5**	**CNP7.5**
Fishmeal[1]	350	350	350	350
Yellow Maize Meal[2]	250	250	250	250
Wheat bran[3]	200	250	200	250
Soybean[4]	200	200	200	200
CNPS	0	2.5	5	7.5
Mineral+vitamin premixes[5]	15	15	15	15
Vegetable oil[6]	20	20	20	20
Salt (NaCl)[7]	5	5	5	5
Binder (cassava starch)	50	50	50	50
Proximate composition (g kg^{-1})				
Crude fibre	45.2	45.2	45.2	45.2
Crude protein	40.67	40.67	40.67	40.67
Ether extract	70.4	70.4	70.4	70.4
Moisture	115.3	115.3	115.3	115.3
Nitrogen free extract	501.8	501.8	501.8	501.8
DE (MJ kg^{-1} DM)	2619.5	2619.5	2619.5	2619.5

[1] Fishmeal (menhaden , 72%, Denmark); [2] milled, (Uyo, Nigeria); [3] milled, (Uyo, Nigeria); [4] toasted (Uyo, Nigeria); [5] Vitamin-mineral mix (Aqua Biomix for catfish): contains 20,000,000 IU vit A; 2,000,000 IU vit D3; 200,000 mg vitamin E 8,000 mg vitamin K_3; 20000 mg of vitamin B; 30,000 mg vit B2; 150,000 mg niacin; 50,000 mg pantothenic acid; 12,000 vit., B6; 50 mg B12; 500,000 mg vitamin C (as monophosphate) 4,000 mg folic acid; 800 mg biotin h_{12}; 600,000 mg choline chloride; 2,000 mg cobalt; 4000 mg copper; 5,000 mg iodine; 200,000 mg inositol; 40,000 mg iron; 30,000 mg manganese; 200 mg selenium; 40,000 mg zinc; 100,000 mg lysine; 1000,000 mg; 100,000 mg methionine; 100,000 mg antioxidant; [6] fresh (PAMOL, Calabar, Nigeria); [7] iodized (Dangote, Nigeria), [8] calculated digestible energy, DE (MJ kg-1 DM) = [(CP x 4) + (EE x 9) + (TC x 4)] x 0.042.

2.5 Diet Preparation for the Experiment

The formula was first converted to weight basis based on a bag size of 20 Kg. Ingredients were weighed individually using an electronic sensitive weighing balance (Model JY10S-01, China). 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+mineral premix was prepared prior to diet preparation to ensure the freshness of ingredients. Chitosan NPs were then added according to the treatments and combined thoroughly to obtain a homogenous mix. Cassava starch (Fisher Scientific, Pittsburgh, PA, USA) was prepared by dissolving the starch in cool water (50 mL water/50 g starch)**.** 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 dried overnight at 40°C to a final moisture of 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^{o}C prior to use.

2.6 Biochemical Analysis

Feed ingredients, prepared diets and fish carcasses were analysed for proximate composition using standard methods by Eurofins Scientific, Des Moines, IA, USA [10]. Moisture content was estimated by drying the samples to constant weight at 95 ^{o}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^{o}C for 6 hours. Crude fiber was determined in a Tecator Fibertec System M/1020 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 [11] and based on the methodology employed by Hassid and Abraham [12].

2.7. Experimental Design

Completely randomized design (CRD) was used. There were four treatments with three replicates. Treatment 1 was Basal diet (BD) which served as control. Fish in treatment 2 were fed basal diet supplemented with chitosan nanoparticles at 2.5 g kg^{-1} diet, fish in treatment 3 were fed basal diet supplemented with chitosan nanoparticles at 5.0 g kg^{-1} diet while fish in treatment 4 were fed basal diet supplemented with chitosan nanoparticles at 7.5 g kg^{-1} diet.

2.8. Experimental Fish Procurement

Two weeks old juveniles of *C. gariepinus* were procured from the University of Uyo Hatchery complex and 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 during which were fed *ad libitum* with Coppen fish diet (45 percent crude protein).

2.9. Tank Preparation, Stocking and Feeding

Four (4) concrete pond of equal sizes were partitioned into three (3) replicates, dressed to prevent leakages and allowed to dry before water was pumped in from bore hole. Water

level was 2 feet from the bottom. Eighteen juvenile *C. gariepinus* of mean weight 23.86±3.11 g were randomly stocked in each of the nine tanks with the same water levels. Feeding was done thrice a day at 8.00, 14.00 and 20.00 hours at 5% fresh body weight for 90 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.

2.10 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 Golterman (1978) for total ammonia. The analyses were carried out in Soil Science Laboratory, University of Uyo-Nigeria following the standard analytical procedures detailed in APHA [13].

2.11 Sampling and Data Collection

Data on fish growth were recorded every two weeks. 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:

2.11.1. 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.

DWG = FE-IW/N……..(1)

DWG= daily weight gain FW=final weight of fish IW=Initial weight of fish N=number of days the fish were culture.

2.11.2. Specific Growth Rate (SGR)

Specific Growth Rate (SGR) was calculated according to Hepher [14], using the formula:

SGR = (ln FBW – ln IBW) x 100) No. of days

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

2.11.3. Feed Conversion Ratio (FCR)

Calculated was done 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 [14] using the formula:

FCR = Dry Weight Feed Fed (g) Weight gain (g)……..(2)

2.11.4. Survival Rate (SR)

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

Survival (%) = Total fish number harvested x 100 Total fish number stocked…(3)

2.11.5. 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:

PER=Weight gain/Protein intake……..(4)

2.12. 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). Where significance existed, post hoc analysis was conducted using Duncan multiple Range test (DMRT) to separate the mean. IBM's SPSS statistical package for window version 20 was used [15].

3. RESULTS

3.1 Water Quality

Physico-chemical parameters of the cultured water are shown in Table 3. And fluctuated slightly. These were within the optimum range recommended for the culture of freshwater fishes in the tropical region (TNAU, 2008).

Table 3: Physico-chemical parameters of tank water during the 90 days culture period

Parameter	CNP00	CNP2.5	CNP5.0	CNP7.5	Optimum
Ammonia (mg l^{-1})	0.04±0.01^{b}	0.02±0.01^{ab}	0.02±0.21^{ab}	0.01±0.01^{b}	**0.02–0.05**
DO (mg l^{-1})	5.11±0.02^{a}	5.32±0.29^{a}	5.31±0.47^{a}	5.34±0.21^{a}	5
Temperature (^{o}C)	22.14±0.11^{a}	22.14±0.03^{a}	23.26±0.21^{a}	22.73±0.26^{a}	23-32
pH	7.12±0.14^{a}	7.34±0.35^{a}	8.01±0.11^{a}	8.27±0.81^{a}	6.5-9.0

Values with the same superscript letter are not significantly different P>0.05
Values are mean±SD

However, a significant (p<0.05) reduction in ammonia was observed in treatment four (CNP7.5), while the ammonia concentration of treatment three (CPN2.5) and treatment three (CNP5.0) were similar and in between treatment one and four.

3.2 Effect of Varying Dietary Levels of Inclusion of Chitosan Nanoparticles on Growth Performance and Feed Utilization of *C. gariepinus* Juveniles

Table 4 shows the growth response and nutrient utilization of *C. gariepinus* fed diets incorporated with varying levels of chitosan nanoparticles (CNP) for 90 days. The highest daily weight gain was recorded in diet CNP**7.5** while the lowest was seen in the control diet (CNP**00**) however, diet **CNP2.5** and **CNP5.0** were similar in terms of weight gain. The specific growth rate also followed the same pattern. Result of the analysis shows that there was significant difference (p<0.05) among the final mean weights of fish in the different treatments. Diet CNP7.5 showed significantly (P<0.05) higher final mean weight as compared with the control while diet CNP2.5 and CNP5.0 showed similar mean weight higher than the control but lower than CNP7.5. The daily weight gain and the specific growth rate also followed the same pattern.

Values for feed conversion ratio were also significantly different (P<0.05). Higher in diet **CNP7.5** while the lowest value was recorded in the control diet **(CNP00)**. The highest protein efficiency ratio was achieved in **CNP7.5** while the lowest was achieved in **CNP00. CNP2.5** and **CNP5.0** were similar and stood in between**.** However, the rates of fish survival in all the experimental treatments were grossly similar.

Table 4: Growth performance and feed utilization of *C. gariepinus* fed different experimental diets for 90 days

	Experimental diets (%)			
Parameter	**CNP00**	**CNP2.5**	**CNP5.0**	**CNP7.5**
Minimum weight (g)	23.17±0.01^{a}	23.06±0.33^{a}	22.84±0.12^{a}	22.83±0.12^{a}
Maximum weight (g)	115.40±8.49^{a}	252.22±8.73^{a}	461.23±11.13^{ab}	836.23±14.46^{b}
Mean weight (g)	68.54±11.79^{a}	118.50±33.26^{a}	169.74±54.90^{ab}	378.34±139.30^{b}
DWG (g $fish^{-1}$)	1.02±0.01^{a}	2.56±0.23^{a}	4.87±0.82^{ab}	6.98±0.81^{b}
SGR	1.79±0.12^{a}	2.67±0.61^{a}	3.35±0.32^{a}	4.20±0.02^{a}
Feed utilization				

FCR	1.31±0.14[a]	1.36±0.01[a]	1.61±0.03[ab]	2.65±0.0311[b]
PER	1.01±0.0215[a]	1.15±0.261[a]	1.47±0.18[b]	2.45±0.261[c]
Survival rate (%)	96.67±5.41[a]	96.27±6.01[a]	98.15±4.35[a]	98.15±6.61[a]

Means in the same row with common letter are not different at P>0.05
DWG=Daily weight gain; FCR=Feed conversion ratio; PER=Protein Efficiency ratio; SGR=specific growth rate

Generally, the fish showed good appetite to all the treatment diets, attested to by the increase in body weight (Fig. 1). The growth trend shows that there was similar growth pattern until the 3th sampling date when the effect on appetite and rapid growth started to manifest. The trend line of CNP7.5 shows that it is possible to raise fish of up to 1kg within four months of culture.

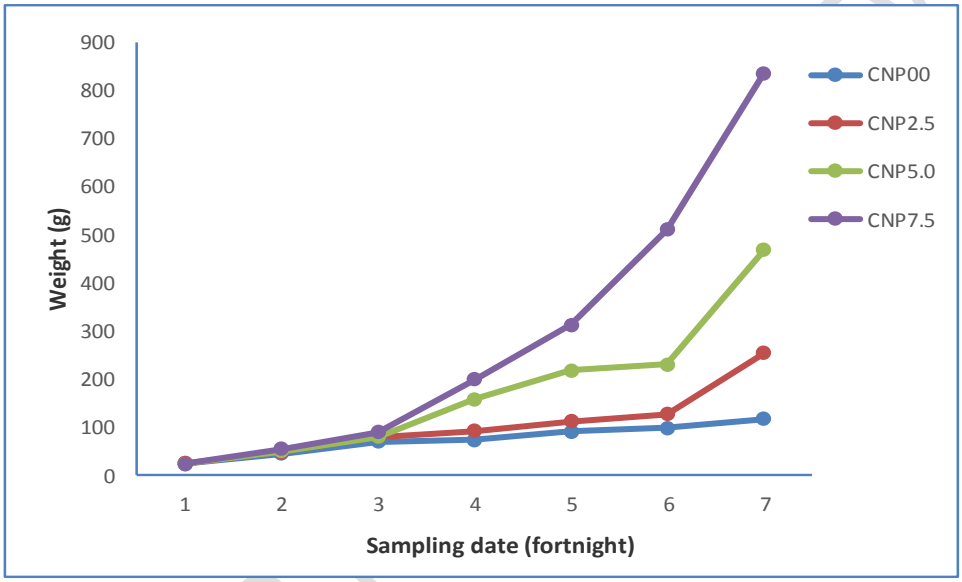

Fig. 1. Growth trend of *Clarias gariepinus* fed varying dietary levels of chitosan nanoparticles for 90 days

4. DISCUSSION

The incorporation of nanoparticle had positive effect on water quality. Ammonia drastically reduced with increase in CNPs concentration. Emerson *et al*. [16], reported that acute exposure of fish to ionized ammonia at a concentration of 0.6 mg/L results in fish killing which chronic exposure at same concentration is capable of causing reduction in growth among other things such as kidney and gill damage. Optimum for tilapia has been fixed by BFAR [17], between 0.02–0.05 mg L^{-1}. Generally, TNAU [18], also reported that the optimal range of un-ionized ammonia is 0.02–0.05 mg L^{-1} in fish ponds. This was observed in the control. However, significant reduction was observed in other treatments. This must have contributed to improved growth performance. This findings agrees with Udo *et al*., [8] who reported that CNPs reduced ammonia concentration of culture water when compared to chitosan. This attribute has made it possible for chitosan to be used in water treatment. Such functional groups as hydroxyl and amino in chitosan make it an excellent adsorbent. Yang *et al*., [19] reported that chitosan-coated nanoparticle membranes were able to remove bacteria much better than uncoated membranes.

Generally, fish growth and yields are greater in ponds with higher DO concentration [20]. In the culture of *C. gariepinus*, a fish farmer's target for DO is 4 mg/l to saturation levels in the pond. However, any level higher is capable of causing a disorder or diseases in fish.

For instance gas bubble disease in fish is a signal that that the DO levels are constantly too high and the water is super-saturated to well above 300 per cent. In the current study, all ponds recorded minimum DO levels of less than 3 mg/L and no significant different was observed. Although no significant difference existed in temperature between the treatments, temperature fluctuated slightly below the optimum ranges. Acceptable temperature range for the culture of C*. gariepinus* is between 23ºC to 32ºC [18]. Consistent low temperature in the ponds (between 16ºC and 26ºC), leads to reduction of feed intake, and there is drastic drag in growth performance rate. High FCR, and stress may also occur in such ponds and prolonged stress can lead to opportunistic infection, stunted growth and death. However, high temperature (above 32^{o}C) will also results in stress and death. The value pH was found to fall within the optimum range in this study. Though there was a gradual rise, the difference was not significant from the control. Mean pH in treatment CNP7.5 was 8.3. This value is not too good for the culture of *C. gariepinus*. High pH values of between 9 and 11 in pond water will result in retarded fish growth. However, death will occur when pH levels rise above 11.

Nano-chitosan incorporation in fish diet significantly affected growth performance. There were significant increase in all growth parameters except SGR in treatment CNP7.5 in terms of feed utilization, FCR and PER were both significantly better in both treatments CNP5.0 and CNP7.5. These results are in agreement with earlier reports. For instance, Wang and Li, [21] reported that dietary CNPs supplementation improved the growth performance and meat quality status of tilapia (*Oreochromis nilotica*). Pandit and Kumara [22] inferred that the chitosan incorporated into diets of bagrid catfish (*Mystus Seenghala*) increased survival rate and improved the growth performance of fish. Dietary supplement containing NAG loaded CSNPs was found to significantly increase immune-modulatory properties, growth performance, and enhanced their disease resistance of Nile tilapia (*Oreochromis nilotica*) [23]. Diets supplemented with nano-chitosan plus clinoptilolite, especially at 0.05 g kg^{-1} nano-chitosan inclusion, improved growth performance and immune parameters of rainbow trout (*Oncorhynchus mykiss*) [24].

Many current work also attest to the fact CNPs promote growth performance and feed utilization of fish. For instance, [25] reported that the optimal level of CNPs to perform the best growth promoting effect depends mainly on animal species. It was found that the supplementation of diets with CNPs did not affect the growth performance of sea bream (*Sparus aurata*) and Japanese eel (*Anguilla japonica*) [26]. Moreover, in *Paramigurunus dabryanus*, the addition of CNPs combined with selenium (Se) with a dose of (0.6, 1.2 and 1.8) mg CS per kg diet for 60 days did not induce any change in fish growth performance [27] It is worthy to mention that CNPs decreased the growth performance in case of using it as a supplemented additive to the feed of *O. niloticus* [28]. Nevertheless, an increase in the growth performance of *Paralichthys olivaceus, Cyprinus carpio* and *Dicentrachus labrax* was detected due to CNPs supplementation [29 – 31]. Zaki *et al*. [31] explained CNPs effect in relation to its ability to induce healthy intestinal epithelium and its potential to promote microvilli growth, which leads to an increase in the absorbance surface of the microvilli. Reversely, the previous authors found that the increase in CNPs concentration in the diets caused a decline in the growth performance due to the intestinal blockage caused by an over growing of the microvilli. It was found that, the growth performance of fish depended mainly on the administered dose of CNPs. Chen et al. [32] fortified diets for gibel carp (*Carasius auratus*) juvenile with different doses of CNPs (1800, 4000, 7500, 10000 and 20000) mg of CNPs per kg diet for 75 days. They found that, 4000 mg CS per kg diet formed the optimum level of CS supplementation. It induced the highest growth performance of fish by influencing the growth of the microvilli and the goblet cells in the intestinal epithelia. Moreover, the use of CNPs supplementation at different levels in O. niloticus enhanced the growth performance by increasing both feed intake and feed utilization [33, 34]. Additionally, dietary fortification of CNPs for *O. niloticus* at a dose of 0.5 g/kg induced a better health and increased fish disease resistance against pathogens [35]. This findings also agrees with our earlier work on nano-chitosan [8], however, all the parameters for growth performance and

feed utilization parameters where significantly better in treatment CNP7.5. Chitosan NPs did not significantly affect fish survival rate across the treatments.

CONCLUSION

This study has demonstrated that chitosan nanoparticles supplemented at the rate of 7.5 g k^{-1} diet can give a better water quality, growth performance and feed utilization of *C. gariepinus* juvenile. However, the mechanism of action by which CPNs improve growth and feed utilization of *C. gariepinus* is phenomenal and is yet to be scientifically elucidated. Incorporation of CNPs in diet of *C. gariepinus* at a concentration of 7.5 g kg-1 is recommended for more profitable and sustainable culture of this fish species. Mass production by various Governments and agencies is also recommended.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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