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Modeling and optimization of effects of chemically and biologically synthesized Nano-Fe_2O_3 supplemented feeds on growth and hematological parameters of African Catfish (*Clarias Gariepinus*)

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ABSTRACT

Effects of chemically and biologically synthesized nano-Fe_2O_3 supplemented fishmeal on the growth and hematological profile of African catfish (*clarias gariepinus*) fingerlings were studied. Sets of ferric oxide nanoparticles (nFe_2O_3) were prepared by chemical and biological methods. The feed diets were prepared in five supplementing levels of 0.02, 0.05, 0.10, 0.15 and 0.20 g of bio-synthesized nano-Fe_2O_3 (N_b) and chemically synthesized nano-Fe_2O_3 (N_c) per Kg of the basal feed. Fish fed with any of the two synthesized nano-Fe_2O_3 supplemented diets showed higher hematological parameter values compared to those fed with the basal diet. Highest weight gain achieved with N_c was 820%, at the supplemental level of 0.15 g Kg^{-1} feed. On the other hand, N_b achieved a maximum weight gain of 826% at level of 0.15 g Kg^{-1} feed inclusion. This indicates that dietary iron supplementation in the basal feed used is necessary for catfish (*C.gariepinus*) fingerlings. It was also observed that diet supplemented with N_c –feed ration performed slightly better than diet supplemented with N_B-feed ration in improving fish weight up to the inclusion level of 0.15 g Fe Kg^{-1} feed. Above this level (0.15 g Kg^{-1}) there was no substantial weight difference between fish fed with N_c and N_b supplemented diets. Suitable Fourier models were developed for predicting weight gain by the catfish at varying levels of supplementation in the range of 0 to 0.2 g.

Keywords: African catfish, nano-Fe_2O_3, fishmeal, haematology, modeling, optimization

1. INTRODUCTION

For some decades now, efforts have been made to increase the efficiency of fishmeal through systematic substitution and reduced levels of fish meal (FM) in the diets of most farmed fish species. This has been reported to result in reduced feed intake, fish growth and nutrient bioavailability in fish body, with consequent negative impacts on the aquatic environment (Bell and Waagbø, 2008; Hardy, 2010).

Iron plays important roles in the life of higher animals, including fish. This is because many metabolic processes in animals such as DNA synthesis, steroid synthesis, drug metabolism, ATP production and electron transport are all iron dependent phenomena. Above all, iron is a structural component of the oxygen-binding protein molecule in red blood cells called hemoglobin, which confers on it the role of oxygen transport. So far, studies evaluating the effect of feed meal replacement by no rumination fish heamatology are very limited (Karapanagiotidis et al., 2019).

Iron exists in abundance in nature but its use in biological systems is challenged by such factors as low solubility and bioavailability of most of its salts (Hilty et al., 2011), as well as generation of toxic oxidants by its free metal ions. Nano materials have novel physicochemical properties which lend them for applications compared to their bulk counterparts. It has, for instance, been shown (Remya et al., 2014; Zanella et al., 2017) that in nano-form, the solubility, bioavailability and reaction kinetics of materials become enhanced on account of their small size, enlarged surface area and altered surface chemistry. Incidentally, their physiological and environmental toxicity and hazards also become enhanced for the same reasons.

Recently there has been global outcry to save the environment through 'green' approaches to experimental procedures. This has given rise to the use of easily degradable and recyclable biological materials as stabilizing agents in experiments (Karthikeyeni et al., 2013). Mineral nanoparticles have also been produced in recent times by the use of biological materials. In this study, iron oxide nanoparticles have been separately produced via chemical and biological routs and their effects on the growth and hematological profiles of African catfish discussed.

2. MATERIALS AND METHODS

Ethical Consideration

The ethical approval was obtained from the Fisheries Department in the Faculty of Agriculture and Fisheries. The functioning ethics committee of the institution covers human experiments and clinical trials.

Materials

Potato extract, ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), ammonia solution, ferric nitrate ($Fe(NO_3)_3$) were taken from open market in Onitsha, Nigeria. African catfish fingerlings, initial weight 5.07g were taken from the hatchery of a commercial, 'Aqua Farms' in Orumba North local government council area in Nigeria. Conventional commercial fish feeds (Skretting and Vital feeds) and vegetable oil was taken from livestock feeds market in Nigeria.

Chemical Synthesis of Fe_2O_3 NPs

A solution of 100 g of ferric nitrate was made in 1000 ml of distilled water in the presence of acetic acid, in an 1800 ml capacity glass beaker. The solution was sonicated on a magnetic stirrer at 50 KHz for 20 minutes and ammonia solution was added until the formation of a brown precipitate was completed. The precipitate was filtered, washed several times in warm water, dried in air oven at 100 ^{O}C for 2 hrs and finally heated in a muffle furnace at 500 ^{O}C for 3 hrs. The resultant product was cooled and ground to fine by mortar and pestle and classified in screen of nano-dimension. This was ferric oxide nanoparticles.

Biological synthesis of Fe_2O_3 NPs

25 ml of potato extract (as starch template) was mixed with 2 g of $FeSO_4.7H_2O$ and stirred for 30 minutes. The temperature of the resultant viscous solution was raised, incrementally at 5 ^{O}C per minute to and maintained at, 60 ^{O}C for 4 hrs. It was subsequently cooled to room temperature at decreasing rate of 10 ^{O}C per minute. Reddish-brown precipitates of iron oxide nanoparticles were obtained. The chemically and biologically prepared particles were tagged N_C and N_B respectively.

Material characterization

N_c and N_b were characterized in the laboratory of Moserbaer India Ltd at 66 Udyog Vihar, Greater Noida, India by means of model ADX 8000 X-ray diffractometer and model FlexSEM 1000 scanning electron microscope. Average crystallite particle size was calculated using Scherrer's formular (equation 1),

$D = (K\lambda)/(\beta cos\theta)$ (1)

Where, D = Size of CuO, K = dimensionless shape factor with a typical value of about 0.94, λ = X-ray wavelength, β = full width at half the maximum intensity (FWHM), denoted as Δ (2θ) and θ is the Bragg angle

Diet preparation

The diets were prepared (each at 5 levels) by differently supplementing levels of 0.02, 0.05, 0.10, 0.15 and 0.20 g of N_c and N_b per Kg of the basal feed. Each amount was first thoroughly dispersed in 20 ml of light vegetable oil, mixed with 1 Kg of the basal feed and allowed to dry at room temperature to form the treatment diets. 1 Kg of the basal feed was also treated with the vegetable oil to serve as the control diet. Diets were stored in cool dry place for use.

Feed administration (Experimental protocol)

Fingerlings of about same size were sorted out of a group that had been acclimatized to the experimental laboratory conditions for 10 days. The selected fish were randomly distributed in groups of 7s into previously weighed plastic bowls which were subsequently reweighed to determine fish total/average weight per group. Fish were finally transferred to flow through plastic culture tanks, each of which was filled with 75 liters of bore-hole water with known parametric values. Diets were randomly assigned to the experimental units (the tanks) and fish were fed to satiation thrice daily at 7:00 am, 12:00 noon and 6:00 pm. Incidence of uneaten feed was minimized by applying feed gradually (a little at a time) till fish were satiated. Water quality kit (PONDLAB 200 from NT Laboratories, Wateringbury, Kent) was used to monitor water quality parameters daily before feed administration. Culture tanks were drained, cleaned and refilled at the end of every week. During this time fish new weights were taken by means of a digital weighing balance that read up to 2 places of decimal. After 7 weeks, fish were harvested and subjected to hematological assays.

Hematological analysis

RBC and WBC were determined using a Futchs-Rosenthal Hemocytometer, Hemoglobin was determined by means of Sigma Diagnostics total hemoglobin colorimeter kit and Hematocrit was measured by the microhematocrit method, in accordance with previous study.

Statistical Analysis

Statistical validation of results (comparison of means) was carried out by online free statistics calculator from *statpac inc., (2017)* and Matlab software 7.5.0 (R 2007b). Experimental data were fitted in Fourier's model and fourth order regression model for the prediction of the effects of different routes of synthesizing nFe_2O_3 (N_c and N_b) on the growth rate of the fingerlings. The Fourier model expression for percent weight gain (%Wt) as a function of N_b and N_c supplementation levels is presented in equation 2.

$\%Wt\ (N_b \text{ or } N_c) = a_0 + a_1 * \cos(x * w) + b_1 * \sin(x * w)$ (2)

Where, a_0, a_1, b_1 and w are model constants and %Wt (N_b or N_c) is % weight gain by fish fed with N_b or N_c treated ration.

On the other hand, the relationships between the supplementation levels and the percentage weight gain by the fingerlings for the two methods of nFe_2O_3 synthesis was also modeled by 4th order regression (quartic) model (Eqn. 3).

$\%Wt\ (N_b \text{ or } N_c) = P_1X^4 + P_2X^3 + P_3X^2 + P_4X + P_5$ (3)

Where, %Wt (N_b or N_c) = % weight gain by fish fed with N_b or N_c treated ration, P_1,…, P_4 = model constants and X = independent variables ($nFe2O_3$ supplementation levels).

Error Analysis

The coefficient of determination (R^2), Sum of Square Error (SSE) and Root Mean Square Error (RMSE) were the primary criteria for selecting the most suitable models to describe the relationships among process variables.

The coefficient of determination (R^2) can be calculated using equation 4:

$$R^2 = \frac{\sum_{i=1}^{N}\left(X_{\exp(j)} - X_{\exp(mean)j}\right)^2 - \left(X_{pre\,(j)} - X_{\exp(j)}\right)^2}{\sum_{i=1}^{N}\left(X_{\exp\,(j)} - X_{\exp\,(mean)j}\right)^2} \quad (4)$$

Where $X_{exp,i}$ stands for the measured value obtained from the experimental investigation, $X_{pre,i}$ is the corresponding predicted value for the same measurement and N is the total number of observations.

Another parameter that is often used in error analysis is sum of square error (SSE), which is calculated from equation 5.

$$SSE = \sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2 \quad (5)$$

where, N is number of observations, n is number of constants in the model, $MR_{exp,i}$ is experimental moisture ratio i^{th}observation, $MR_{pre,i}$ is predicted moisture ratio at the i^{th}observation.

The root mean square error (RMSE) can be computed from equation 6.

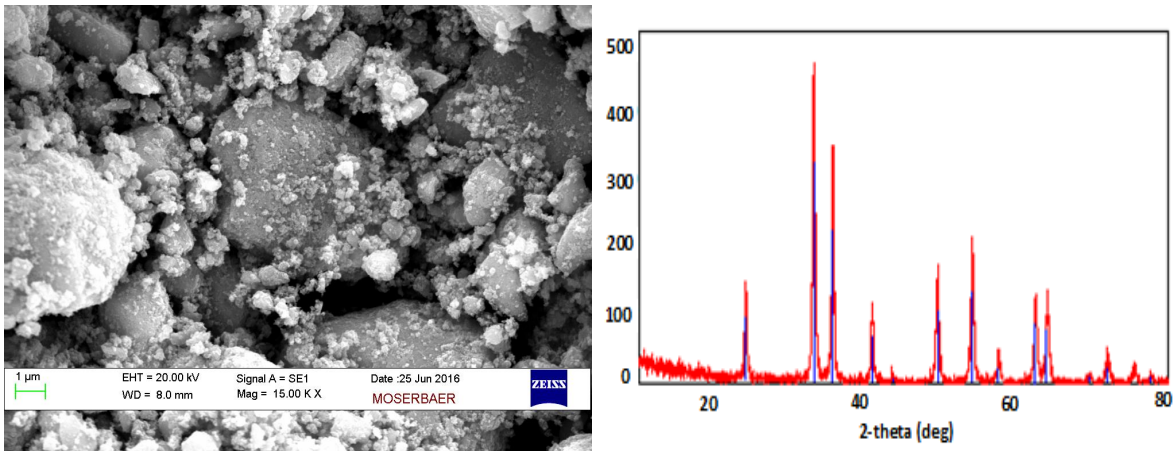
$$RMSE = \frac{\sqrt{\left[\sum_{i=1}^{N}(X_{exp,j}) - \sum_{i=1}^{N}(X_{pre,j})\right]^2}}{N} \quad (6)$$

The decision criteria are that, the higher the R^2 value and the lower SSE and RMSE values the better the model (Turan and Firatligil, 2019, Nag and Dash, 2016).

3. RESULTS AND DISCUSSION

Material Characterization

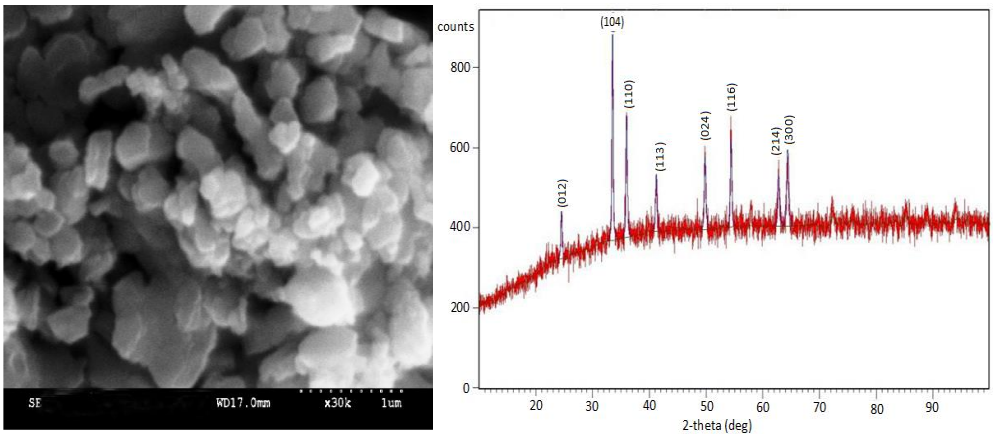
Figures 1(a) and (b) are the SEM image and XRD pattern of the chemically prepared Fe_2O_3 nanoparticles (nFe_2O_3). The SEM image shows a heavy cluster/aggregation of very fine particles while analysis of the X-ray diffraction (Figure 1(b)) indicates average crystallite size of 23.94±0.88nm.



(a) (b)

Figure 1 (a) SEM image; (b) XRD pattern of chemically prepared Fe_2O_3-NPs

The SEM micrograph and XRD of the biologically synthesized nanoparticles (nFe_2O_3) are in (Figures 2(a), (b)). SEM image studies revealed clusters of hexagonally shaped nanoparticles, while the analysis of the XRD indicated average crystallite particle size of 45.21 ± 0.32 nm.



(a) (b)

Figure 2 (a) SEM image; (b) XRD pattern of biosynthesized Fe_2O_3-NPs

Fish weight gain

After 7 weeks of culture, percent weight gain in fish fed the control, N_c and N_b supplemented diets are in (Figure 3).

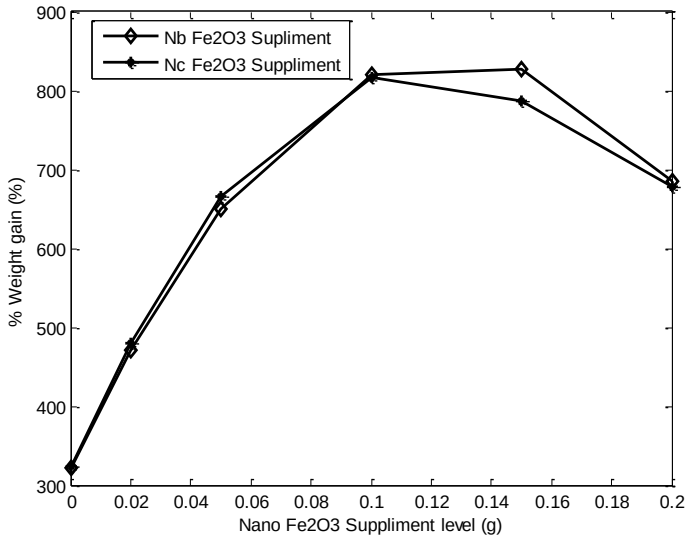


Figure 3 Graph of Nano-materials supplement levels against fish weight gain

The model constants, goodness of fit expressed in terms of the coefficient of determination (R^2) and Root Mean Square Error (RMSE) for Fourier and 4th order regression models are in (Table 1, 2).

Table 1 Constants and fit parameters of the Fourier models

Model	**Model constants**				**Goodness of fit at 95% CI**	
	a_0	a_1	b_1	W	R^2	RMSE
%Wt (N_c)	-1.53E9	1.53E9	1.23E6	0.00638	0.9926	25.86
%Wt (N_b)	-6.48E+9	6.48E9	2.6E6	0.00311	0.9999	2.952

As in Table 1, the coefficients of determination of 0.9926 and 0.9999 for %Wt (Nc) and %Wt (Nb) respectively, show that the Fourier's model adequately fits the experimental data. Furthermore, RMSE of 25.86 and 2.952 representing Nc and Nb respectively, indicate good predictive efficiencies.

Table 2 Constants and fit parameters of the 4th order regression models

Model	Model constants					Goodness of fit at 95% CI		
	P_1	P_2	P_3	P_4	P_5	R^2	SSE	RMSE
%Wt (N_c)	6.38E5	-1.82E5	-2.18E4	8305	323.1	1.0000	1.196E-25	3.46E-13
%Wt (N_b)	1.47E5	-5.25E4	-2.61E4	7965	322.6	0.9999	5.17E-26	2.27E-13

The coefficients of determinations (R^2) of 1.0000 and 0.9999 for %Wt (Nc) and %Wt (Nb) respectively, indicate good relationships. The low values of SSE and RMSE as in Table 2 supports the high values of R^2 which shows good relationships among the variables. It can therefore be deducted that the two models can very adequately predict the percentage weight gain for given levels of nFe2O3 supplementation in fish feed formulation. The final Fourier equation in terms of the actual values of the variable (N_c and N_b) levels are given in equations 7 and 8.

$$\%Wt\ (N_c) = -1.53E9 + 1.53E9 * \cos(0.00638 * X) + 1.23E6 * \sin(0.00638 * X) \quad (7)$$

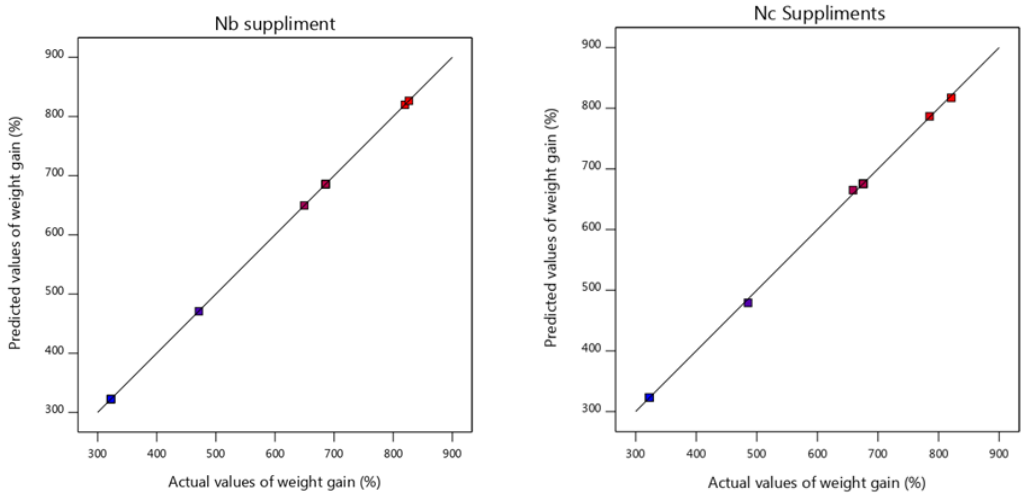
$$\%Wt\ (N_b) = -6.482E9 + 6.482E9 * \cos(0.00311 * X) + 2.6E6 * \sin(0.00311 * X) \quad (8)$$

Similarly, the 4th order regression equations in terms of the actual values of N_c and N_b levels are presented in equations 9 and 10.

$$\%Wt\ (N_c) = 6.38E5 * X^4 - 1.82E5 * X^3 - 2.18E4 * X^2 + 8305 * X + 323.1 \quad (9)$$

$$\%Wt\ (N_b) = 1.47E5 * X^4 - 5.25E5 * X^3 - 2.61E4 * X^2 + 7965 * X + 322.6 \quad (10)$$

The calibration graph of observed against predicted data are in (Figure 4). This helps to explain how close the predicted and observed data are. The scatter points are expected to align with the trend lines from the origin to the upper right of the graph. The more closely aligned to the trend line the better the prediction of the experimental (observed) data.



(a) (b)

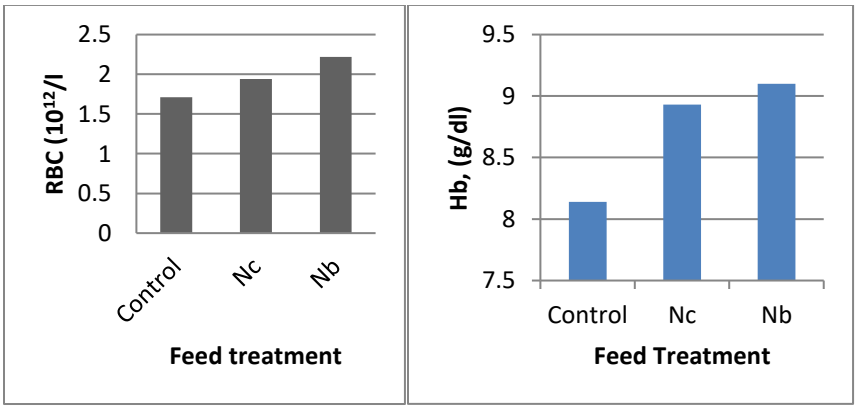
Figure 4 Calibration graphs of predicted versus actual values: (a) Nb supplement; (b) N_c supplement

Optimization of nFe_2O_3 Supplementation Process

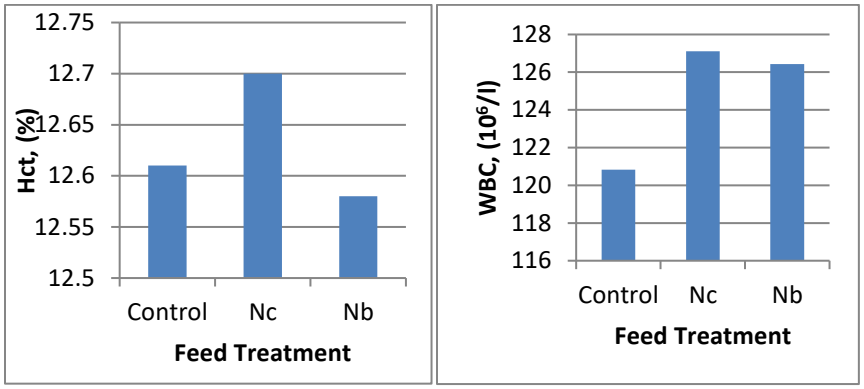
The objective of nFe_2O_3 supplementation study was to determine the level at which the percent weight gain by the fingerlings was optimal. To achieve this, the objective functions were set to maximize weight gain and minimize nFe_2O_3 quantity. At factor level of 0.15 and two-sided 95% confidence interval, the predicted optimal percent weight gains were 826.468 and 786.552 for Biosynthesized and Chemically synthesized nFe2O3 supplementation respectively at the optimal desirability of 0.815 and standard error of 0.236. At this level continued increase in the amount of the supplements will decrease the percent weight gain. However, from Figure 3 it the decreasing weight gain is more pronounced in chemically synthesized nonmaterial (nFe_2O_3).

Hematological analysis

Harvested fish were subjected to analysis of basic hematological parameters and the results obtained are in (Figures 5a, 5b, 5c, 5d).



(a) (b)



(c) (d)

Figure 5 Effects of feed treatments on hematological profile of Catfish samples: (a) RBC; (b) Hb; (c) Hct; (d) WBC

Weight Gain

There was a remarkable difference in weight between fish fed the control diet compared to those fed the nano-Fe_2O_3 (nFe_2O_3) supplemented diets. This clearly indicates that dietary iron supplementation in the basal feed has effect on the growth rate of catfish (*C.gariepinus*) fingerlings. It was also observed that diet supplemented with chemically synthesized nanoparticles (N_c) was slightly more effective, than diet supplemented with biosynthesized nanoparticles (N_b), in improving fish weight up to the inclusion level of 0.15 g Fe Kg^{-1} feed. Above this level of supplementation, the reverse became the case.

At the inclusion level of 0.15 g Kg^{-1}, there was no significant weight difference between fish fed N_c and N_b supplemented diets. This finding could be due to the fact that N_c, having smaller particle size and larger exposed surface area, interacted better with feed nutrients and was more effective in transporting them across gastrointestinal, tissue and cellular barriers. This would result in higher cellular activities and increased cell division and multiplication, leading to higher weights (Onuegbu et al., 2018). Above the inclusion level of 0.15 g Kg^{-1}, properties of chemically prepared nanoparticles such as surface charge may have induced toxicity, via reactive oxygen species (ROS) generation and so produce more negative impacts on fish physiology in contrast to biologically prepared nanoparticles.

On account of its larger particles (in this case), N_b would interact with other organic and inorganic feed nutrients and transport them across barriers less readily compared to N_c (Onuegbu et al., 2018). This could be the reason for its initial apparent lag behind N_c in improving fish weight gain. Same reasons of its larger particle size limiting the number of particles crossing cellular barriers and its biological nature reducing the potential of ROS generation, toxicity inducement could be the explanation for the sustained weight gain in fish fed diet supplemented with N_b beyond the inclusion level of 0.15 g Kg^{-1} in contrast to those fed diet supplemented with N_c.

Hematological parameters

Fish fed iron supplemented diets showed higher hematological parameters values compared to those fed the basal diet. This may be on account of higher iron intake from supplemented diets against basal diet. Iron is a structural component of the blood and higher iron intake necessitates higher blood production. Hemodilution can be caused by nanoparticles in blood stream adsorbing water and lowering blood concentration. The adsorption capacity of particles is a direct function of exposed surface area which, in turn, is an inverse function of particle size. This may explain the lower RBCs count and hemoglobin (Hb) concentration in fish fed N_c against those fed N_b supplemented diets.

Conversely, the larger particle size, combined with, possibly, less reactive surface structure of the biosynthesized nanoparticles in the experiment would mean lower water adsorption and higher RBCs count and Hb concentration in the presence of N_b. This is so because blood iron is actually the structural component of the protein, hemoglobin in the red blood cells. There is higher WBCs count in fish fed N_c supplemented diet against those fed N_c supplemented diet. It is possible that the chemically prepared N_c, by its chemical nature, induced a comparatively higher level of some undetermined stress in fish compared to the biologically prepared N_b, causing fish system to produce more WBCs to fight such stress.

4. CONCLUSION

Iron supplementation is important in conventional basal feed for *C. gariepinus* fingerlings. Since nano form of iron is more soluble and has higher bioavailability in fish than most chemical forms of iron, it is preferred as dietary iron source for the fish. Nano iron materials by bio-synthesis (green routes) is more eco-friendly and the product has less adverse effects on the exposed animal. Biologically prepared iron oxide is therefore recommended against its chemical counterpart, as dietary iron source for supplementation for *C. gariepinus* fingerlings if sustainability and environmental friendliness are desired. In so doing, it will solve the problem of agro waste in potato processing industries.

Ethical issues

Not applicable.

Informed consent

Not applicable.

Funding

This study has not received any external funding.

Conflict of Interest

The author declares that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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