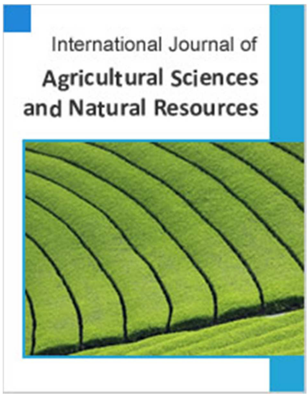
International Journal of Agricultural Sciences and Natural Resources
2018; 5(1): 6-11
http://www.aascit.org/journal/ijasnr
ISSN: 2375-3773

American Association for Science and Technology



Assessment of the Optimal Inclusion Level of Dietary Zinc Requirement for Catfish *Clarias gariepinus* Production

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Keywords

Clarias gariepinus,
Dietary Zinc,
Optimal Growth,
Plant Base Diet

Received: October 4, 2017
Accepted: December 11, 2017
Published: February 12, 2018

Citation

Umma Samuel Bem, Doro-Adeyemi Omoniyi, Oshimagye Michael Ibagye, Igbani Flourizel, Uruku Ndekimbe Mamndeyati, Dauda Abdullahi Kida. Assessment of the Optimal Inclusion Level of Dietary Zinc Requirement for Catfish *Clarias gariepinus* Production. *International Journal of Agricultural Sciences and Natural Resources*. Vol. 5, No. 1, 2018, pp. 6-11.

Abstract

Aquaculture requires adequate food in the form of artificial diet to furnish fish with nutrients for optimal growth. Trace mineral are recognized for their important roles in presenting proteins bioavailable in diets. Hence, zinc offers some solutions against the many inhibitory actions of plant base materials; however, its deficiency perturbs the general wellbeing of the fish. The study attempts to ascertain the required optimum level of dietary zinc essential for juvenile African catfish (*Clarias gariepinus*) growth and health performance. Fish were fed practical diet with graded levels of zinc at 0 to 27.33 mg Zn kg^{-1} respectively. Broken-line quadratic model showed the optimum dietary supplementation of zinc in fish growth performance at 13.67 mg Zn kg^{-1}. Mean weight gain showed a significant difference at $P<0.05$, with the least value in the control diet (41.08±0.34) and highest value in diet 2 (52.53±0.15). SGR, ADG, PER and FCR had significant values. The serum profile indicated significant variations in the Total Protein, AST, ALT, Creatinine and Ca^{2+} while Urea showed no significant effect in all the diets as recorded in Table 5. The investigation indicates optimum growth response. Therefore, emphasis should be placed on supplementing adequate zinc minerals in diets to reduce anti-nutritional factors and boost protein bioavailability**.**

1. Introduction

Aquaculture contributes significantly to food security and livelihood, providing about 3.0 billion people with almost 20 percent of their average per capital intake of animal protein [1] Fish and fish products are projected as being among the most widely traded foods internationally [2]. Fish requires essential nutrients in order to thrive and in the wild such essential foods are available and as the fish forage for food they are able to meet their body need by feeding extensively on these foods [3]. Hence, enough food in the form of artificial diet to furnish all the nutrients requirement of the fish must be supplied to fish out of their natural environment in order to enable fish grow at an optimal rate [4; 3] Trace minerals which are needed in minute quantity at a time for the general health maintenance, growth and other biochemical functions in the body of animal support such great benefit

[5]. However, their deficiency stresses the general health status of fish in most areas such as skeletal formation, transmission of nerve impulses and muscle contraction, acid-base equilibrium of the body, and absence of cofactors in metabolism. Zinc has been recognized to play a vital role in almost every aspect of living system either directly or indirectly [6]. Some of the importance of zinc in diet include cell division, cell growth, wound healing, breakdown of carbohydrate, increases in senses of smell and taste and also functional defensive system. Normal zinc levels in freshwater and seawater are said to be insufficient to meet the requirement of growing aquatic species [7; 8]. [9] reported that when catfish diets were low in zinc, appetite was reduced, resulting in low growth, low bone zinc, calcium levels and serum zinc concentration. Zinc is an important trace element in fish nutrition as it is involved in various metabolic pathways and serves as a specific cofactor of several enzymes. Therefore, any biochemical components of fish diet that needs varying in terms of proportion of inclusion, which will render the protein in feed more bioavailable for promoting fast growth of healthy fish and that is relatively cheap should be considered, adopted and incorporated in diets. Most often attention is centered on substituting macronutrients for growth performance in fish while neglecting the important roles played by micronutrient, therefore, the study aims at investigating and ascertaining the optimal growth, serum biochemical profile and haematological responses of African catfish *Clarias gariepinus* fed varied zinc practical diet.

2. Materials and Methods

2.1. Experimental Procedure

This study was performed in a hygienic condition by grading dietary zinc and incorporating into 40% crude protein practical diets using the following ingredients Table 1. Clarias gariepinus juvenile fish were obtained from Aquatech Fisheries Institute Ibadan. A total of 180 juvenile fish of average weight 12.5g were sorted out on the third day and were placed into 6 equal groups in triplicate of 10 fish per tank containing 25 litres of water. They were fed control diet for 14 days in order to condition them for the study [10]. Fish were fed graded levels of zinc base diet at 5.46 and 21.86 mg Zn kg-1dry diets as lower and higher concentrations respectively for 12 weeks as described [11]. Feeding of the experimental fish was at 5% body weight twice daily (morning and evening), while water exchange was twice in a week with freshwater from deep well. Zinc concentrations of the freshwater from the deep well before and after use were measured. Growth performance in the different treatments was measured bi-weekly throughout the duration of the study.

2.1.1. Diet Preparation

Zinc supplemented diets Table 1, was formulated by dissolving the minerals in warm water, and mixed homogenously to the feed ingredients respectively. The required graded levels of zinc in milligrams (zinc sulphate, reagent grade) were weighed using the sensitive balance (PW 124 Ǽ Adams$^{(R)}$) from the University of Ibadan central laboratory at 15, 30, 45, 60, 75 mg respectively. The actual zinc concentration in zinc sulphate was calculated from the above measurement as presented below Table 1. The zinc contents of the practical diets were determined by Buck 211 Atomic Absorption Spectrophotometer at a wavelength of 232 nm (nanometer) and a slope of 1.

Table 1. *Gross composition of compounded experimental diets (40% CP).*

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet$_5$	Diet 6
	Control	5.46mg/kg	10.93mg/kg	16.40mg/kg	21.86mg/kg	27.33mg/kg
Fish meal	25.84	25.84	25.84	25.84	25.84	25.84
Soya bean meal	25.84	25.84	25.84	25.84	25.84	25.84
G/nut cake	12.92	12.92	12.92	12.92	12.92	12.92
Yellow Maize	25.40	25.40	25.40	25.40	25.40	25.40
Vegetable oil	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50	0.50
Zinc-free mineral mix*	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin mix*	2.00	2.00	2.00	2.00	2.00	2.00
Binder (Cassava)	1.50	1.49454	1.48907	1.4836	1.47814	1.47267
Zinc mineral	0.00	0.00546	0.01093	0.0164	0.02186	0.02733
Total%	100.00	100.00	100.00	100.00	100.00	100.00

* Zinc-free Biovita fish vitamin and minerals providing per kg of diet: 20,000 i.u., Vitamin A, 300 i.u, Vit. E 800mg, Ascorbic acid 100mg, Vit. D3 200mg Vit. E 8 mg, Vit. K3 20mg, Vit. B3 60mg, Vit. B6 300mg, Biotin 15 mg, Vit. K 200mg, Cobalt 40mg, Iron 5.0mg, Iodine 30mg, Manganese 5mg, Copper 5mg, Lysine 10mg, Methionine 10mg.

Table 2. *Proximate composition of diet (%).*

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet$_5$	Diet 6
	Control	**5.46 mg/kg**	**10.93 mg/kg**	**16.40 mg/kg**	**21.86 mg/kg**	**27.33 mg/kg**
Protein	36.89	36.89	36.89	36.89	36.89	36.89
Fat	7.81	7.81	7.81	7.81	7.81	7.81
Ash	6.77	6.77	6.77	6.77	6.77	6.77
Fibre	6.49	6.49	6.49	6.49	6.49	6.49
M.C	9.67	9.67	9.67	9.67	9.67	9.67
NFE	32.40	32.40	32.40	32.40	32.40	32.40
Zn	1.18	6.64	12.11	17.58	23.04	28.51

Zn = mg kg^{-1}

2.1.2. Growth and Nutritional Performance

The fish weight gain were measured bi-weekly, and other growth parameters such as Specific growth rate, SGR, Feed conversion ratio, FCR, Protein efficiency ratio, PER, Average daily gain, ADG were calculated as described by IAF, (2013) as follows: Average weight gain, AWG (g/fish) = [Average final weight (g) - Average initial weight (g)], SGR (%/day) = 100 [In final body weight - In initial body weight] / experimental period in days (d), FCR = Feed Intake (g) / Live weight gain (g), PER = Live weight gain (g) / protein intake (g), ADG (g/fish/day) = [AWG (g) / experimental period in days (d)].

2.1.3. Water Quality Assessment

The water quality parameters such as DO, pH, Temperature were measured using dissolved oxygen kit (K-7152), pH meter and thermometer, while zinc and calcium ions concentrations were determined following the methodology described in [12].

2.2. Statistical Analysis

Data obtained in the study were subjected to ANOVA and further comparison between pairs of means was determined using fisher's least significant difference (LSD) as described by [13].

3. Results

3.1. Fish Growth Parameter

The results of the growth parameters for Clarias gariepinus juvenile fed graded levels of dietary zinc in the practical diet Table 3, showed significant difference at P>0.05, the control diet for final weight reflected a lower value (26.21±0.21) while highest value was recorded in diet 2 (30.02±0.14), the weight gain also reflected the same trend, showing lowest value in the control diet (41.08±0.34) and highest value in diet 2 (43.97±0.18), SGR, ADG, PER and FCR also recorded significant values Table 3.

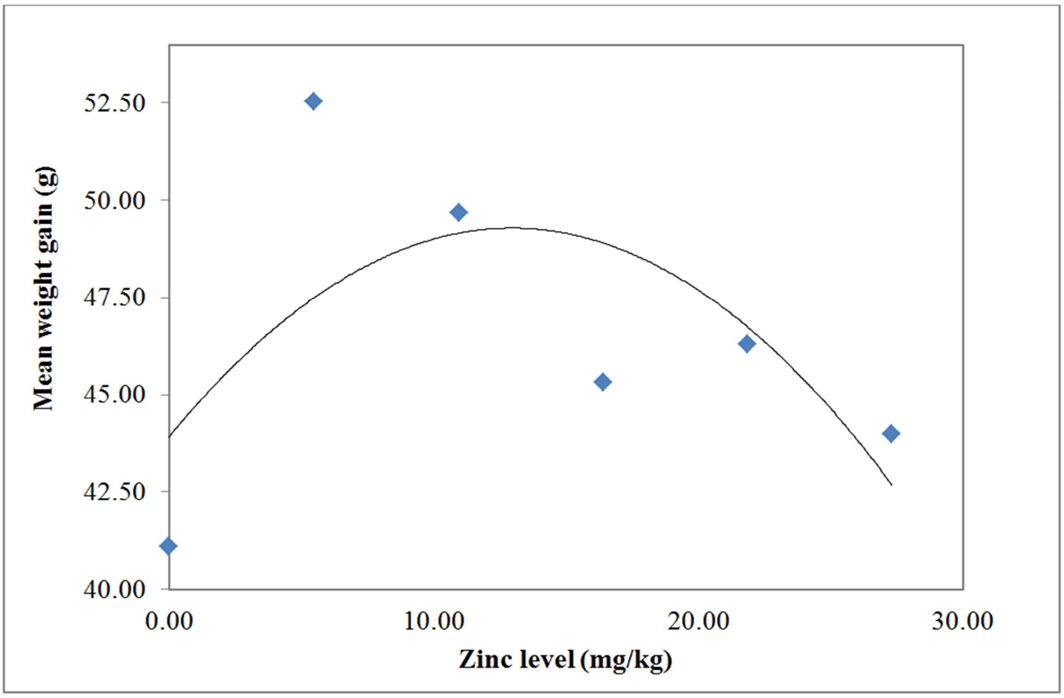

Figure 1. *Trend in mean weight gain with respect to dietary zinc level (mg/kg).*

Table 3. *Growth and nutritional parameters of Clarias gariepinus fed dietary zinc for 12 weeks.*

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	Control	5.46mg/kg	10.96mg/kg	16.40mg/kg	21.86mg/kg	27.33mg/kg
Initial weight (g)	12.52±0.10^{a}	12.51±0.01^{a}	12.46±0.05^{a}	12.50±0.02^{a}	12.45±0.06^{a}	12.49±0.07^{a}
Final weight (g)	26.21±0.21^{a}	30.02±0.14^{d}	29.02±0.24^{d}	27.61±0.53^{c}	27.88±0.23^{c}	27.15±0.10^{b}
Weight gain (g)	41.08±0.34^{a}	52.53±0.15^{f}	49.68±0.24^{e}	45.32±0.51^{c}	46.29±0.16^{d}	43.97±0.18^{b}
SGR	1.38±0.02^{a}	1.63±0.11^{d}	1.57±0.03^{c}	1.47±0.22^{b}	1.50±0.01^{bc}	1.44±0.31^{ab}
ADG	0.20±0.01^{a}	0.25±0.00^{e}	0.24±0.03^{d}	0.22±0.01^{c}	0.22±0.02^{c}	0.21±0.00^{b}
PER	0.34±0.01^{a}	0.44±0.00	0.41±0.01	0.38±0.01	0.39±0.00	0.37±0.00
FCR	0.41±0.10^{a}	0.37±0.05^{a}	0.39±0.01^{a}	0.43±0.03^{b}	0.42±0.02^{a}	0.44±0.06^{b}

N.B: Means with the same alphabets (a b or c) as superscripts on the same row are not significantly different from each other (P>0.05)
Legend: SGR =Specific growth rate, FCR = Feed conversion ratio, PER = Protein efficiency ratio,
ADG= Average daily gain

Quadratic graph Description:
Quadratic Equation: $y = 0.032x^2 + 0.8293 + 43.90$
Optimum value = 13.67
R^2 Value = 0.4207

3.2. Haematology

The values recorded for the haematology were significant at P>0.05 showing a rising trend in values to diet 2 for PCV (30.67±0.47), HB (10.30±0.22), RBC (3.44±0.06) and diet 3 for WBC (17.02±1.27), before leveling to diet 6,

Table 4. *Haematological profile of Clarias gariepinus fed zinc practical diet.*

	Haematological indices									
Parameter	Pcv	Hb	Rbc	Wbc	Platelets	Lymp	Hetero	Mono	Eosi	Baso
Initial Diet 1	19.78±0.69^{a}	6.12±0.59^{a}	1.49±0.77^{a}	12.65±3.29^{a}	44758.33±77.55^{b}	64.22±3.02^{b}	30.33±0.58^{c}	2.00±0.00^{a}	3.33±2.08^{c}	0.33±0.58^{b}
(control)	26.67±0.47^{b}	8.65±0.53^{b}	3.07±0.65^{b}	17.08±1.35^{b}	31461.33±44.52^{a}	55.00±16.97^{a}	18.00±11.31^{a}	2.33±0.47^{a}	4.33±0.47^{d}	0.33±0.47^{b}
Diet 2	30.67±0.47^{c}	10.30±0.22^{c}	3.44±0.06^{b}	16.75±4.32^{b}	25506.33±35.41^{a}	69.00±2.94^{c}	23.00±3.56^{a}	3.67±0.47^{b}	3.67±0.47^{d}	0.33±0.47^{b}
Diet 3	30.00±0.82^{c}	9.57±0.58^{b}	3.69±0.06^{c}	17.02±1.27^{b}	33465.00±47.35^{a}	64.00±1.41^{b}	30.67±1.70^{c}	3.00±0.00^{b}	2.33±0.47^{a}	0.00±0.00^{a}
Diet 4	29.67±1.25^{c}	9.43±0.61^{b}	3.67±0.10^{c}	15.68±0.46^{b}	32799.00±46.07^{a}	65.67±4.50^{c}	30.33±4.50^{a}	2.33±0.47^{a}	1.67±0.47+	0.00±0.00^{a}
Diet 5	30.33±0.47^{c}	10.00±0.41^{c}	3.45±0.08^{c}	20.97±1.66^{c}	36839.67±51.17^{a}	68.00±4.32^{c}	24.00±4.97^{a}	3.33±0.94^{b}	4.00±0.00^{d}	0.33±0.47^{b}
Diet 6	27.33±0.47^{b}	8.83±0.12^{b}	3.58±0.07^{c}	16.37±3.59^{b}	34482.33±48.31^{a}	68.67±4.78^{c}	25.00±4.97^{b}	3.00±0.82^{b}	3.33±0.47^{c}	0.00±0.00^{a}

N.B: Means with the same alphabets (a b or c) as superscripts on the same row are not significantly different from each other (P>0.05).
Legend: Hb = Haemoglobin, RBC = Red blood cell, MCV = Mean cell volume, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell haemoglobin, WBC = White blood cell.

3.3. Serology

The values for total protein was significantly different among diets with lowest value in diet 3 (3.19±0.18) and highest value in diet 6 (4.13±0.56), ALB levels were lowest in diet 4 (0.49±0.28), and highest in diet 2 (0.81±0.12), AST. level was lower in diet 3 (176.00±1.73) and higher in diet 2 (179.00±4.58), ALT lowest in diet 3 (18.00±1.00) and higher in diet 1 (23.67±3.21).

Ca^{2+} lower in diet 3 (175.67±54.72) while diet 6 (232.33±19.76) was highest, Urea showed no effect in all the diets, Creatinine values where significantly different with lowest recorded values in diet 2 (0.57±0.23) and higher values in diet 5 (0.73±0.15) and Glucose level were lowest in diet 2 (194.33±47.06) and highest in diet (223.67±9.45) as recorded in Table 6.

Table 5. *Serum biochemical profile of Clarias gariepinus fed zinc practical diet.*

	Serum indices								
Parameters	T.P	ALB	AST	ALT	Ca	UREA	CREAT.	GLUCOSE	CHOLEST
Initial	4.10±0.30^{c}	0.82±0.24^{b}	187.14±1.03^{b}	29.52±13.07^{c}	104.36±80.80^{a}	6.86±5.18^{a}	0.65±0.05^{b}	145.39±60.82^{a}	137.70±32.45^{a}
Diet 1 (Control)	3.84±0.49^{c}	0.54±0.06^{a}	179.00±1.73^{b}	23.67±3.21^{b}	189.67±12.50^{b}	10.13±0.38^{b}	0.67±0.21^{b}	198.00±19.16^{b}	116.67±4.73^{a}
Diet 2	3.81±0.12^{c}	0.81±0.12^{b}	179.00±4.58^{b}	20.33±2.89^{b}	188.67±19.63^{b}	9.80±0.35^{b}	0.57±0.23^{b}	194.33±47.06^{b}	116.33±9.87^{a}
Diet 3	3.19±0.18^{a}	0.56±0.43^{a}	176.00±1.73^{a}	18.00±1.00^{a}	175.67±54.72^{b}	10.07±0.35^{b}	0.60±0.10^{a}	207.33±9.07^{b}	113.67±4.73^{a}
Diet 4	3.29±0.12^{b}	0.49±0.28^{a}	178.33±3.06^{a}	19.33±1.53^{a}	215.67±18.45^{c}	10.15±0.23^{b}	0.64±0.05^{a}	219.33±10.12^{b}	132.67±25.42
Diet 5	3.68±0.13^{c}	0.68±0.13^{b}	179.67±1.53^{b}	22.33±2.52^{b}	196.00±10.58^{b}	10.03±0.35^{b}	0.73±0.15^{c}	210.33±18.50^{b}	120.00±4.36^{b}
Diet 6	4.13±0.56^{c}	0.54±0.20^{a}	177.67±4.16^{a}	21.00±3.00^{b}	232.33±19.76^{c}	10.12±0.25^{b}	0.65±0.05^{b}	223.67±9.45^{b}	149.33±28.22^{b}

N.B: Means with the same alphabets (a b or c) as superscripts on the same row are not significantly different from each other (P>0.05)
Legend: T.P = Total protein, Alb. = Albumin, Creat. = Creatinine, AST = Aspartate aminotransferase, ALT. = Alanine aminotransferase, Chol. = Cholesterol, Gluc. = Glucose, Ca = Calcium, Zn = Zinc

3.4. Water Quality Parameter

The values for zinc concentration in water were not significant at P>0.05 with the exception of diet 5 (0.05±0.00), Ca^{2+} were significantly different with lower and higher values as 30.20±0.13 and 31.01±0.24 in diet 4 and diet 3 respectively. DO, Temperature and pH values were also recorded as shown in Table 6.

Table 6. *Water quality analysis.*

Parameters	Ca (mg/l)	Zn (mg/l)	DO (mg/l)	TEMP. °C	Ph
Initial	34.67±0.15^c	0.03±0.00^a	4.20±0.09^c	30.83±0.57^b	7.27±0.31^a
Diet 1	31.32±0.47^b	0.05±0.00^a	4.07±0.31^b	27.23±3.26^a	7.33±0.15^a
Diet 2	30.25±0.05^a	0.05±0.00^a	4.02±0.16^a	30.30±0.61^{ab}	7.37±0.06^b
Diet 3	31.01±0.24^b	0.05±0.00^a	3.83±0.48^a	30.80±0.44^b	7.39±0.20^b
Diet 4	30.20±0.13^a	0.04±0.00^a	4.03±0.21^a	30.40±1.04^b	7.54±0.09^c
Diet 5	30.11±0.02^a	0.19±0.24^b	4.14±0.22^b	31.77±1.33^c	7.42±0.08
Diet 6	30.83±0.21^{ab}	0.05±0.00^a	3.94±0.07^a	30.38±0.57^b	7.38±0.17^b

N.B: Means with the same alphabets (a b or c) as superscripts on the same row are not significantly different from each other (P>0.05)

4. Discussion

The use of dietary supplement in recent times is seen to cut across human, livestock and fish nutrition, reproductive enhancement and diseases resistance [14], growth boosting [15] and chemotherapy [16]. This study investigates the African catfish Clarias gariepinus juvenile growth, serum and haematological responses to fed graded levels of zinc incorporated practical diet. The mean weight gain of fish fed diet-borne zinc nutrients revealed that there were significant differences in growth responses of catfish juvenile fed practical diet with graded levels of zinc inclusions of 0, 5.46, 10.96, 16.40, 21.86, and 27.33 mg Zn kg-1. The assessment of the mean weight indices agrees with the results of [17] where the diet with much higher dietary zinc (900 mg Zn/kg) did not actually reflected the highest weight, indicating that at a particular feeding regime, it’s only what the body needs at the time that can be put into use for weight or carcass increase. It also showed a relationship with other research works on zinc requirements; for a wide range of fish species, diet-borne zinc concentrations approximately 20 mg Zn kg-1 diets were sufficient in a semi purified diet as long as the daily ration provided zinc doses of approximately 0.3 - 4 mg kg-1 body weight-1 [18]. [19] reported an average daily weight gain of 22.52, 54.95 and 38.74% respectively in fish fed practical diets supplemented at an inclusion levels of 25, 50 and 100 mg Zn kg-1 of diet, with the highest weight gain in Nile tilapia fed practical diet at 50mg Zn kg-1 dry diet showing variance in dietary absorption of zinc for metabolic functions by different species of fish. The reasons for the high dietary zinc inclusions in fish feed by some of the researchers as mentioned above may be as a result of the use of plant materials having high percentage of phytic acid content, hence, showing the tendency of inhibiting the bioavailability of zinc in the diets [20]. Therefore, diet with less anti-nutritional activities will intrinsically render the supplemented minerals more bioavailable for functional health of the fed fish species.

5. Conclusion

African catfish Clarias gariepinus fed practical diet with graded levels of zinc at concentrations ranging within 0 to 27 mg Zn kg-1 showed significant variations in growth responses. The serology and haematology did not indicate values showing toxic or adverse effect of zinc in the diets however, there were some variances in the result trend. Growth responses with respect to dietary zinc inclusions indicates an optimum value at 13.67 mg Zn kg-1, higher weight gain value of 52.53 ± 0.15 mg Zn kg-1, and least value of 43.97 ± 0.18 mg Zn kg-1. The growth responses were quite slow indicating some anomaly in the diets.

Recommendation

The above result study suggested that plant base diet should be carefully and properly selected and evaluated to ascertain the micronutrient base levels before supplementation, as well proper pre-treatment of plant material should be recommended and research work be focus on the factors that arrest the bioavailability of micronutrients that would be required by catfish in a plant based diet to reduce the effect of anti-nutritional factors and permit micronutrients in fish diet to be metabolize for optimum growth performances of fish.

Acknowledgements

I will like to extend my deepest gratitude for the many technical support received from Dr. Oyin Olukunle and Adeshina Ibrahim, field support from Adams Ovie, Mrs. Winifred Tyokumbur, Cynthia and also laboratory support from the Department of Aquaculture and Fisheries management, University of Ibadan.

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