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Mineral and Heavy Metal Content of African Catfish (*Clarias Gariepinus*) Fed with Combination of Black Soldier Fly Larvae (*Hermetia Illucens L.*) and Commercial Feed in Port Harcourt, Nigeria

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Abstract:- The aim of this research was to investigate the mineral and heavy metal contents in field trials of catfish fed with the combination of commercial feed and BSFL meal of different proportions. Group A were fed with 100% commercial feed, group B 60% commercial feed and 40% black soldier fly larvae (BSFL), group C; 55% commercial feed and 45% BSFL, and group D 50% commercial feed and 50% BSFL. Mg, Ca, Na, and Cu were highest in group D in comparison to the control group A, and the other experimental groups B and C; while the control group A, recorded the highest mineral contents in K, Fe, and Zn. Heavy metal contents for Pb, Cd, Co, Hg, and As were all below the equipment detection limit being all below 0.01 mg/100g. It is therefore recommended that BSFL of up to 50% inclusion combined with commercial feed is free of heavy metals and contamination free.

Keywords:- Black Soldier Fly Larvae (BSFL), African Catfish, Mineral Composition and Heavy Metal Contents.

I. INTRODUCTION

Food and Agriculture Organization [1] noted that the supply of fish globally is expected to increase with aquaculture contributing up to 62% and Nigeria regarded as one of the major producers in Africa [2]. Catfish (*Clarias gariepinus*) are primarily freshwater fish that are well adapted to confined environments and are not easily suspensible to manipulation and disease [3]. African catfish farming has increase in production and has recently gained significant importance in many African countries. One of the major challenges to the growth of aquaculture in Nigeria and catfish in particular is the very high cost of fish feed; which has necessitated a paradigm shift toward the search to sustainable aquafeed. Hence, the need for alternative aquafeed such as mealworms, grasshoppers, crickets, locust, house fly larvae, silkworms, and black soldier fly (BSF) [4]. Of all the alternative aquafeeds that have been tried, black soldier fly larvae (BSFL) stand out. Some of the major protein sources for aquatic feed production and aquafeed include fishmeal, fish oil and soybean meal. Fishmeal and fish oil contain protein and essential fats [5]. On the other hand, soybean meal contain mainly protein, with some limiting factors such as poor content of methionine, lysine and the presence of anti-nutritional factors [6, 7]. It has been reported that processed animal proteins (PAPs) that may be allowed in fish feed, are not included in many of the aquafeeds found in the market today [5]. BSFL protein is similar to PAPs thus providing a good, sustainable alternative. It can be processed into defatted BSFL with 60% protein and 10-12% lipid contents [8]. It can also be processed into full-fat BSFL with 42% crude protein and 30% lipid content [9]. The BSFL convert wastes into useful biomass rich in protein and lipid. During its developmental stage, these larvae make use of the nutrients present in the waste for its body fat, and in the process, accumulate minerals and toxic substances which may pose a risk in aquafeed and safety of fish. Though, the minerals have an important role in animal nutrition, studies have shown that larvae produced solely with animal manure contain calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P) in amounts exceeding the maximum tolerance levels [10]. Few studies have recorded successes in the inclusion of BSFL as feed component for catfish [11], but information on the mineral and toxic contents of the inclusion of BSFL as feed is lacking. Therefore, the aim of this research was to investigate the mineral and heavy metal contents of field trials of catfish fed with commercial feeds and BSFL meal at different proportions.

II. LITERATURE REVIEW

➢ *Mineral and Heavy Metal Accumulation in Black Soldier Fly Larvae and Catfish Composition*

In a study that did not specify the feed components used, the composite analyses of catfish revealed 304.82 mg/100g of Calcium (Ca), 11.45 mg/100g of Magnesium (Mg), 142.42 mg/100g of Sodium (Na), 279.45 mg/100g of Phosphorus (P), 17.03 mg/100g of Iron (Fe), 2.92 mg/100g of Zinc (Zn) and 1.17 mg/100g of Manganese (Mn) [3]. The inclusion of BSFL as fish feed components in several field trials ranged from 13% in Rainbow trout (*Oncorhynchus mykiss*) [12] to 50% in Rainbow trout (*Oncorhynchus mykiss*) [13]; 40% in Eurasian perch (*Perca fluviatilis*) [14]; 100% in Atlantic salmon (*Salmo* salar) [15]; 100% replacement of soybean in Juvenile Jian Carp (*Cyprinus carpio var. Jian*) [16]; 75% in African catfish (*Clarias gariepinus*) [11]; 30% in Siberian sturgeon fingerlings (*Acipenser baerii*) [17]; 19.5% in European seabass (*Dicentrsrchus labrax*) [9]. These studies recorded some level of successes. In terms of mineral found in BSFL due to the substrate fed to it, there are variability in Potassium (K), Calcium (Ca), Sodium (Na), Manganese (Mn) and

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Magnesium (Mg) in BSFL [18]. When the substate of sludge aquaculture waste was fed to BSFL, some mineral contents of BSFL such as Ca, K, Mg, P, Na, Fe, Zn, and Mn ranged from 68.0-82.9 g kg^{-1} on dry matter (DM) for Ca, 9.8-9.9 g kg^{-1} DM for K, 3.9-4.1 g kg^{-1} DM for Mg, 16.7-19.2 g kg^{-1} DM for P, 1.9-2.2 g kg^{-1} DM for Na, 0.5-0.6 g kg^{-1} DM for Fe, 0.2-0.3 g kg^{-1} DM for Zn, and 0.2 g kg^{-1} DM for Mn respectively [10]. On the other hand, the value of heavy mental contents in BSFL, ranged from 2.1-3.3 g kg^{-1} DM for Arsenic (As), 1.0-1.4 g kg^{-1} DM for Cadmium (Cd), 0.2 g kg^{-1} DM for Mercury (Hg) and 0.3 for Lead (Pb) [10]. Many countries of the world are yet to regulate the use of BSFL as feed component for fish while some have already. The USA allows for dried BSFL-fed "feed grade materials" to be used in food for salmon fishes only [19] while the European Union's Regulation (EC) No 767/2009 and Regulation (EC) No 1069/2009 prohibit the use of manure or any digestive tract content or excrement as insect feed [20].

III. MATERIALS AND METHODS

This research design was exploratory as it was a field trial with African Catfish (*Clarias gariepinus*). The field trial included control and treatment applications in Plastic tanks of $1m^3$ to enable comparison. Melange sized catfish with average weight of 113-315g were purchased from a private fish hatchery in Port Harcourt, Rivers State, Nigeria. The fish were acclimatized to the ambient temperature of the experimental tanks for 1 week before the experiment commenced. The 600 Melange size catfishes were thereafter placed in four treatment groups, each with three replicates fed with the experimental nonconventional feed. The schools of fish were labelled as Group A (the control group), Groups B, C, and D (treatment groups). Each replicate contained 50melange in the tank measuring 1 m^3 (1 × 1 × 1 m) and filled with 500-700 L of water. The field trial was carried out for 90 days; recording their weights and lengths bi-monthly. Three sample each from the control and treatment groups were taken to the laboratory for composite sample analyses at the department of food science and technology laboratory, faculty of agriculture, Rivers State University. Catfish were analysed in line with the Association of Official Analytical Chemist (AOAC, 2006). Elementary assay using Atomic Absorption Spectrophotometer (Buck Scientific 210VP was utilized. Data were analysed with Statistical Package for Social Science (SPSS Version 21) as well as Microsoft Excel 2019. Content of the element was calculated based on the derivatives:

$$\text{Metal (\%)} = \frac{\text{Concentration (ppm) x solution volumes}}{10^4 \text{ x sample weight.}}$$

$$\text{Metal (mg/100g sample)} = \text{metal (\%)} * 1000$$

IV. RESULTS

➢ *Contents of Minerals in African Catfish*

Results of laboratory analysis for content of magnesium in African catfish feed with different field trial is shown in Figure 1.

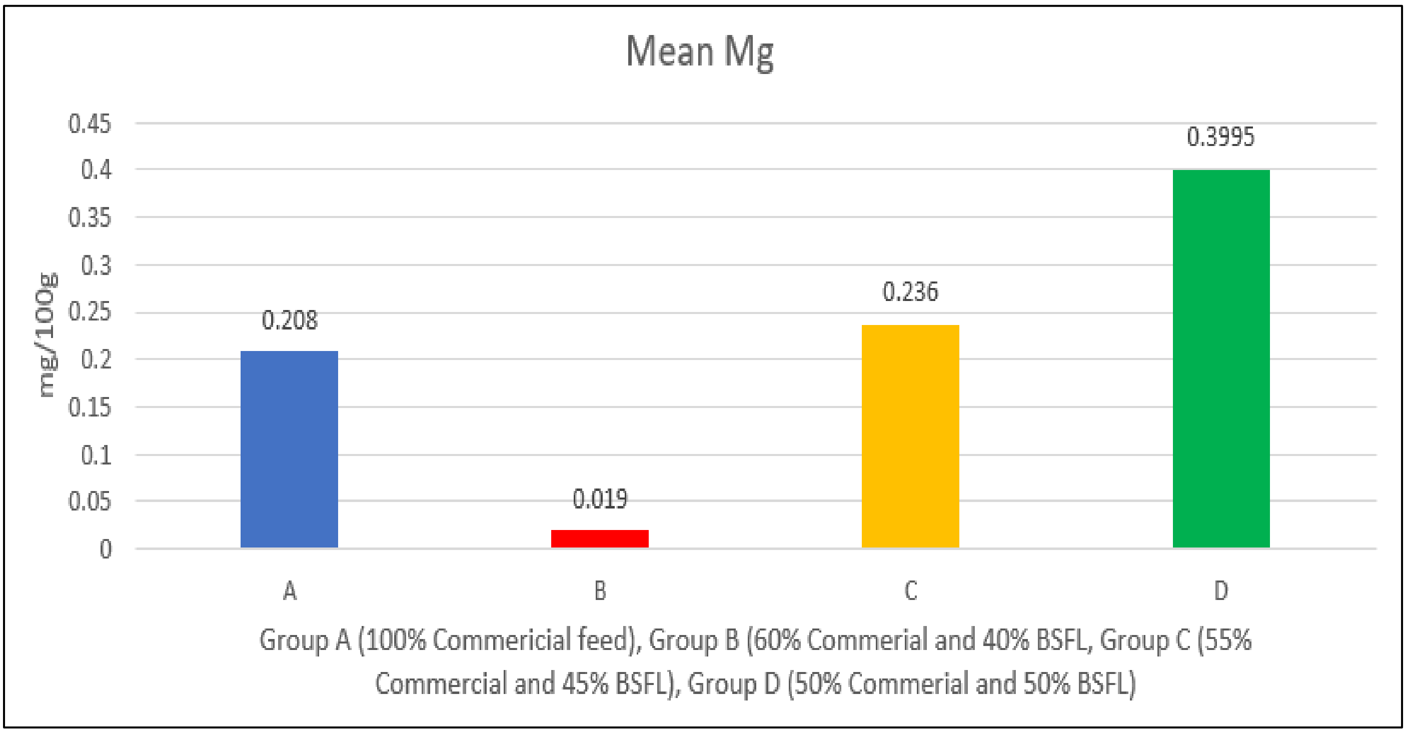

Fig 1 Content of Magnesium in Different Field Trials

- *The Content of Calcium in the Laboratory Result is shown in Figure 2.*

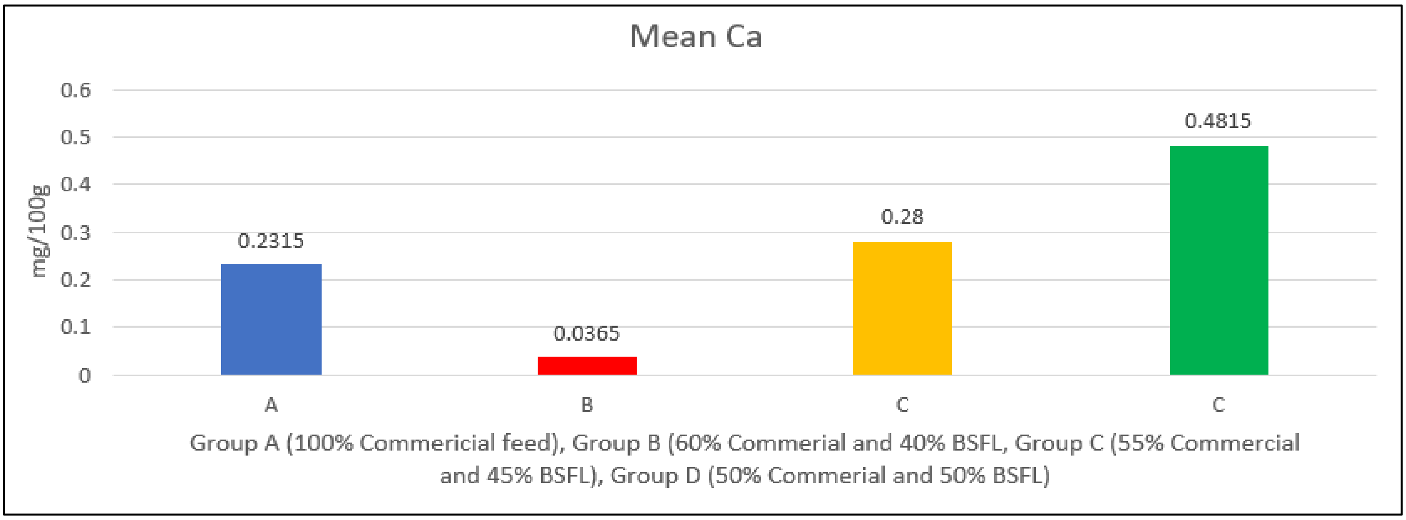

Fig 2 Content of Calcium in Different Field Trials

- *The Result of the Potassium Content Found in the Different Field Trial is shown in Figure 3.*

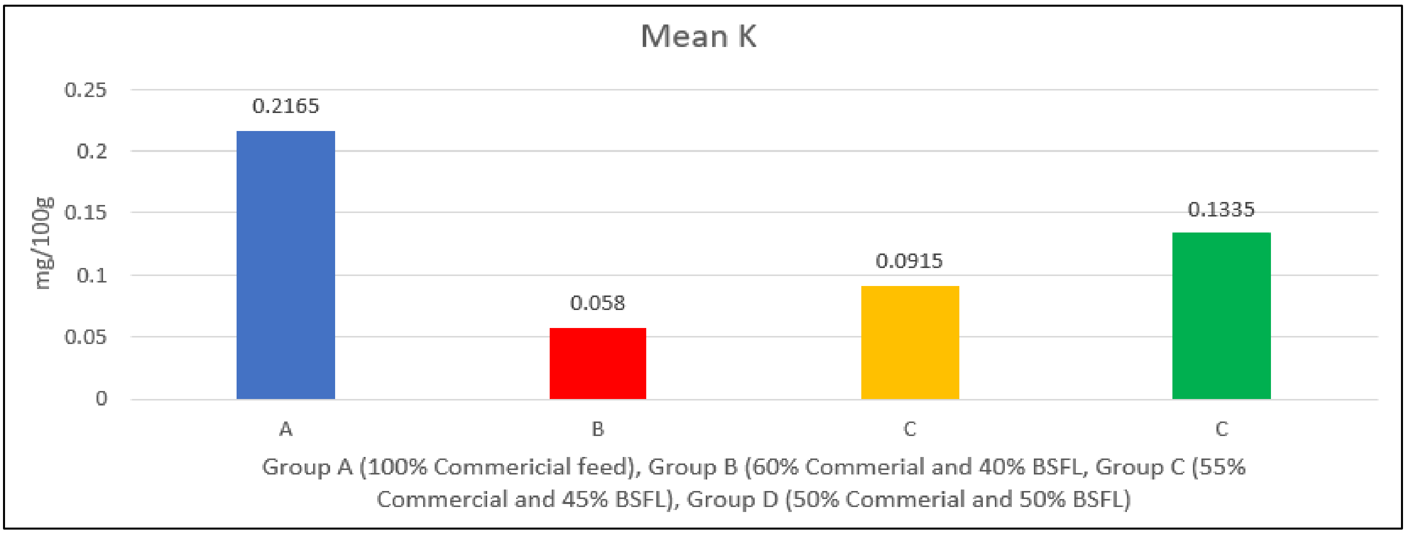

Fig 3 Content of Potassium in Different Field Trials

- *Laboratory Result of the Sodium Content in Field Trials is shown in Figure 4.*

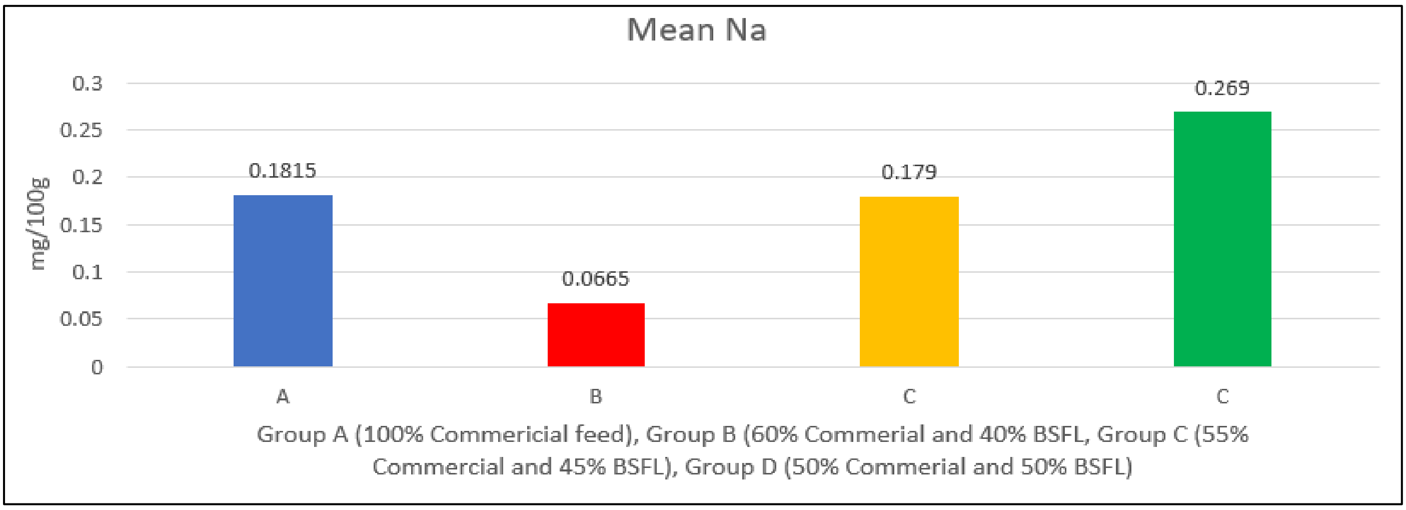

Fig 4 Content of Sodium in Different Field Trials

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- *The Laboratory Result for the Content of Iron in the Field Trial is shown in Figure 5.*

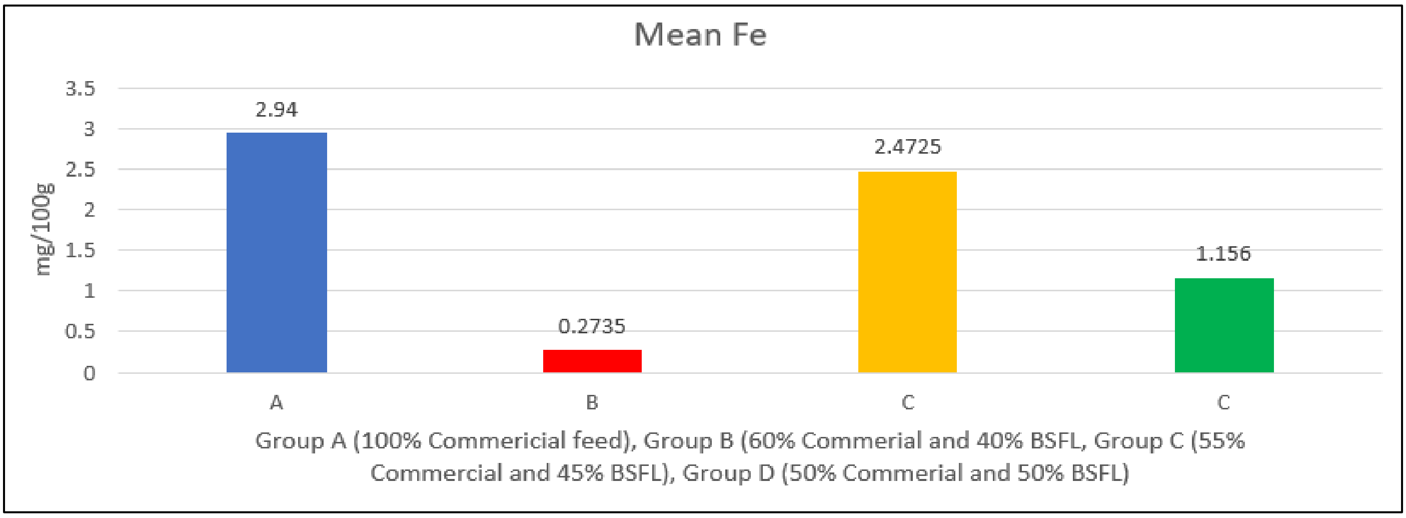

Fig 5 Content of Iron in Different Field Trials

- *Laboratory Result for the Content of Copper is shown in Figure 6.*

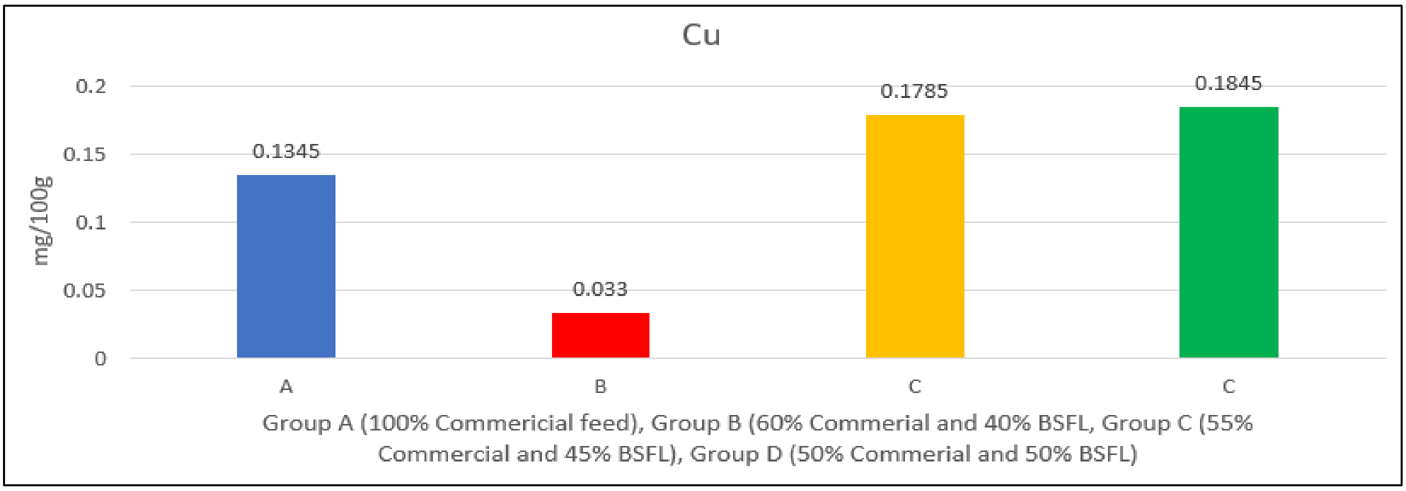

Fig 6 Content of Copper in Different Field Trials

- *The Laboratory Result for the Content of Zinc (Zn) is shown in Figure 7.*

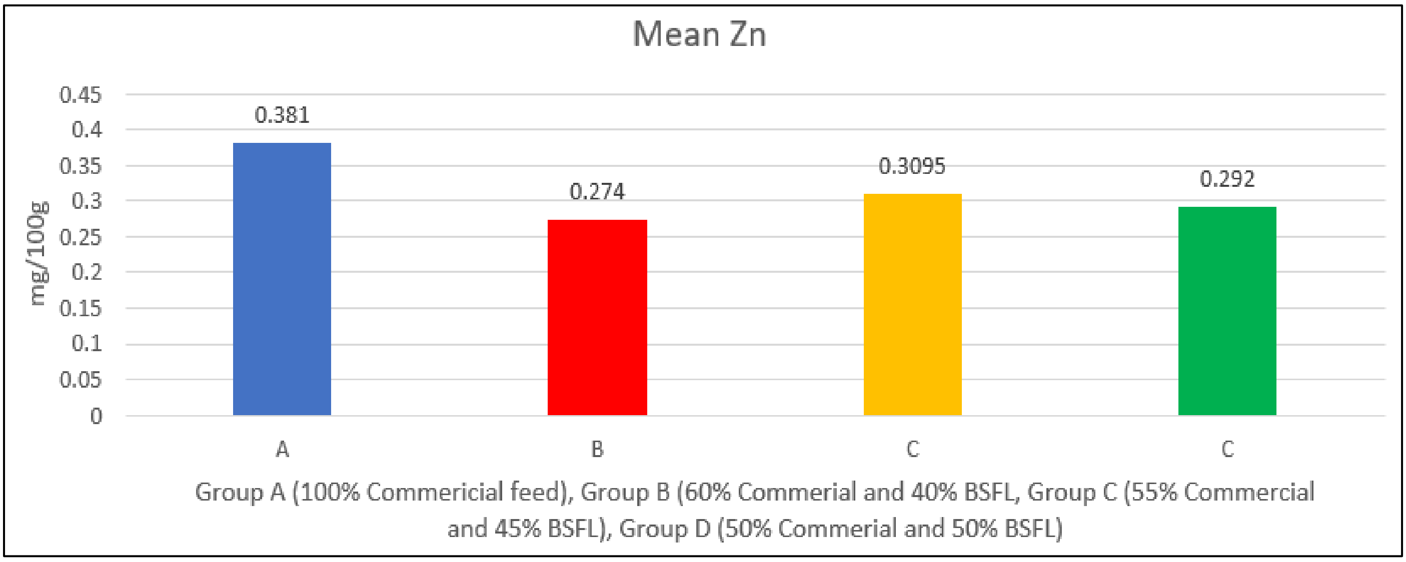

Fig 7 Content of Zinc in different field trials

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Analysis of variance for the content of minerals in the trial of African Catfish fed with feeds as grouped in A, B, C and D is shown in Table 1.

Table 1 Analysis of Variance

		Sum of Squares	df	Mean Square	F	Sig.
Mg	Between Groups	.146	3	.049	162.846	.000
	Within Groups	.001	4	.000		
	Total	.147	7			
Ca	Between Groups	.200	3	.067	4207.840	.000
	Within Groups	.000	4	.000		
	Total	.200	7			
K	Between Groups	.028	3	.009	6814.879	.000
	Within Groups	.000	4	.000		
	Total	.028	7			
Na	Between Groups	.041	3	.014	2900.000	.000
	Within Groups	.000	4	.000		
	Total	.041	7			
Fe	Between Groups	8.930	3	2.977	19422.527	.000
	Within Groups	.001	4	.000		
	Total	8.930	7			
Cu	Between Groups	.029	3	.010	2908.481	.000
	Within Groups	.000	4	.000		
	Total	.029	7			
Zn	Between Groups	.013	3	.004	717.626	.000
	Within Groups	.000	4	.000		
	Total	.013	7			

The multiple comparison (Bonferroni) test is shown in Table 2.

Table 2 Multiple Comparison (Bonferroni) Test

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Mg	1.0000	2.0000	.1890000*	.0172808	.002	.105171	.272829
		3.0000	-.0280000	.0172808	1.000	-.111829	.055829
		4.0000	-.1915000*	.0172808	.002	-.275329	-.107671
	2.0000	1.0000	-.1890000*	.0172808	.002	-.272829	-.105171
		3.0000	-.2170000*	.0172808	.001	-.300829	-.133171
		4.0000	-.3805000*	.0172808	.000	-.464329	-.296671
	3.0000	1.0000	.0280000	.0172808	1.000	-.055829	.111829
		2.0000	.2170000*	.0172808	.001	.133171	.300829
		4.0000	-.1635000*	.0172808	.004	-.247329	-.079671
	4.0000	1.0000	.1915000*	.0172808	.002	.107671	.275329
		2.0000	.3805000*	.0172808	.000	.296671	.464329
		3.0000	.1635000*	.0172808	.004	.079671	.247329
Ca	1.0000	2.0000	.1950000*	.0039843	.000	.175672	.214328
		3.0000	-.0485000*	.0039843	.002	-.067828	-.029172
		4.0000	-.2500000*	.0039843	.000	-.269328	-.230672
	2.0000	1.0000	-.1950000*	.0039843	.000	-.214328	-.175672
		3.0000	-.2435000*	.0039843	.000	-.262828	-.224172
		4.0000	-.4450000*	.0039843	.000	-.464328	-.425672
	3.0000	1.0000	.0485000*	.0039843	.002	.029172	.067828
		2.0000	.2435000*	.0039843	.000	.224172	.262828
		4.0000	-.2015000*	.0039843	.000	-.220828	-.182172
	4.0000	1.0000	.2500000*	.0039843	.000	.230672	.269328
		2.0000	.4450000*	.0039843	.000	.425672	.464328
		3.0000	.2015000*	.0039843	.000	.182172	.220828
K	1.0000	2.0000	.1585000*	.0011726	.000	.152812	.164188
		3.0000	.1250000*	.0011726	.000	.119312	.130688
		4.0000	.0830000*	.0011726	.000	.077312	.088688

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	2.0000	1.0000	-.1585000*	.0011726	.000	-.164188	-.152812
		3.0000	-.0335000*	.0011726	.000	-.039188	-.027812
		4.0000	-.0755000*	.0011726	.000	-.081188	-.069812
	3.0000	1.0000	-.1250000*	.0011726	.000	-.130688	-.119312
		2.0000	.0335000*	.0011726	.000	.027812	.039188
		4.0000	-.0420000*	.0011726	.000	-.047688	-.036312
	4.0000	1.0000	-.0830000*	.0011726	.000	-.088688	-.077312
		2.0000	.0755000*	.0011726	.000	.069812	.081188
		3.0000	.0420000*	.0011726	.000	.036312	.047688
Na	1.0000	2.0000	.1150000*	.0021794	.000	.104427	.125573
		3.0000	.0025000	.0021794	1.000	-.008073	.013073
		4.0000	-.0875000*	.0021794	.000	-.098073	-.076927
	2.0000	1.0000	-.1150000*	.0021794	.000	-.125573	-.104427
		3.0000	-.1125000*	.0021794	.000	-.123073	-.101927
		4.0000	-.2025000*	.0021794	.000	-.213073	-.191927
	3.0000	1.0000	-.0025000	.0021794	1.000	-.013073	.008073
		2.0000	.1125000*	.0021794	.000	.101927	.123073
		4.0000	-.0900000*	.0021794	.000	-.100573	-.079427
	4.0000	1.0000	.0875000*	.0021794	.000	.076927	.098073
		2.0000	.2025000*	.0021794	.000	.191927	.213073
		3.0000	.0900000*	.0021794	.000	.079427	.100573
Fe	1.0000	2.0000	2.6665000*	.0123794	.000	2.606447	2.726553
		3.0000	.4675000*	.0123794	.000	.407447	.527553
		4.0000	1.7840000*	.0123794	.000	1.723947	1.844053
	2.0000	1.0000	-2.6665000*	.0123794	.000	-2.726553	-2.606447
		3.0000	-2.1990000*	.0123794	.000	-2.259053	-2.138947
		4.0000	-.8825000*	.0123794	.000	-.942553	-.822447
	3.0000	1.0000	-.4675000*	.0123794	.000	-.527553	-.407447
		2.0000	2.1990000*	.0123794	.000	2.138947	2.259053
		4.0000	1.3165000*	.0123794	.000	1.256447	1.376553
	4.0000	1.0000	-1.7840000*	.0123794	.000	-1.844053	-1.723947
		2.0000	.8825000*	.0123794	.000	.822447	.942553
		3.0000	-1.3165000*	.0123794	.000	-1.376553	-1.256447
Cu	1.0000	2.0000	.1015000*	.0018371	.000	.092588	.110412
		3.0000	-.0440000*	.0018371	.000	-.052912	-.035088
		4.0000	-.0500000*	.0018371	.000	-.058912	-.041088
	2.0000	1.0000	-.1015000*	.0018371	.000	-.110412	-.092588
		3.0000	-.1455000*	.0018371	.000	-.154412	-.136588
		4.0000	-.1515000*	.0018371	.000	-.160412	-.142588
	3.0000	1.0000	.0440000*	.0018371	.000	.035088	.052912
		2.0000	.1455000*	.0018371	.000	.136588	.154412
		4.0000	-.0060000	.0018371	.185	-.014912	.002912
	4.0000	1.0000	.0500000*	.0018371	.000	.041088	.058912
		2.0000	.1515000*	.0018371	.000	.142588	.160412
		3.0000	.0060000	.0018371	.185	-.002912	.014912
Zn	1.0000	2.0000	.1070000*	.0024749	.000	.094994	.119006
		3.0000	.0715000*	.0024749	.000	.059494	.083506
		4.0000	.0890000*	.0024749	.000	.076994	.101006
	2.0000	1.0000	-.1070000*	.0024749	.000	-.119006	-.094994
		3.0000	-.0355000*	.0024749	.001	-.047506	-.023494
		4.0000	-.0180000*	.0024749	.011	-.030006	-.005994
	3.0000	1.0000	-.0715000*	.0024749	.000	-.083506	-.059494
		2.0000	.0355000*	.0024749	.001	.023494	.047506
		4.0000	.0175000*	.0024749	.013	.005494	.029506
	4.0000	1.0000	-.0890000*	.0024749	.000	-.101006	-.076994
		2.0000	.0180000*	.0024749	.011	.005994	.030006
		3.0000	-.0175000*	.0024749	.013	-.029506	-.005494

*. The mean difference is significant at the 0.05 level.

➢ *Heavy Metals Contents in African Catfish*

Results of the heavy metal contents is shown in table 3.

Table 3 Heavy Metal Content in African Catfish

Sample Catfish Groups	Pb(mg/100g)	Cd(mg/100g)	Co(mg/100g)	Hg(mg/100g	As(mg/100g)
A	<0.01	<0.001	<0.01	<0.01	<0.01
✓	<0.01	<0.001	<0.01	<0.01	<0.01
B	<0.01	<0.001	<0.01	<0.01	<0.01
✓2	<0.01	<0.001	<0.01	<0.01	<0.01
C	<0.01	<0.001	<0.01	<0.01	<0.01
✓2	<0.01	<0.001	<0.01	<0.01	<0.01
D	<0.01	<0.001	<0.01	<0.01	<0.01
✓2	<0.01	<0.001	<0.01	<0.01	<0.01

V. DISCUSSION

This study revealed that the mean Mg content of group D (0.3995 mg/100g) was highest and more than that of group A (0.208 mg/100g). The mean group C (0.236 mg/100g) was even higher than mean group A, which was 100% commercial feed. Earlier study reported 11.45 mg/100g for Magnesium (Mg) content of catfish [3]. The mean Ca content of group D (0.4815 mg/100g) was also highest while groups C and A recorded 0.28 mg/100g and 0.2315 mg/100g respectively. Earlier reported study showed 304.82 mg/100g of Calcium (Ca) content in catfish [3]. The mean K of group A (0.2165 mg/100g) was the highest from this study while group D and C recorded 0.1335 mg/100g and 0.0915 mg/100g respectively. Mean Na of group D (0.269 mg/100g) was highest and more than group A (0.1815 mg/100g) and group C (0.179 mg/100g) respectively. Previous study reported 142.42 mg/100g of Sodium (Na) [3]. The mean Fe content of this study showed group A (2.94 mg/100g) was highest followed by group C (2.4725 mg/100g) while group D recorded 1.156 mg/100g. Prior study of Fe content of catfish revealed 17.03 mg/100g) [3]. Mean Cu of group D (0.1845 mg/100g) was highest and more than group C (0.1785 mg/100g) and group A (0.1345 mg/100g). Mean Zn in group A (0.381 mg/100g) was the highest and more than group C (0.3095 mg/100g) and group D (0.292 mg/100g) while reported value from another study revealed 2.92 mg/100g of (Zn).

The values that were earlier reported for Mg, Ca, Na, Fe, and Zn were not in agreement with this study as they were higher. This disagreement may have arisen as the study referred to did specify the feed component used. Analysis of variance indicated that there were significant differences within and between groups A, B, C, and D in all the parameters analysed for mineral contents. All the parameters analysed for heavy metal contents in catfish showed no detection from the equipment as they were all <0.01.

VI. CONCLUSION AND RECOMMENDATION

This study considered mineral and heavy metal contents in catfish when fed with a combination of commercial feed with BSFL at different inclusion levels. Mg, Ca, Na, and Cu were highest in group D compared to control group A and other experimental groups B and C, while the control group A, recorded the highest mineral content of K, Fe, and Zn. Heavy metal contents for Pb, Cd, Co, Hg, and As were all below the equipment detection limit as they were all below 0.01 mg/100g. To the best of the researchers' knowledge, no study has been conducted on the mineral and heavy metal content of catfish, fed with the combination of commercial feed and BSFL meal.

It is therefore recommended that BSFL of up to 50% inclusion with commercial feed is free of heavy metal and contamination risk-free and therefore safe for consumption.

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