

EVALUATION OF GRADED QUANTITATIVE PARTIAL REPLACEMENT OF BASARIA (SAND SMELT) LOCAL FISH MEAL AND BIOGEN, WITH BIOSYNTHESIS TO CHITOSAN NANOPARTICLES AND *SPIRULINA PLATENSIS* ALGAE IN DIETS FOR AFRICAN CATFISH (*CLARIAS GARIEPINUS*) REARED IN TANKS

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(Received 8/11/2023, accepted 10/12/2023)

SUMMARY

In the present study, African catfish *Clarias gariepinus* one of the most important cultured fish in tropical and subtropical regions and is an economically important fish species, the need to reduce cost of feeding, become more popular among world fish farmers and drive to increase fish productivity that motivated the present research. The present practical study was conducted out at the experimental fish farm, fish production department, faculty of agriculture, Al-Azhar University, Cairo, Egypt. The experimental period (98 days, 14 weeks) started during first 1st April to 15th July 2023. A 98-day feeding trial was conducted in four tanks system has consist of a four series of rectangle concrete tanks (in each divided two basins). 240 *C. gariepinus* post fingerlings (Sixty experimental fish in each treatment) each of average initial weight $38.87 \pm 1.14 - 39.75 \pm 1.19$ g and Initial Fish length $19.2 \pm 1.07 - 20.1 \pm 1.12$ (cm) to check the effect of graded quantitative levels of partial replacement of local Basaria (Sand Smelt) meal (SSM) and Biogen as a main source of protein with chitosan nanoparticles biosynthesis and powder *Spirulina platensis* in diets for African catfish (*clarias griepinus*) reared in tanks , on water quality, growth performance , feed utilization ,chemical body compositions parameters. Four experimental diets were prepared by T1, at a rate of 0% (control, basal diet, contain 40% crude protein, local Sand Smelt meal and Biogen), T2 (chitosan nanoparticle biosynthesis(CSNPs) of 10 g kg^{-1} and powder of *S.platensis* a rate of 50 g kg^{-1} , T3(CSNPs) of 20 g kg^{-1} and 100 g kg^{-1} powder of *S.platensis*, and T4(CSNPs)of 30 g kg^{-1} and 150 g kg^{-1} powder of *S.platensis*, respectively .These were replicated twice. Chitosan nanoparticles biosynthesis (20 g kg^{-1}) and powder *Spirulina platensis* (100 g kg^{-1}) in diets for *C. griepinus* reared in tanks was found to significantly ($P < 0.05$) the best level improved water quality daily weight gain, survival, feed utilization and meat quality as well as body composition ($P < 0.05$) of *C. gariepinus* post fingerlings which lay waste globally has the potentials to revolutionize aquaculture.

Keywords: *C. griepinus*, Chitosan nanoparticles biosynthesis, powder *Spirulina platensis*, Water quality, Feed utilization, Growth Performance, Body Composition.

INTRODUCTION

In the current global situation, which is characterized by climate change, the destruction of ecosystems and the associated extinction of species and the excessive use of natural resources, the topic of sustainability is increasingly becoming the focus of public discussion. In the context of the need to ensure an adequate supply of food for the constantly growing world population, approaches for more sustainable production are being discussed, particularly in the area of agricultural and animal production (Alexandratos and Bruinsma, 2012).

In the future, the availability of (FM)for aqua feeds can no longer be guaranteed because the fishery stocks in the oceans have been depleted, and sustainability of FM production from wild fish catch is question able (Naylor *et al.*, 2000). Therefore, extensive research has been done to find alternative protein sources of either animal or vegetable origin for improving the growth and health status of farmed fish for sustainable aquaculture (Abdel-Tawwab *et al.*, 2020).

In the present study, African catfish (*Clarias gariepinus*)is one of the most important cultured fish in tropical and subtropical regions and represent the most cultivated fish in Egypt (FAO, 2014). Has rich nutritional content, delicious taste, palatability, absence of intramuscular spine, along with a high market value (Adewolue *et al.*, 2008). This species is known for its high growth rate, resistance to handling and

stress, relatively low requirements for water quality, amenability to high stocking densities, excellent meat quality and preference amongst consumers in many African countries (Hecht *et al.*, 1996).

According to Anoop *et al.* (2009), it provides food for the populace, it allows for improved protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein.

Feed additive sectors are expanding day after day to achieve better growth and health of fish and shrimp and to meet the potential requirements of the culturists (Ibrahim, 2013). Fish feed generally constitutes 60–70% of the operational cost in intensive and semi-intensive aquaculture system (Singh *et al.*, 2006) and the cost of fishmeal is about 80% of aquaculture industry operating costs where protein is the controlling factor, which determines the cost of fish diet (Shepherd and Jackson, 2013). However, it is not only expensive but the supply is stagnated due to overexploitation of the natural resources and competition from humans and other livestock ventures (Jabir *et al.*, 2012).

The increasing cost of feed because of the high cost of fishmeal is the major limitation in the culture of African catfish. Therefore, the national organics standards board (NOSB) has proposed limiting the use of fishmeal and oil in organically certified aquaculture products with a 12-year phase-out schedule (NOSB, 2008). Consequently, efforts have been made to replace fishmeal partially or wholly with alternative protein sources in the diet of fish species. These efforts have sought to achieve similar or better output like fishmeal at a reduced cost (Ojewole *et al.*, 2022).

Elsadek *et al.* (2016) concluded that, in short the results concerned with the effect of replacing fish meal protein with sand smelt meal (SSM) protein indicate that fish meal protein could be replaced up to 75% sand smelt higher than effects on growth performance, feed utilization and chemical composition parameters of Nile Tilapia compared with control and no significant difference between the control diet and diet replacement 100% with SSM. On the other hand, used biogen in diets replacement at a rate of 0.3g/kg it led to an improvement in all measurements in substitution treatment compared to the control. EL-Haroun (2007), and he was concluded that the addition of probiotic Biogen® as a feed additive in African catfish *Clarias gariepinus* diets is recommended to improve growth performance and nutrient utilization as well as improve the economic efficiency.

Due to its valuable bioactive compounds, algae culture has been one of the fastest-growing aquaculture industries, developing global aquaculture economic production, and playing a key role in several bioindustries, like fish feed additives, human food supplements, and organic agriculture (Ashour *et al.*, 2019).

From the large number of microalgae species, *Spirulina*, *Arthrospira platensis* (SP), in particular has proven to be a promising substitute for fishmeal. *Arthrospira* (formally named *Spirulina*) is a filamentous cyanobacterium, which is not only characterized by a high protein content (50%–70%), essential amino acids, essential fatty acids, vitamins, minerals, and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007), but also has a balanced pattern of fatty acids and amino acids (Becker, 2007).

Nanotechnology is a process or technique that work on materials at the nanoscale, mostly between 1 and 100 nm, and scientifically, these materials are known as nanoparticles. Chitosan nanoparticles (CSNP) attracted attention due to their distinctive properties and interesting applications (Radhika-Rajasree and Gayathri, 2014).

Marwa *et al.* (2022) reported that CS and CSNP are good feed additives that promote the growth performance and stimulate the immune system, especially the innate immune response. In addition, they are used as drug carriers, antioxidants and water treatment agents. Moreover, in fish aquaculture, CSNP proved to be favored by the recent authors than CS due to their larger surface area, bioavailability and deep penetration to the target sites. Therefore, CSNP is recommended to be used worldwide, and particularly in Egypt to enhance fish aquaculture quality.

There is no previous literature about the Evaluation of partial quantitative levels of replacement of Basaria (Sand Smelt) local fish meal as main source protein and Biogen with chitosan biosynthesis and *Spirulina platensis* powder in diets for African catfish (*clarias griepinus*) reared in tanks.

In general, the major research objective of this study is to examine the evaluation and suitability of different partial levels replacement of local Basaria (Sand Smelt) fish meal and Biogen, with chitosan nanoparticles biosynthesis (CSNPs) and powder *S. platensis* and to find out the optimum level of replacement without negatively affecting their on some parameters such as water quality, fish growth

performance, feed utilization ,proximate composition, survivability , and Economic evaluation, in diets for *clarias griepinus* post fingerling reared in tanks.

MATERIALS AND METHODS

The present practical study was conducted out at the experimental fish farm, fish production department, faculty of agriculture, Al-Azhar University, Cairo, *Egypt*. The actual experimental period extended for 14 weeks (98 days) started during first 1th April to 15rd July 2023.

Description of experimental aquaculture units:

The experimental rearing tanks system has consisted of a series of rectangle concrete tanks (3m x 1.0 m x1.0 m) of 1.0 m³ water; each were used to store the experimental fish until they acclimatized and execute this study. The tanks were supplied with freshwater (dechlorinated tap water). All experimental tanks were supplied with air through an aeration system which connected with air pump (5 hp).

Experimental fish:

The fishes used in this study were Post Fingerling African Catfish (*Clarias gariepinus*) and local Basaria (Sand Smelt (*Atherina boyeri*) fish meal (SSM)dried was obtained from a commercial supplier (Elobor market) Cairo, Egypt.

The experimental fish were transported at early morning using a special fish transport car with aeration facilities. They seemed healthy and were acclimated to the experimental system condition 15 days before starting the experiment thereafter fish and were fed a manufactured fish basal diet (BD) 40% CP, dried local Basaria (Sand Smelt) fish meal (SSM) as a source of protein with 3 gm Biogen / kg.) diet thrice a day. The turnover rate of water was 0.5 m³ day⁻¹ tank⁻¹. *Clarias gariepinus* were randomly distributed in four experimental dietary groups (duplicated) and carefully stocked into 4 tanks 3.00 x 1.00 x 0.35 m³ (1m³ water each, divided into two basins) and 1.0 m depth, in each basins fish African Catfish *Clarias gariepinus* were stocked at a density of 30 fish / basin (60 experimental fish in each treatment).

In 15 March 2023, 240 African catfish were equally divided into 4 groups The average initial body weight for African Catfish *Clarias gariepinus* ranged from 94.75±1.19 to 95.87±1.14g and average initial body length ranged from 34.1±1.12 to 35.6±1.07cm.

Feeding rate and techniques:

The experiment was conducted in a Randomized Completely Block Design (RCBD) with four different treatments in duplicates. Feeding ration amounted to 5% of total body weight daily at the first period (2 weeks adapted to experimental conditions) and then reduced to 3% of total body weight daily for (6 days) a week during the experimental period (14 weeks). Diets were fed to each group of fish in the form of dried pellets suitable to the fish size. Fish were hand fed thrice a day at 3% fresh body weight for 98 days. The daily diets were divided into three equal amounts and offered three times a day (0900, 1200 am and 1700 pm). A random sample of fish from each treatment was weighed every fourteen days using hand net, the fish in each tank was weighed and the amount of daily diet was adjusted according to the new fish biomass throughout the experimental period (Annet, 1985).

Experimental ingredients:

The artificial diet was prepared by using the ingredients dried Basaria (Sand Smelt) fish meal (SSM) as a source of protein with (0.3g Biogen/kg.), powder of *S. platensis*, chitosan nanoparticle biosynthesis (CSNPs), soybean meal, wheat bran, yellow corn, Vitamin and mineral premix, sun flower oil and carboxy methyl cellulose(CMC).

The soybean meal, wheat bran, yellow corn and Vitamins and minerals premix, sun flower oil and (CMC) were purchased from the Islamic Company, Nasr City, Cairo, Egypt. Biogen®, powder of spirulina *Spirulina platensis* and powder of chitosan nanoparticle biosynthesis (CSNPs) (Mahatani Chitosan Pvt. Ltd., Veraval, India) used in this experiment were purchased from National Research Centre (NRC), Cairo, Egypt.

S.platensis powder and all experimental diets was analyzed and determination at the Central Laboratory conducted in the laboratory of the Department of Chemistry, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, and National Research Centre (NRC), Dokki, Cairo, Egypt according to the

methods described by the Association of Official Analytical Chemists (A.O.A.C, 2010).

Preparation of chitosan nanoparticles (CNPs):

Chitosan nanoparticle biosynthesis CNPs were produced using ionic gelation method; incorporating a polyanion, TPP (tripolyphosphate) into the crude chitosan solution under constant stirring as reported by Masarudin *et al.* (2015).

The obtained chitosan nanoparticle biosynthesis (CNPs) were freeze-dried for further use in fish feed as an additive. The size, shape, and surface morphology of CNPs were determined using a scanning electron microscope (SEM) (JSM-6490-A, JEOL Ltd. Tokyo, Japan) at $\times 5000$ magnification.

Preparation of sand smelt meal:

Sand smelt was sun-dried and then at 70°C for 24 h, ground with a hammer mill, packed in plastic bags, sealed, and stored at -20°C until used in diet production.

Proximate compositions of feed ingredients:

Proximate compositions (Table 1) of feed ingredients were analyzed before feed formulation. Experimental diets were prepared by partial quantitative replacement of Sand Smelt fish meal with chitosan nanoparticle biosynthesis and *Spirulina platensis* powder to assess the practicability of their use in *C. gariepinus* feed.

Ingredients of each diet were ground using a laboratory grinder and then separately blended to form homogenous dough by adding 100 mL water per one kg diet dried *S.platensis* and (CNPs) were added as an partial quantitative levels replacement of sand smelt meal and combined thoroughly to obtain a homogenous mix.

The dough was passed through a meat chopper (Brand-Filizola) to obtain pellets of 3 mm diameter and sun-dried for two days. The pellets were then stored in plastic containers and refrigerated (-4°C) for further use. After preparation of the experimental diets, the proximate composition was analyzed (Table 2) according to A.O.A.C. (2010) specifications.

The proportion and proximate composition of different ingredients in experimental and control diets has been incorporated in Table (1).

Table (1): Proximate composition of feedstuff (% of dry matter basis).

Ingredients	(DM)	(CP)	(EE)	(CF)	(CA)	(NFE¹)	(GE²)
Sand smelt meal	87.39	65.3	4.72	0.66	17.49	11.83	461.47
Soybean meal	88.5	44	4.82	7.3	6.75	37.13	446.27
Yellow corn	87.5	8	5.27	2.3	2.91	81.52	429.92
Wheat bran	88.62	15.31	8.86	9.9	5.27	60.66	419.3
Spirulina(g/kg)	89.8	66.8	9.3	7.6	6.5	9.8	504.82

¹(NFE) was estimated as = 100 - (% ash + %fiber+ % crude fat + % crude protein).

²Gross energy value was calculated from their chemical composition, Estimated according to Jobling, (1983). As 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively

Preparation of the experimental diets:

Four different isonitrogenous (39.63 \pm 0.657-39.66 \pm 0.599)) crude protein and isocalours (3490.0 \pm 40.9 - 3544.6 \pm 41.5) kcal/kg gross energy, experimental diets were formulated, and used in the feeding trials. The diets were processed by blending the dry ingredients into a homogenous mixture and then passing the mixed. All diets were reformulated on dry matter basis. Dry ingredients were weighed, combined and mixed together by using a mixer. Diets were single extruded through a 1-cm die air-dried in a convection oven. After drying, all diets were broken into pellets. Diets were reformulated from purified diet ingredients to contain 40% crude protein diets as shown in (Table 2).

Table (2): Feed ingredients and proximate composition (% on dry matter basis) of the experimental diets

Ingredients	T1 (control)	T2	T3	T4
Sand Smelt fish meal (SSM) (g/kg) (65.3%CP)	375	315	255	195
Soybean meal(g/kg) (44% CP)	320	320	320	325
Yellow corn g/kg) (8% CP)	130	130	130	130
Spirulina(g/kg) (66.8% CP)	---	50	100	150
Chitosan nanoparticles (CSNPs) (g/kg)	---	10	20	30
Wheat bran(g/kg) (15.31% CP)	105	105	105	100
Vitamin and mineral premix ¹ (g/kg)	20	20	20	20
Biogen• (g/Kg)	0.3	0.3	0.3	0.3
Sunflower oil(g/Kg)	40	40	40	40
Carboxy methyl cellulose(CMC) (g/Kg)	10	10	10	10
Total (g/kg)	1000.3	1000.3	1000.3	1000.3
Mean (± SE) chemical compositions (% dry matter basis) of treatment diets used in the study.				
Dry matter (DM) (%)	88.59±0.426	88.93±0.441	89.62±0.167	89.08±0.577
Crude protein (%)	39.66±0.599	39.65±0.524	39.64 ±0.059	39.63±0.657
Lipid (%)	9.34±0.305	10.26 ±0.567	10.53±0.526	10.86±0.779
Ash (%)	9.67±0.440	9.02±0.370	10.18±0.681	9.35±0.367
Fiber (%))	4.95±0.210	4.40±0.450	4.22±0.421	4.18±0.247
The Nitrogen Free Extract (NFE)	26.50±1.041	25.64±1.450	25.07±1.348	24.59±0.720
Gross energy(Kcal/kg diet ³)	3544.6±41.5	3524.4±47.3	3492.1±43.1	3490.0±40.9
Gross energy (kJ g-1) ⁴	17.87	18.18	18.11	18.48
Protein energy ratio(mg CPkcal ¹)	11.31	11.49	11.52	11.92

¹Vitamin and mineral mixture each 1 kg of mixture contains: retinyl acetate (vit. A), 3000 IU; cholecalciferol (vit.D), 2400 IU; all-race-in-tocopheryl acetate (vit. E), 60 IU; menadione sodium bisulfite (vit. K), 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid, vit. G), 120mg; cyanocobalamin (vit. B12), 0.024mg; d-biotin, 0.168mg; choline chloride, 1200mg; folic acid, 1.2mg; niacin, 12mg; d-calcium pantothenate, 26mg; pyridoxine.HCl, 6mg; riboflavin, 7.2mg; thiamin.HCl, 1.2mg; sodium chloride fNaCl, 39% Na, 61% Cl, 3077mg; ferrous sulfate fFeSO .7H₂O, 20% Fe, 65 mg; manganese sulfate fMnSO., 36% Mn, 89 mg; zinc sulfate (ZnSO.7H₂O, 40% Zn), 150 mg; copper sulfate (CuSO .5H₂O, 25% Cu), 28 mg; potassium iodide fKI, 24% K, 76% I, 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000mg.

²The Nitrogen Free Extract (NFE) was estimated as = 100 - (% ash + % fiber + % crude fat + % crude protein).

³ Gross energy (Kcal/kg diet), E (kcal /100 g.) value was calculated from their chemical composition, Estimated according to Jobling, (1983). As 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

⁴Gross energy (kJ g-1) were calculated using the physiological values, CP x 23.9 + lipid x 39.8 + carbohydrates x 17.6

(Schulz et al., 2005).

The dough was pelleted using a Hobart A-200T mixing and pelleting machine. Long strings of pellets were created with a 3 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%.

Formulated diets and fish carcasses were analyzed for proximate composition using standard methods by (A.O.A.C, 2010).

Fish in treatment (1): were fed basal diet (BD) was formulated of Basaria (Sand Smelt) fish meal with 0 g chitosan nanoparticle biosynthesis and 0g *Spirulina platensis* algae powder

Fish in treatment (2): were fed BD with partial quantitative replacement of Basaria (Sand Smelt) fish meal by chitosan nanoparticle biosynthesis (10 g kg⁻¹) and *Spirulina platensis* algae powder (50 g kg⁻¹).

Fish in treatment (3): were fed BD with partial quantitative replacement of Basaria (Sand Smelt) fish meal by chitosan nanoparticle biosynthesis (2 g kg^{-1}) and *Spirulina platensis* algae powder (10 g kg^{-1}).

Fish in treatment (4): were fed BD with partial quantitative replacement of Basaria (Sand Smelt) fish meal by chitosan nanoparticle biosynthesis (30 g kg^{-1}) and *Spirulina platensis* algae powder (150 g kg^{-1}).

At the beginning of feeding trial, a total number of five experimental fish *C. gariepinus* post fingerling were netted, weighed and immediately kept in a deep freezer (-18°C) for chemical analysis (as zero group). A similar procedure was applied at the end of such experimental period (five experimental fish *C. gariepinus* as final samples of each treatment (group). Zero group and the final samples of each treatment were separately dried at 65°C for 24 hrs. Then ground in a mixer. Representative samples were chemically analyzed according to A.O.A.C. (2010) methods, while their energy contents were calculated according to NRC (1993).

Water quality measurement:

Physicochemical parameters (water temperature, dissolved oxygen (DO), pH, and total ammonia) of the experimental tanks water were recorded weekly, in situ in each tank to observe the overall culture environment, where there was fluctuation, 10% of the cultured water was replaced with fresh water from the reservoir.

Temperature was measured every day, dissolved oxygen and pH was measured weekly. The temperature was measured directly in the water column two time every day (minimum and maximum) after feeding by thermometer.

Dissolved oxygen (DO) was measured directly in the water column of tanks every week by using oxygen meter. pH was measured of tanks every week during experiment by pH meter.

Total ammonia was determined by an ammonia measuring kit (HANN instrument test kit). The analyses were carried out in the tanks, following the standard analytical procedures detailed in (APHA,1988).

Fish growth and feed utilization performance:

Data on growth of fish were gathered. Fish were weighed to the gram using an electronic balance. Fish lengths were measured in centimeters by using measuring scale. All fish growth parameters were calculated on performance such as mean final fish weight, daily weight gain (g/f/d), percentage of weight gain (%) and specific growth rate, SGR (%/day), survival rates and condition factor(K) of fish.

Sampling and data collection:

Weighing and length determination of fish was carried out prior to commencement of feeding trial and fortnightly throughout the period of the experiment and fish were not fed but feeding rate was recalculated to accommodate weight changes. At the end of the feeding trial, fish were starved of feed for 24 hours captured and weighed individually in all the treatments. Data on fish growth were recorded two weeks. The experimental tanks were inspected daily to remove dead fish, if any.

Growth performance parameters:

Fish growth performance, mean daily weight gain (g), mean weight gain, average body weight gain, condition factor, specific growth rate and survival rate, protein productive Value (PPV) and energy retention (ER) as described in Jimoh *et al.* (2019) by the following equations:

Mean 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.

$$\text{DWG} = \text{Fw} - \text{IW} / \text{N}$$

Where: DWG= daily weight gains FW=final weight of fish

IW=Initial weight of fish.

N=number of days the fish were cultured

Mean weight gain (MWG) = (W1) – (W0)

Where: W1: mean final weight W0: mean initial weight

Condition factor(K) = [FW / FL³] x 100

Where: FW: Final body weight (g) FL³: Final body length (cm³)

Specific Growth Rate (SGR %):

Specific Growth Rate (SGR) was calculated using the formula:

Specific growth rate (SGR, % /day) = ((ln FBW – ln IBW) x 100) / No. of days, Where;

FBW =Final Body Weight at each harvest, IBW=Initial Body

Weight ln=Natural logarithm.

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.

Feed utilization:

Feed intake (FI) , daily feed intake, feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), protein productive value (PPV%) and energy retention (ER).

Feed efficiency (FE):

(FE %) = [Weight gain (g) / Feed intake (g)] x100

Feed conversion ratio (FCR):

This is the numerical value used to measure the gross utilization of feed for growth in fish. It is also, a measure of efficiency or suitability of a feed. This ratio shows the amount of feed required to achieve a unit weight increase in the product. Feed conversion ratio (FCR) is calculated from the number of kilos of feed that are used to produce one kilo of whole fish. Feed conversion ratio (FCR) were determined at the end of the experiment as follow (Tacon, 1987):

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed supplied (g) (dry weight basis) Total}}{\text{weight gained by fish (g)}}$$

Protein Efficiency Ratio (PER):

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

Protein productive value (PPV%):

(PPV %) = [(PR1– PR0) / PI] x 100

Where:

PR1: is the total fish body protein at the end of the experiment.

(On dry matter basis)

PR0: is the total fish body protein at the start of the experiment.

(On dry matter basis)

PI: Protein intake.

Protein retention (PR%):

$$\text{Protein retention (\%)} = \frac{\text{Total protein gain (g)}}{\text{Total protein fed (g)}} \times 100$$

Energy retention (ER %) = [E- E0 / EF] X 100:

Where:

E= the energy in fish carcass (kcal) at the end of experiment.
E₀= the energy in fish carcass (kcal) at the start of experiment.
E_F = the energy (kcal) in feed intake.

Lipid retention (LR%):

$$\text{Lipid retention (\%)} = \frac{\text{Total lipid gain (g)}}{\text{Total lipid fed (g)}} \times 100$$

Energy value (E):

Calculating the approximate composition data. Energy (kcal) was calculated according to the following equation:

$$E \text{ (kcal /100 g.)} = \text{CP} \times 4 + \text{CHO} \times 4 + \text{fat} \times 9.$$

Values were expressed in kcal/100g.

Proximate and chemical scrutiny:

The proximate composition:

Whole bodies of fish from the feeding trial (n = 5 fish before; n = 3 fish per replicate tank after) were compared for proximate composition, which was calculated as follows:

Biochemical analyses of fish diets and dried *Spirulina platensis*:

Sample preparation:

After 98 days of rearing, the fish samples were collected, measured and weighted. Then the samples were taken for laboratory analysis to estimate the whole body percentages of moisture, crude protein, crude fat, crude ash, crude fiber and Nitrogen Free Extract (NFE), were chemically analyzed according to A.O.A.C. (2010) methods, the samples were then weighted and minced in a chemical tissue grinder.

Carbohydrates (CHO): they were quantified according to the method in (Duchonová et al., 2013), by a difference of all other components as weighting rams minus water, fiber, ash, fat, and protein content.

Energy value (E): it was calculated according to the method in (Duchonová et al., 2013), by calculating the approximate composition data. Energy (kcal) was calculated according to the following equation:

$$E(\text{kcal}) = \text{CP} \times 4 + \text{CHO} \times 4 + \text{fat} \times 9.$$

Values were expressed in kcal/100g.

The fish were also analyzed before commencement of experiment for initial body composition.

Statistical analysis:

Data were tested for distribution normality, linearity and homogeneity of variance. Log-transformation of the raw data was used for some measured parameters because of the large range across the data. Data were analyzed and visualized in GraphPad Prism 6 and all results were reported as means with SEM. A two-way ANOVA was used to evaluate and suitability of fed on diets contain different ratios of replacement of Basaria (Sand Smelt) fish meal with chitosan nanoparticles (CSNPs) biosynthesis and *Spirulina platensis* algae as an alternative supplementation.

The interaction of the two factors was tested using Tukey's multiple comparison test as a post hoc test where appropriate. The level of significance was set at $p \leq 0.05$ followed by the Duncan's post hoc multiple test at a 5% probability level. Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was applied to compare means for detection of the level of variation among treatments.

All analysis was performed using SAS (version 6, 2004 SAS Institute, Cary, NC, USA), SAS (2004). Considering the control group with and without probiotic supplementation, the used model for analysis was:

$$X_{ij} = \mu + T_i + E_{ij}$$

Where:

- μ is the overall mean.
- T_i is the effect of all treatments including the control.
- E_{ij} is the experimental random error

RESULTS AND DISCUSSION

The present study reports the first use of partial replacement of dried *Spirulina platensis* and Chitosan Nanoparticles biosynthesis by local sand smelt meal in the African catfish (*C. gariepinus*) post fingerling diets.

Effect of experimental diets on water quality:

Water quality parameters influence the growth and survival of different developmental stages of fishes and hence determining the optimal water quality variables is greatly important for any aquaculture farming.

The water quality parameters (temperature (°C), hydrogen ion concentration (pH), dissolved oxygen concentration (DO mg L⁻¹) and ammonia nitrogen (mg L⁻¹) were carefully monitored throughout the experiment in order to know, manage and to ensure good and appropriate water quality conditions at all time during the study period were president in (Table 3). The range of values of the water quality parameters during the experimental period for, pH, dissolved oxygen, temperature and fall within the range recommended by Boyd and Tucker (1998), for freshwater fish production. All fish irrespective of the feeding frequency fed actively and appeared healthy. There was significant difference (p<0.05) among the different treatments in the morning temperature. However, pH and DO (mg L⁻¹) were significantly (p<0.05) higher in T2, D3 and T4 than in (T1) Control, (Table 3).

There was a significant difference (p<0.05) among the different treatments in the morning temperature. However, pH and DO (mg L⁻¹) were significantly higher in T2, T3 and T4, respectively, than in T1(Control), (Table 3).

While Ammonia nitrogen (mg L⁻¹) was significantly(p<0.05) lower in T2 (0.03 ± 0.01), T3 (0.018 ± 0.01)and T4(0.021± 0.03)than in T1(Control)(0.042 ± 0.02 mg L⁻¹), respectively, (Table 3).

Table (3): Effect of experimental diets on physicochemical parameters of experimental tank water of the African Catfish (*C. gariepinus*) grow out Stage. (Means±SE, 14 weeks).

Parameters of experimental water	T1 control	T2	T3	T4
Water temperature(°C)	27.11±1.49 ^c	27.35±1.49 ^c	29.69 ±1.49 ^a	28.96 ± 1.2 ^b
pH	6.54±0.25 ^c	7.38±0.51 ^b	7.96±0.24 ^a	7.59 ± 0.32 ^a
Dissolved oxygen (mg/L)	6.82± 0.34 ^c	7.15±0.24 ^b	7.73±0.24 ^a	7.26±0.24 ^b
Ammonia nitrogen (mg/L)	0.042±0.02 ^a	0.03 ±0.01 ^b	0.018 ±0.01 ^c	0.021 ± 0.03 ^c

Mean with the same superscript letter are not significantly different (P<0.05).

Values are mean±SD . *S. platensis* and chitosan nanoparticles biosynthesis (CSNPs).

While Ammonia nitrogen (mg L⁻¹) was significantly lower in T2, T3 and T4 than in T1 (Control), respectively (Table 3). In general, averages of morning water temperature ranged from 27.11±1.49 to 29.69 ±1.49°C during the experimental period. Although no significant difference (p<0.05) existed in temperature between the treatments. The African catfish has an optimal temperature which varies from 20 °C to 30 °C with 25 °C and 27 °C as the most favorable for adults and juveniles respectively (Viveen *et al.*, 1985). On the other hand, Isyagi *et al.* (2009) reported the ideal temperature range for pond production of African catfish was 26°C to 32 °C. Among the variables, water pH is considered the key factors and plays an important role in the maintenance of the homeostasis in fishes. The value pH was found to fall within the optimum range in this study. Though there was a gradual rise, the difference was significant(p<0.05) difference from the control. Mean pH in treatment T3 was 7.96±0.24. This value is too good for the culture of *C. gariepinus* (Table 3). Dissolved Oxygen (DO mg L⁻¹) ranged between 6.82 T1 and 7.73 mgL⁻¹ in T4 (Table 3). The temperature and dissolved oxygen levels were within the acceptable limits for rearing African catfish (Ogunji and Awoke, 2017). Averages of water ammonia nitrogen (mg L⁻¹) in water experimental tanks ranged from 0.042±0.02 (T1) to 0.018 ± 0.01 mg L⁻¹ (T3) during the experimental period (Table 3).

From Table 3 , the incorporation of *S. platensis* and chitosan nanoparticles biosynthesis (CSNPs) had positive effect on water quality. Ammonia drastically reduced with increase in *S. platensis* and chitosan nanoparticles concentration. Isyagi *et al.* (2009) suggested the range of 0.3 to 2 mg/l of toxic form of ammonia is acceptable or optimal for African catfish production. Generally, TNAU (2008), also reported

that the optimal range of un-ionized ammonia is 0.02–0.05 mg L⁻¹ in fish ponds. This finding agrees with Udo *et al.* (2018) 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.

The present finding showed that effect of experimental diets, T2, T3 and T4 on Physicochemical parameters of experimental tank water of the African Catfish (*C. gariepinus*) grow out stage significantly improved the quality of the cultured water by increasing the pH concentration of the cultured water and significantly ($p < 0.05$) improved the quality of the cultured water by reducing the ammonia concentration of the cultured water. These findings agree with that of (Wang and Li, 2011) who reported improved water quality as a result of chitosan and chitosan nanoparticles supplementation in the diet of *Oreochromis niloticus* and *Clarias gariepinus* individually.

The proportion and proximate chemical composition of different ingredients in experimental and control diets:

The proportion and proximate chemical composition of different ingredients and partial replacement of dried *Spirulina platensis* and Chitosan Nanoparticles biosynthesis by local sand smelt meal (SSM) (%) on a DM basis) in experimental and control diets has been incorporated in Tables (1 and 2).

From Table (1), the dry matter (DM), crude protein (CP), Crude Lipid (EE), Crude Fibers (CF), Crude Ash (CA), Nitrogen Free Extract (NFE) and gross energy (Kcal/kg diet) levels content of dried *Spirulina platensis* were (89.8, 66.8, 9.3, 7.6, 6.5, 9.8, and 504.82), respectively, were higher than that of sand smelt meal by product (87.39, 65.3, 4.72, 0.66, 17.49, 11.83 and 461.47), respectively. However, the levels of crude ash levels were found to be lower than that of *S. platensis* (6.5%) to local Sand smelt meal (17.49%).

Carbohydrate as nitrogen-free extract (NFE) in *S. platensis* content (9.8%) was lower than that of Sand smelt meal (11.83%). While, the sand smelt meal by product contains (461.47 Kcal/kg diet) gross energy while *S. platensis* contains (504.82 Kcal/kg diet) gross energy. Results indicated that, the chemical composition of *S. platensis* was found to be best than local Sand smelt meal.

The CP levels of the formulated African Catfish (*C. gariepinus*) grow out stage diets attained the minimum nutritional requirements of 35-40% of catfish (Adewolu and Aro, 2009). The diets in all the treatments had a crude protein of between 39.63±0.657 - 39.66±0.599 %, Lipid % (9.34±0.305-10.86±0.779), Ash (%) (9.02±0.370-10.18±0.681), Fiber (%) (4.18±0.247-4.95±0.210), Nitrogen Free Extract (NFE) (24.59±0.720-26.50±1.041) and Gross energy (Kcal/kg diet³) (3490.0±40.9-3544.6±41.5) as shown in table 2. The results of proximate and chemical analysis showed that the four treatment diets were similar in nutrient composition although they had slight non-significant variations. On the other hand, there was no significant difference ($P > 0.05$) among the proximate composition of the formulated experimental diets (Table 2).

Effect of experimental diets on growth performance of the African Catfish (*C. gariepinus*) grow out Stage:

The present study showed that the mean final weight (g), final weight gain (WG) (g), daily weight gain (DWG)(g) and specific growth rate (SGR (%/ day)) of the fish fed diets containing partial replacement up to 100 g dried *S. platensis* and + 20 g chitosan biosynthesis by sand smelt meal (T3) were significantly higher ($p < 0.05$) than that of fish fed with the T1, T2 and T4, respectively, while fish fed the control diet had the significant differences ($p < 0.05$) lowest FBWG (404.94±14.4^d), BWG (309.07±14.39) and SGR% (2.39±0.09) (Table 4). This trend was significantly noticeable ($p < 0.05$) for WG and SGR, in which there was obvious increases as the Effect of partial replacement of dried *Spirulina platensis* and chitosan biosynthesis by local sand smelt meal on growth performance of the African Catfish (*C. gariepinus*) grow out stage. Rapid growth rate is one of the most favorable aspects of the biology of African catfish culture in terms of its aquaculture potential (Haylor, 1992a). Under optimal management conditions, they grow to over 10 g at an age of two months and more than 200 g in 5-7 months in tanks (Huisman and Richter, 1987).

Final Body length were observed in significantly differed ($p < 0.05$) between groups (T2, T3 and T4) in *C. gariepinus* grow out stage compared with control diet (T1). Maximum final Body length were significant differences ($P > 0.05$) achieved in the experimental fish fed diets T3 (74.8±1.25 cm) compared to the control diet T1 (60.2±0.61 cm). Size variation in fish length was observed in all the treatments. However, the lowest growth performance was significant differences ($P > 0.05$) noted in experimental fish fed the experimental diet (T1; control) compared to the other experimental diets (Table 4). These might be related to the chitosan nanoparticles and *S. platensis* which might be enhance the digestive enzymes such as protease, amylase, and lipase, which has the potential to increase the value of digestive enzymes

in the gut and/or its effect for also improving digestive activity by synthesis of vitamins and co-factors or enzymatic improvement for WG and SGR (Gatesoupe, 1999). African catfish grow out Stage are highly efficient feed converters showing very good Feed Conversion Ratios (FCRs) in culture systems (1:1) (Uys, 1989) when fed on commercial pelleted food, but also grow very well when fed low cost feeds manufactured from agricultural by-products (Michiels, 1987). Trials indicate that the specific growth rate (SGR) of small fish (0.3 - 3 g) was 11% per day whereas for large (95 - 200 g) fish SGR is around 2% (Hogendoorn, 1983). Condition factor (K) of African catfish culture fed diets (T3) (2.427±0.05) was higher significantly (p<0.05) compared to fish fed the control diet, and was higher significantly (p<0.05) than that the fish fed with the other diets. While fish fed the control diet (1.856±0.01) had the lowest significantly (p<0.05) condition factor (K) (Table 4). The present study reveals that T2, T3 and T4 on growth performance of the African Catfish (*C. gariepinus*) grow out Stage diets showed better Fulton's condition factor and survival rate when compared to the fish fed control diet. This further corroborates the high growth performance exhibited by fish fed dried *S. platensis* and Chitosan biosynthesis diets and hence, it can be suggested that together these factors signify the good health condition of the fish. There was a significant difference (P>0.05) in survival % were confirmed on the experimental groups of African catfish culture fed diets containing partial replacement up to 100 g dried *Spirulina platensis* and+ 20g chitosan biosynthesis by sand smelt meal (T3,96.7 %) compared to fish fed the control diet, were higher (p<0.05) than that the fish fed with the other diets. While fish fed the control diet had the lowest survival % (91.7 %) (Table 4).

Table (4): Effect of partial replacement of dried *Spirulina platensis* and Chitosan biosynthesis by sand smelt meal on growth performance of the African Catfish (*C. gariepinus*) grow out Stage (Means ±SE, 98 days).

Parameters	Graded levels of dried <i>Spirulina platensis</i> and Chitosan biosynthesis by sand smelt meal (SSM) in experimental diets (%)			
	(T1) Control	T2	T3	T4
Initial. Fish weight (g)	95.87±1.14 ^a	94.75±1.19 ^a	94.88±1.10 ^a	95.34±1.10 ^a
Final fish weight (g)	404.94±14.4 ^d	676.71±18.82 ^c	759.88±17.44 ^a	697.16±17.95 ^b
Initial. Fish length (cm)	35.4±1.12 ^a	35.6±1.07 ^a	34.1±1.12 ^a	35.5±1.14 ^a
Final fish length (cm)	60.2±0.61 ^b	71.1±1.16 ^a	74.8±1.25 ^a	72.8±1.34 ^b
Final weight gain (g)	309.07±14.39 ^c	581.96±15.40 ^b	665±12.90 ^a	601.82±17.26 ^b
AV. Daily gain (g)	3.15±2.67 ^c	5.938±2.22 ^b	6.786±1.35 ^a	6.15±1.49 ^b
Specific growth rate (SGR%/day)	2.39±0.09 ^c	2.75±0.05 ^b	3.39±0.14 ^a	3.11±0.15 ^a
Condition factor (K) ^a	1.856±0.01 ^c	1.883±0.03 ^b	2.427±0.05 ^a	2.273±0.03 ^b
No. of fish at Start.	60	60	60	60
No. of fish at end.	55	57	58	57
Survival ratio (SR %)	91.7 ^c	95 ^b	96.67 ^a	95 ^b

Values are mean±SD from three replicates. Mean with the same superscript letter are not significantly different (P<0.05).

These results are in accordance with Moe (2011) who noted that growth performance and the average of survival rate of *Litopenaeus vannamei* fed with 0.05% dietary *S. platensis* and chitosan was higher (100%) than the control group (80%). Jana *et al.* (2014) also found that *Spirulina* improves the survival rate of Pangus fish *Pangasius sutchi* it was found to be 94% and 80% survival rate, respectively, for the fodder impregnated with *spirulina* and chitosan at the end of the experiment. Zaki *et al.* (2015) 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.

Generally, the best response was observed when *C. gariepinus* grow out stage were fed diet by partial replacement of dried *Spirulina platensis* and Chitosan biosynthesis by sand smelt meal (SSM) on growth performance of the African Catfish (*C. gariepinus*) grow out stage the basal diet (T1, control). Moreover, these data were in accordance with the finding by Abdel-Warith and Elsayed (2019) who found that the possibility of adding *A. platensis* as a protein source fed to *O. niloticus* improves the overall feed utilization, growth performance, survival rate and body composition. Carnevali *et al.*, (2006) found that weight gain for black tiger shrimp was significantly higher for the treated groups compared to the control group when *S. platensis* were treated by chitosan nanoparticles and used for 70 days. Al-Dohail *et al.*

(2009) also indicated that African catfish *Clarias gariepinus* fed the combination of *S. platensis* with chitosan showed better growth performance compared to the control fish group.

Effect of experimental diets on Feed utilization:

All variables related to feed utilization efficiencies such as Feed intake (g/fish) (FI), Daily Feed intake, Feed conversion ratio (FCR), Feed efficiency (FE), Protein efficiency ratio (PER), Productive protein value (PPV %) and Energy retention (ER %) in all experimental diets were influenced by dietary treatments (Table 5).

There are significant differences Feed intake (FI), daily feed intake, feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), protein productive value (PPV%) and energy retention (ER)($P>0.05$) shown in Table (5). As can be seen in (Table 5), average amounts of Total feed intake (FI) (g/fish) were significant differences ($P>0.05$) found to be 827.99 ± 15.89 , 1149.79 ± 21.34 , 1324.37 ± 20.41 and 1246.76 ± 17.15 , for fish fed T1 until T4, respectively, which indicate increases in feed consumption in fish fed T3 group compared to other diets groups with a significant difference ($P < 0.05$) between control and other groups. There was a noticeable effect of the dietary inclusion of alternative protein sources on feed intake (Table 5). Feed intake for catfish fed on the control diet containing the highest amount of sand smelt meal and T3 containing (100 g Sp.pl. +20 g(CSNPs) were significantly better than those observed for fish fed T2 and T4 (Table 5).

Table (5): Effect of partial replacement of dried *Spirulina platensis* and Chitosan biosynthesis by sand smelt meal on feed efficiencies of the African Catfish (*C. gariepinus*) grow out stage (Means \pm SE, 98 days).

Parameters	Graded levels of dried <i>Spirulina platensis</i> and Chitosan biosynthesis by sand smelt meal (SSM) in experimental diets (%)			
	T1 control	T2	T3	T4
Total feed intake (FI) (g/fish)	827.99 \pm 15.89 ^c	1149.79 \pm 21.34 ^b	1324.37 \pm 20.41 ^a	1246.76 \pm 17.15 ^{ab}
Feed conversion ratio (FCR)	1.46 \pm 0.05 ^a	1.40 \pm 0.06 ^a	1.18 \pm 0.03 ^a	1.39 \pm 0.07 ^a
Feed conversion efficiency (FCE%)	68.37 \pm 3.34 ^b	71.23 \pm 2.95 ^a	73.46 \pm 3.01 ^a	72.09 \pm 2.83 ^a
Protein feed intake (PFI) (g/fish)	328.38 \pm 8.65 ^c	455.89 \pm 7.59 ^b	524.99 \pm 9.37 ^a	494.09 \pm 8.19 ^{ab}
Energy feed intake (EFI) (Kcal/fish)	2934.8 \pm 17.96 ^c	3052.3 \pm 25.63 ^b	4628.6 \pm 22.79 ^a	4354.9 \pm 26.14 ^{ab}
Protein efficiency ratio(PER%)	1.73 \pm 0.08 ^b	1.79 \pm 0.11 ^{ab}	1.83 \pm 0.10 ^a	1.82 \pm 0.14 ^a
Energy efficiency ratio(EER)	1.9 \pm 0.06 ^b	2.02 \pm 0.09 ^{ab}	2.07 \pm 0.07 ^a	2.06 \pm 0.09 ^a
Protein Retention ratio (%)	32.77 \pm 0.84 ^b	37.27 \pm 0.75 ^a	39.83 \pm 0.67 ^a	38.19 \pm 0.84 ^a
Energy Retention ratio (%)	25.23 \pm 0.67 ^b	28.49 \pm 0.91 ^a	30.71 \pm 0.73 ^a	28.85 \pm 0.69 ^a

Values are means of two replicates per diet \pm SE. Mean with different superscript in the same row are significant ($p<0.05$).

The amount of daily feed intake, frequency and timing of the feedings and presentation of the predetermined ration are the key factors of feed management strategies influencing the growth and feed conversion (Goddard, 1995). Concerning the Total feed intake (FI) (g/fish) values, the groups fed T2(1149.79 ± 21.34), T3 (1324.37 ± 20.41) and T4(1246.76 ± 17.15) recorded the highest significant ($P<0.05$) values as compared to other tested group T1(control, 827.99 ± 15.89) (Table 5). FCR values also differed significantly ($P < 0.05$) between the control group (1.46 \pm 0.05) and T2 (1.40 \pm 0.06) when compared to fish fed on T3 and T4 (1.18 \pm 0.03 and 1.39 \pm 0.07). On the other hand, the best FCR% (lowest) values were obtained by the T4, T3 and T2 groups, respectively, followed in an significant ($P<0.05$) increasing order (worth) by T1 group, respectively. All other diets except (T1) control had similar feed conversion ratio.

Diets, T2, T3 and T4 had significantly lower ($P < 0.05$) FCR% than other diet (T1). Diets T2, T3 and T4 had significantly ($P < 0.05$) higher protein efficiency ratio value than other diet which had a lower protein efficiency ratio. In this study partial replacement of dried *Spirulina platensis* and chitosan biosynthesis by sand smelt meal had significantly ($P < 0.05$) higher improved the feed utilization parameters. The protein efficiency ratio (PER%) was noticeably differed significantly ($P < 0.05$) between treatments and supported the same trend, with the fish fed the control diet PER% (1.73 ± 0.08) and fish receiving the experimental diets displaying a superior exhibiting a PER% of T2(1.79 ± 0.11), T3(1.83 ± 0.10) and T4(1.82 ± 0.14), respectively.

As presented in the same Table, the highest significant differences ($P > 0.05$) of energy efficiency ratio (EER) value (2.07 ± 0.07) was recorded by the T3 group followed with significantly ($P < 0.05$) decreasing order by T1(1.9 ± 0.06), T4(2.06 ± 0.09) and T2(2.02 ± 0.09), respectively.

Protein Retention ratio (%) values also showed increase when sand smelt meal was replaced by the graded levels of dried *Spirulina platensis* and chitosan biosynthesis increase. These values were 32.77 ± 0.84 for fish fed T1 control diet and 37.27 ± 0.75 , 39.83 ± 0.67 and 38.19 ± 0.84 for T2, T3 and T4, respectively (Table 5).

Energy Retention ratio (%) values was differed significantly ($P < 0.05$) between treatments and supported the same trend, with the fish fed the control diet exhibitin energy retention ratio (%) (25.23 ± 0.67) and fish receiving the experimental diets displaying a superiora (E R R; %), these values were 28.49 ± 0.91 , 30.71 ± 0.73 and 28.85 ± 0.69 for T2, T3 and T4, respectively. Both feed conversion ratio and protein efficiency ratio were significantly ($p < 0.05$) improved. This is in agreement with the findings of Wang *et al.* (2008). Feed utilization of African Catfish (*C. gariepinus*) grow out stage was improved slightly when fed with diets containing partial replacement of dried *Spirulina platensis* and chitosan biosynthesis by sand smelt meal with significant difference among them ($P > 0.05$), while significant ($P > 0.05$) decreases were obtained with the (T1 control).

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 *et al.* (2008). These might be related to the chitosan nanoparticles and *S. platensis* which might be enhance the digestive enzymes such as protease, amylase, and lipase, which has the potential to increase the value of digestive enzymes in the gut and/or its effect for also improving digestive activity by synthesis of vitamins and co-factors or enzymatic improvement for WG and SGR (Gatesoupe, 1999).

Effect of experimental diets on initial and final muscles composition of African catfish (C.gariepinus) grow out stage receiving the basal diet and the experimental diets:

The proximate composition of fishes is an important ecological measure of condition that in tegrates both feeding condition and habitat quality. Proximate composition can also have important implications in the study of fish bioenergetics as well as the study of contaminants, given the propensity of many compounds to be related to lipid levels (Lanno *et al.*, 1989).

Further, certain components such as fat levels have also importance in aquaculture and food technology, where the fish grading, fish quality and value are linked to fat levels in the tissue (Rasmussen, 2001). Fish body composition receiving the experimental diets are significant differences ($P > 0.05$) shown in table (6). From (Table 6), the initial and final muscles composition showed little significantly ($P < 0.05$) variation in proximate composition as a result of the diet formulations.

Fish fed the experimental diets were yield variations in their muscles moisture, crude protein, lipid, ash, and gross energy content ($P > 0.05$) whereas, there was a significant difference ($P < 0.05$) between the control group and T2 compared with T3 and T4 in moisture and protein contents (Table 6). Also, there was a significant ($P < 0.05$) increase in percentage of protein in muscle composition from 71.57 ± 1.32 % as dry weight basis and 18.97 ± 0.35 as wet weight basis for T1 to 72.84 ± 1.81 as dry weight basis and 21.65 ± 0.54 as wet weight basis for T3. Also, ash content in muscles showed slight significant differences ($P < 0.05$) among groups (Table 6).

Fish fed (T1) control (basal diet) and T2 recorded had significantly ($P < 0.05$) high (73.64 ± 0.96 , 71.19 ± 1.49) moisture content when compared with T3 and T4 (70.03 ± 1.74 and 71.11 ± 1.66) feeding trials respectively. A significant difference ($P < 0.05$) in crude protein level was obtained when T2, T3 and T4 feeding experiment were compared to T1 control (basal diet). Analysis of variance showed that body composition was significantly ($P < 0.05$) affected by chitosan nanoparticles biosynthesis using *S. platensis* diets for all incorporation ratios.

Table (6): Effect of partial replacement of dried *Spirulina platensis* and Chitosan biosynthesis by sand smelt meal on proximate chemical composition of muscle the African Catfish (*C. gariepinus*) grow out stage. (Means \pm SE, 98 days).

Proximate composition	Initial muscles composition	Final muscles composition(g/ 100 g as dry matter basis)			
		T1 Control	T2	T3	T4
Moisture	74.21 \pm 1.51 ^a	73.50 \pm 1.96 ^a	71.19 \pm 1.49 ^{ab}	70.03 \pm 1.74 ^b	71.11 \pm 1.66 ^{ab}
Crude protein(g/ 100 g as dry weight basis).	71.45 \pm 1.16 ^a	71.57 \pm 1.32 ^a	71.80 \pm 2.04 ^a	72.94 \pm 1.81 ^a	72.39 \pm 1.50 ^a
(g/100 g as wet weight basis).	18.42 \pm 0.30 ^b	18.97 \pm 0.35 ^b	20.59 \pm 0.59 ^a	21.65 \pm 0.54 ^a	20.91 \pm 0.43 ^a
Ash	8.43 \pm 0.76 ^a	8.10 \pm 0.85 ^a	7.19 \pm 0.72 ^b	6.31 \pm 0.57 ^{bc}	6.10 \pm 0.93 ^c
Ether Extract (g/ 100 g as dry weight basis).	20.12 \pm 2.30 ^b	20.33 \pm 2.90 ^{ab}	21.01 \pm 1.79 ^a	21.75 \pm 2.43 ^a	21.51 \pm 1.71 ^a
(g/ 100 g as wet weight basis)	5.19 \pm 0.60 ^b	5.39 \pm 0.77 ^{ab}	6.05 \pm 0.52 ^a	6.43 \pm 0.73 ^a	6.21 \pm 0.49 ^a
Gross energy*(Kcal GE/100g).	592.78 \pm 27.56 ^b	595.53 \pm 23.32 ^b	603.35 \pm 25.67 ^a	609.88 \pm 23.51 ^a	611.47 \pm 19.32 ^a
KJGE/100g)*	63.73 \pm 2.98 ^b	65.81 \pm 3.74 ^b	72.46 \pm 3.35 ^a	76.22 \pm 4.10 ^a	73.61 \pm 2.93 ^a

Values are mean \pm SD from three replicates. Mean with the same superscript letter are not significantly different ($P < 0.05$).

* Gross energy value was calculated from their chemical composition, Estimated according to Jobling, (1983), as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively

**KJGE/100g) $CP \times 23.41 + lipid \times 39.71$ kJ g⁻¹, According to (Shiau and Liang, 1994).

In most bony fish, fat and water content make up to 80% of the fresh weight. In simple terms, the high water content can be held responsible for the perishability of fish (Clucas and Ward, 1996). The highest crude protein level (as dry weight basis) was observed in T3 (72.94 \pm 1.81, as dry weight basis and 21.65 \pm 0.54, as wet weight basis) feeding closely followed by T4 (72.39 \pm 1.50, as dry weight basis, and 20.91 \pm 0.43, as wet weight basis), T1 recorded the least (71.57 \pm 1.32, as dry weight basis and 18.97 \pm 0.35, as wet weight basis) crude protein among the experimental fish.

The highest Ether Extract (g/ 100 g as dry weight basis and g/ 100 gas wet weight basis) (lipid) (20.33 \pm 2.90 and 5.39 \pm 0.77, respectively) content was observed in fish fed T3 (21.75 \pm 2.43 and 6.43 \pm 0.73) closely followed by T4 (21.51 \pm 1.71 and 6.21 \pm 0.49), respectively with significant difference ($P < 0.05$) when compared to other experimental feeding trials while T1 had the lower (20.33 \pm 2.90 and 5.39 \pm 0.77), respectively, lipid content. The proximate composition of fish in the experiment shows a significant difference ($P < 0.05$) increase in values of crude protein, lipid, ether extract, and ash at the end of the experiment over the initial fish samples. The result follows this trend because the experimental fish convert and utilized the nutrient in the diet into their body nutrient. The higher protein content of the experimental fish means that, they converted and utilized the protein from the feed into their body protein. This result is similar with that of Marimuthu *et al.* (2010) and Ali and Onder (2011). The findings were consistent with those of (Nubia *et al.*, 2014) who found that, the protein content of wild catfish was 19.33 \pm 0.25%. However, (Oladipo and Bankole, 2013) discovered that the dried *C. gariepinus* had the highest fat content (11.02%) and the highest protein content (42.88%). While, Zhu *et al.* (2015) reported that the protein and fat content of untreated raw channel catfish fillets was 16.01 \pm 0.35% and 13.47 \pm 0.81% respectively. The ash content of catfish meat was 8.10 \pm 0.85, 7.19 \pm 0.72, 6.31 \pm 0.57 and 6.10 \pm 0.93 for T1, T2, T3 and T4, respectively, according to the data in Table 6. The results were inconsistent with those stated by (Deng, 2018) who found that the ash content in *Clarias gariepinus* was 1.7 \pm 0.06%. The proximate composition of fish in the experiment shows a significant difference ($P < 0.05$) increase in values of gross energy (Kcal GE/100g) (Jobling, (1983) at the end of the experiment over the initial fish samples. The

result follows this trend because the experimental fish convert and utilized the nutrient in the diet into their body nutrient. The higher Gross energy(Kcal GE/100g) content of the experimental fish means that, they converted and utilized the protein from the feed into their body Gross energy (Kcal GE/100g). The gross energy (Kcal GE/100g) content of catfish meat was 595.53 ± 23.32 , 603.35 ± 25.67 , 609.88 ± 23.51 and 611.47 ± 19.32 for T1,T2,T3 and T4 as (Kcal GE/100g)(Jobling, (1983) , respectively and gross energy as (KJGE/100g) were 65.81 ± 3.74 , 72.46 ± 3.35 , 76.22 ± 4.10 and 73.61 ± 2.93 for T1,T2,T3 and T4 , respectively.

CONCLUSION

This study investigated the effect of graded quantitative levels for partial replacement of dried *S.platensis* and chitosan biosynthesis by sand smelt meal on growth performance of the African Catfish (*C. gariepinus*) grow out Stage diets. The experimental diets, **T2** (50 g *Sp.pl.*+10 g CSNPs/kg) ,**T3**(100 g *Sp.pl.*+20 g CSNPs/kg) and **T4**(150 g *Sp.pl.*+30 g CSNPs /kg) in fish diet by sand smelt meal can lead to an increase in the African Catfish (*C. gariepinus*) grow out Stage improve the water quality, growth performance, feed utilization and improve the body composition of especially the protein and lipid content thereby improving their production and nutritional value on African catfish (*C. gariepinus*) post fingerlings. Although this increase show T3 (100 g *Sp.pl.* + 20 g CSNPs / Kg) in fish diet was the optimum inclusion level.In this work, protein levels of fish carcasses decreased as the inclusion of *S. platensis* increased while fat increased in a dose dependent manner.

The outcome of this study shows that *C. gariepinus* post fingerlings readily accepted the experimental diets as they exhibited good growth. This study further reveals that T2 ,T3 and T4 , positively correlated to the growth of the post fingerlings resulting in higher weight gain and SGR and FCR , while higher PER may be due to high protein digestibility.

The proximate composition of fish in the experiment shows an a significant difference ($P < 0.05$) increase in values of crude protein, lipid, ether extract, and ash at the end of the experiment over the initial fish samples. The result follows this trend because the experimental fish convert and utilized the nutrient in the diet into their body nutrient. The higher protein content of the experimental fish means that, they converted and utilized the protein from the feed into their body prtein.

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تقييم الاستبدال الجزئي الكمي المتدرج لمسحوق سمك البساريا المحلي *Basaria (Smelt)* والبيوجين، مع التخليق الحيوي لجسيمات الكيتوزان النانوية وطحالب *Spirulina Platensis* في العلائق الغذائية لسمك القرموط الأفريقي (*Clarias gariepinus*) الذي يتم تربيته في التناكات.

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في هذه الدراسة يعد سمك القرموط الأفريقي *Clarias gariepinus* أحد أهم الأسماك المستزرعة في المناطق الاستوائية وشبه الاستوائية وهو من الأنواع السمكية ذات الأهمية الاقتصادية، مما أدى إلى الحاجة إلى تقليل تكلفة التغذية، وأصبح أكثر شعبية بين مزارعي الأسماك في العالم ودفع إلى زيادة إنتاجية الأسماك. التي حفزت البحث الحالي. أجريت الدراسة العملية الحالية في المزرعة السمكية التجريبية، قسم الإنتاج السمكي، كلية الزراعة، جامعة الأزهر، القاهرة، مصر. بدأت الفترة التجريبية (98 يوماً، 14 أسبوعاً) خلال الفترة من 1 أبريل إلى 15 يوليو 2023. تم إجراء تجربة التغذية لمدة 98 يوماً في نظام أربعة تناكات يتكون من أربع سلاسل من التناكات الخرسانية المستطيلة (كل تناك مقسم إلى حوضين) .

240 من أسماك القرموط الأفريقي بعد الإصبعيات من نوع *C. gariepinus* (ستون سمكة تجريبية في كل معاملة غذائية) وكان متوسط الوزن الأولي 95.87±1.14 g - 94.75±1.19 جم وطول السمكة الأولي (cm) 35.6±1.07 - 34.1±1.12 (سم) للتحقق من تأثير المستويات الكمية المتدرجة لـ الاستبدال الجزئي لمسحوق سمك البساريا (*Smelt*) المحلية (*SSM*) والبيوجين كمصدر رئيسي للبروتين مع التخليق الحيوي لجسيمات الكيتوزان النانوية ومسحوق طحلب سبيرولينا بلانتنيسيس في النظام الغذائي لسمك القرموط الأفريقي (*Clarias gariepinus*) الذي يتم تربيته في التناكات، على جودة المياه، وأداء النمو، و الاستفادة من الأعلاف. في تكوين الجسم الكيميائي. تم تحضير أربع علائق تجريبية بواسطة T1 بمعدل 0% (الكنترول ، العليقة الأساسية تحتوي على 39% بروتين خام، من مسحوق سمك البساريا المحلية و بيوجين 3%)،

T2 - الأحلل الكمي لمسحوق سمك البساريا لمسحوق طحلب الإسبيرولينا والتخليق الحيوي لجسيمات الكيتوزان النانوية بمعدل (100 جرام / CSNPs) كجم ومسحوق طحلب *S. platensis* بمعدل 50 جم / كجم ،

T3 - الأحلل الكمي لمسحوق سمك البساريا لمسحوق طحلب الإسبيرولينا والتخليق الحيوي لجسيمات الكيتوزان النانوية بمعدل (20 جرام / كجم ومسحوق طحلب *S. platensis* بمعدل 100 جم / كجم ،

T4 - الأحلل الكمي لمسحوق سمك البساريا بمسحوق طحلب الإسبيرولينا والتخليق الحيوي لجسيمات الكيتوزان النانوية بمعدل (30 جرام / كجم ومسحوق طحلب *S. platensis* بمعدل 150 جم / كجم عليقة . ، على التوالي. وقد تم تكرار هذه المعاملات مرتين.

وقد وجد أن الاستبدال الكمي بالتخليق الحيوي لجسيمات الكيتوزان النانوية (جم 20 / كجم) ومسحوق أسبيرولينا (100 g / كجم) في علائق سمك القرموط الأفريقي *C. griepinus* المرعى في التناكات أدى إلى تحسين مستوى جودة المياه بشكل ملحوظ ($P < 0.05$) و من حيث زيادة الوزن اليومي والبقاء على قيد الحياة وكفاءة استخدام العلف وجودة اللحوم. وكذلك تكوين الجسم ($P < 0.05$) لسمك القرموط الأفريقي بعد الإصبعيات *C. gariepinus* والتي يكون لديها القدرة على إحداث ثورة في تربية الأحياء المائية على مستوى العالم .