Assessment of growth performance, nutrient utilization and haematological profile of *Clarias gariepinus* fed with nanoselenium formulated diets

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Abstract
A 63-day experiment was conducted to determine the effects of nanoselenium formulated diets on growth performance, nutrient utilization and haematological profile of *Clarias gariepinus*. Fish were acclimatized for 14 days, 10 fish each were randomly introduced into different troughs. Feed supplemented with nanoselenium at 2 mg/kg, 4 mg/kg and the control without nanoselenium were fed to group of fish each in triplicate. The feed intake, weight gain, food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate (SR) were determined, also the haematological profile of *Clarias gariepinus* fed were analyzed. There were significant increase (p<0.05) in the weight gain of fish fed 2 mg/kg of nanoselenium as compared with those fed 4 mg/kg of nanoselenium and the control. Similarly, PER and SR were significantly higher (p<0.05) in 2 mg/kg when compared with other formulated diets. The FCR of fish fed 2 mg/kg was significantly lower compared with 4 mg/kg nanoselenium formulated diets. WBC, RBC, haemoglobin, and haematocrit significantly increased in fish fed 2 mg/kg of nanoselenium as compared with those fed 4 mg/kg nanoselenium and the control group while there was variation in the values of other haematological parameters such as platelets, MCV, MCH and MPV. The research indicated that dietary inclusion of nanoselenium at 2 mg/kg may enhance better growth performance and nutrient utilization of *Clarias gariepinus*.

Keywords: *Clarias gariepinus*, nanoselenium, growth performance, nutrient utilization, haematology

1. Introduction
Minerals are essential micronutrients needed for the normal functioning of the body system. For the enzymatic system of fish to undergo catalytic reaction, adequate amount of micronutrients is needed. Fish cannot synthesis nutrients on their own; therefore, the need for supplementary micronutrients such as
selenium in the fish diets is essential. Selenium is an important dietary micronutrient for animals required for the normal body functions and metabolisms [1, 2]. It has also been known that selenium plays important roles in antioxidant defense systems, regulation of thyroid hormone metabolism and cell growth [3]. Csilla and Miklos (2013) reported that selenium plays vital roles in antioxidant defence systems, prevents cell damage and is necessary for growth, fertility, and immune system in farm animals. Nanoparticles are known to be playing additive and functional roles in fish when incorporated in feed and fed directly or included in the culturing media of fish. Nanoparticles have an inherent tendency to grow into larger particles [4], which is an evidence that nanoparticles such as nanoselenium when use as feed additives are more active and potent than selenium at the same concentration.

*Clarias gariepinus* is of the family Clariidae, genus *Clarias* [5]. They are omnivorous in nature, efficient in feed utilization and have ability to withstand adverse environmental condition. It is popularly refer to as African catfish because of its hardness and ability to withstand stress to a tolerable range [6]. It is the most cultured fish in Nigeria and is widely distributed throughout the country. It is an important aquaculture candidate with faster growth rate and global market demands. In spite of the adaptability of the catfishes to cultivations and commercial importance attached to its culture in developing countries like Nigeria, there are still some gaps in meeting the demand of the consumers. This is associated with the increasing population and anthropogenic effects on water bodies causing population decline of this important species of fish in most of their natural habitat. To overcome these challenges, there is need to technologically improve the aquaculture practice of *Clarias gariepinus*.

Most of the earlier research revealed the role that nanoselenium played on growth performance in animal and various fish species [4, 7, 8], while insufficient literature is available on the role that dietary nanoselenium supplementation played on nutrients utilization and growth in *Clarias gariepinus* which led to this study. The study, therefore, aimed to assess growth performance, nutrient utilization and haematological profile of *Clarias gariepinus* fed different dietary inclusions of nanoselenium formulated diets.

## 2. Materials and methods

### 2.1 Fish diets and experimental design

Healthy and uniformly sized 150 post-fingerlings (average weight of 12.30±0.01 g and average length of 6.8±1.0) were procured from a commercial fish farm and were transferred to the laboratory. The fish were acclimatized for 14 days and fed with a commercial feeds. After acclimatization, 10 fish were randomly selected and stocked per trough. Already engineered nanoselenium of size 90 nm was procured from Hebei Tianyin Biotech Co. Ltd. (Hebei, China). Then it was mixed with other feed additives at the required inclusion level, before mixing it with the variable ingredients. Three diets, consisting of control diet (without
nanoselenium) and two treatment diets at 2 mg/kg nanoselenium inclusion level and 4 mg/kg nanoselenium inclusion level were compounded (Table 1). The fish were fed twice daily (08:00 h and 17:00 h) at 3% body weight for 63 days [9, 10].

2.2 Water quality parameters
The water qualities were monitored and measured weekly. The water temperature was measured using mercury-in-glass thermometer (100 °C), pH was measured using Jenway pH meter (model E 512) and the dissolved oxygen (DO) was measured using Milwaukee DO2 meter (model MW600). The water in the rearing media was changed weekly.

2.3 Growth parameter and nutrient utilization
The weekly weight gain (WG) was measured using Camry sensitive digital scale (model SF 400), feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR), protein intake (PI), protein efficiency ratio (PER) and survival rate (SR) were also determined [11, 12].

2.4 Samples collection
After 63 days of the experiment, fish blood was collected. Blood samples of fish from each treatment were collected through tail ablation method [8, 11]. One ml of blood was collected in sampling EDTA bottle and shaken together to allow the blood mix with the EDTA anticoagulant and labeled for identification for further analysis.

2.5 Haematological parameters
The blood samples of fish fed with nanoselenium formulated diets were analyzed for haematological parameters. Blood parameters such as red blood cell (RBC), white blood cell (WBC), plateletcrit, mean platelet volume (MPV), hemoglobin (Hb), mean corpuscular volume (MCV), lymphocytes, and hematocrit (HCT) were determined using Diatek vet haematological analyser (model-D-3125plus).

2.6 Statistical analysis
The data obtained from the study were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) and expressed as mean ± SE [13]. Statistical significance for all the tests was set at p<0.05. All statistical analyses were done using Microsoft Excel 2007 and IBM SPSS statistics 20.0.
Table 1. Percentage composition (%) of the formulated diets

| Ingredient* | Inclusion level of dietary nanoselenium (mg/kg) |
|-------------|-----------------------------------------------|
|             | 0 (Control) | 2   | 4   |
| Fish meal   | 220         | 220 | 220 |
| Toasted soya bean | 220 | 220 | 220 |
| Groundnut cake (GNC) | 220 | 220 | 220 |
| Maize       | 200         | 200 | 200 |
| Wheat offal | 90          | 89.98 | 89.96 |
| Salt        | 5           | 5   | 5   |
| Fish premix | 10          | 10  | 10  |
| Vitamin     | 10          | 10  | 10  |
| Lysine      | 10          | 10  | 10  |
| Methionine  | 10          | 10  | 10  |
| Bone meal   | 5           | 5   | 5   |
| Nanoselenium | 0.00   | 0.02 | 0.04 |
| Total       | 1000        | 1000 | 1000 |

*All the ingredients are measured in gram

3. Results and Discussion

3.1 Water quality parameters

The water temperature in the rearing media ranged from 26.4 to 27.5 °C, the pH ranged from 7.2 to 7.9, while the dissolved oxygen ranged between 6.01 and 6.37 mg/l. The normal physiological profile of fish may be subjected to changes due to the alterations in the physicochemical setup of the rearing media. Fish is known to experience different environmental diseases, stress and haematological changes as a result of physicochemical changes of certain water parameters. As a result, physicochemical water parameters were studied and well monitored in order to nullify their effects on survival, growth as well as their effect on the blood composition of the experimental fish. Casillas and Smith [14] and Tomasso et al. [15] reported that haematological variables are stress indicators which are caused by environmental changes. From the findings and effective management of the water quality revealed that the temperature, pH and DO are all at the tolerable range throughout the experimental period.
3.2 Growth performance and nutrient utilization parameters

The growth performance and nutrient utilization parameters of *Clarias gariepinus* fed different inclusions level of dietary nanoselenium formulated diets is presented in Table 2. Supplementation of dietary nanoselenium in the fish diets showed significant effects on growth and nutrient utilization of *Clarias gariepinus*. Higher weight gain (WG), better feed conversion ratio (FCR), lower feed intake (FI), high survival rate (SR) and protein efficiency ratio (PER) were observed in fish fed 2 mg/kg nanoselenium formulated diets as compared with those fed 4 mg/kg nanoselenium formulated diets and the control diets. Selenium and nanoselenium are of great importance to fish [8, 16, 7], although inclusion concentration of both selenium and nanoselenium varies with fish species. The better nutrient utilization in fish fed 2 mg/kg nanoselenium which positively increased the growth and feeding profile of *Clarias gariepinus* may be that at this concentration their system make up responded well to the feed as compared with 4 mg/kg formulated diets as revealed in the feed intake and weight gain.

Dietary selenium (Se) is very important. It has been demonstrated that the growth performance of channel catfish (*Ictalurus punctatus*) was affected by Se level [17]. Very similar results were observed in this present study. It clearly indicated that the supplementation of nanoselenium (Nano-Se) in the fish diets could improve the final weight, weight gain and the survival rate of African catfish, *Clarias gariepinus*. Selenium deficiency generally resulted in growth depression and in some cases reproduction associated problems when not managed well [18, 19, 20]. The significance increase in the weight gain of *Clarias gariepinus* fed 2 mg/kg nanoselenium formulated diets align with the statement of [8, 21, 22] and that adequate amount of dietary selenium is essential for the proper body growth of fish and other animals [12].

3.3 Haematological parameters

Haematological parameters of fish fed different inclusions levels of nanoselenium formulated diets are presented in Table 3. The RBC, WBC, haemoglobin and haematocrit of fish fed 2 mg/kg nanoselenium formulated diets significantly increased as compared to fish fed 4 mg/kg nanoselenium formulated diets and fish fed the control diets. The nutritive status of fish can be linked to the health status of animal and potential way they handle stress resulting from their surrounding environment [23, 24]. In this study, there is reasonable interrelationship between the increased weight gain and increased number of RBC. The fish fed 2 mg/kg nanoselenium formulated diet exhibited the highest RBC and weight gain as compared with fish fed 4 mg/kg nanoselenium formulated diet. This possible correlation between RBC and weight gain may be due to the fact that the supplementation of dietary nanoselenium at 2 mg/kg concentration enhance better performance and growth. This is in agreement with previous findings [9] which reported that supplementation of selenium in tilapia feed at the rate of 2 mg/kg of feed does not alter its haematological profile of the fish as well as normal
physiological activities rather promotes better performance and productivity. This is also backed up by [25] which stated that RBC counts are mostly affected by dietary treatments fed to fish because the composition of the blood can change during malnutrition and/or stress condition [26].

Table 2. Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* fed different inclusions level of dietary nanoselenium formulated diets

| Parameter                              | Inclusion level of dietary nanoselenium (mg/kg) |
|----------------------------------------|-----------------------------------------------|
|                                        | 0 (Control) | 2 | 4 |
| Mean Initial weight (g)                | 124.50± 0.01<sup>a</sup> | 124.00± 0.01<sup>b</sup> | 121.50± 0.01<sup>a</sup> |
|                                        | 177.00± 0.05<sup>a</sup> | 180.50± 0.01<sup>b</sup> | 175.50± 0.02<sup>a</sup> |
| Mean final weight (g)                  | 52.50± 0.03<sup>a</sup> | 56.50± 0.01<sup>b</sup> | 54.00± 0.02<sup>a</sup> |
| Mean weight gained (g)                 | 45.21± 0.58<sup>a</sup> | 44.41± 0.58<sup>a</sup> | 45.03± 0.58<sup>a</sup> |
| Feed Intake (g)                        | 0.86± 0.05<sup>a</sup> | 0.79± 0.02<sup>a</sup> | 0.83± 0.03<sup>a</sup> |
| Food Conversion Ratio (FCR)            | 0.34± 0.01<sup>a</sup> | 0.37± 0.01<sup>a</sup> | 0.40± 0.01<sup>a</sup> |
| Protein Intake                         | 15.22± 0.04<sup>a</sup> | 16.36± 0.04<sup>a</sup> | 17.85± 0.02<sup>a</sup> |
| Protein Efficiency Ratio (PER)         | 3.45± 0.01<sup>a</sup> | 3.45± 0.01<sup>a</sup> | 3.02± 0.01<sup>a</sup> |
| Specific Growth Rate (SGR)             | 0.24± 0.02<sup>a</sup> | 0.26± 0.02<sup>a</sup> | 0.26± 0.02<sup>a</sup> |
| Survival Rate (%)                      | 95.00± 0.10<sup>a</sup> | 95.00± 0.01<sup>a</sup> | 90.00± 0.01<sup>a</sup> |
| Mean final standard length (cm)        | 13.25± 0.50<sup>a</sup> | 13.78± 0.01<sup>a</sup> | 13.05± 0.01<sup>a</sup> |

Means within the row with different superscripts are significantly different (P<0.05)
Table 3. Haematological profile of *Clarias gariepinus* fed different inclusion levels of nanoselenium formulated diets for 9 weeks (63 days)

| Parameter | 0 (Control) | 2 | 4 |
|-----------|-------------|---|---|
| RBC       | 2.62±0.40a  | 3.89±0.33a | 3.30±0.99a |
| HGB       | 14.65±0.35a | 16.25±0.49a | 11.15±0.21a |
| HCT       | 40.40±5.09a | 58.60±0.85b | 48.40±14.42a |
| MCV       | 154.85±4.03a | 151.55±15.20a | 146.55±0.07a |
| MCH       | 44.45±0.07a | 41.95±2.33a | 42.90±0.57a |
| MCHC      | 287.00±7.07a | 277.50±12.02a | 293.00±4.24a |
| WBC       | 107.95±5.44a | 115.85±10.25a | 93.20±9.19b |
| LYM       | 79.25±1.06a | 67.60±6.51b | 65.25±1.63c |
| PLT       | 18.00±4.24a | 43.00±7.07b | 49.00±12.73c |
| MPV       | 7.85±0.35a | 7.30±0.14a | 7.80±0.28a |
| PCT       | 0.02±0.01a | 0.04±0.01a | 0.04±0.01a |

RBC, Red blood cell; PLT, Platelets; HGB, Hemoglobin; MPV, mean platelet volume; HCT, Haematocrit; WBC, White blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; PCT, Plateletcrit; LYM, Lymphocytes; MCHC, Mean corpuscular hemoglobin concentration; Means within the rows with different superscripts are significantly different (p<0.05).

The main function of white blood cell (WBC) is to defend the body against foreign pathogenic organisms such as disease and stress. The fish fed 4 mg/kg nanoselenium formulated diets have low immune system and vulnerable to pathogenic activity and which possibly led to death of some of the fish. The amount of WBC, RBC and haemoglobin (HGB) concentration were greater in fish fed 2 mg/kg nanoselenium formulated diet which affirm that this could be the possible reason for the enhanced growth. WBC result provide vital information that dietary 2 mg/kg nanoselenium formulated diet possibly provided the fish fed with defensive mechanism making them as empowered body guards against the impending possible
pathogenic attacks or any possible ailment that might arise, which were evident in the survival rate of the fish. As a result of this, for better defensive mechanism, dietary inclusion of nanoselenium at 2 mg/kg nanoselenium in the fish diets of African catfish, *Clarias gariepinus* will be ideal. The significant reduction in WBC in fish fed 4 mg/kg nanoselenium formulated diet may not possibly be able to withstand stress experienced by the fish which reflected in the survival rate. The significant reduction of the lymphocytes of fish fed 2 mg/kg nanoselenium formulated diets may be one of those factors responsible for an enhanced growth which possibly made them secured from the impending ailment that might rise.

4. Conclusion

In conclusion, the study indicated that dietary inclusion at 4 mg/kg nanoselenium formulated diets is good as supplementary concentration but 2 mg/kg nanoselenium formulated diets enhanced better fish growth performance and nutrient utilization of *Clarias gariepinus*. Further research work need to be carried out on the two dietary concentrations (2 mg/kg and 4 mg/kg nanoselenium formulated diets) on *Clarias gariepinus* for a prolong period to reveal their impacts more on growth performance, nutrient utilization and organ function.

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