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PROXIMATE, MINERAL AND VITAMIN COMPOSITION OF FISH FEED SUPPLEMENTED WITH *MORINGA OLEIFERA* LEAF AND SHRIMP MEALS AS PROTEIN SOURCES

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ABSTRACT

Objective: Conventional fish meal makes up about 60% of the total protein source of aquaculture diet; it is very expensive and difficult to get. This study was aimed at utilizing *Moringa oleifera* leaf and shrimp meals as alternative protein sources in fish feed production. **Method:** Graded levels of *Moringa oleifera* leaf meal (MLM) (0, 10, 20 and 30%) and same quantity of shrimp meal were added as replacements in a standard formulation to produce fish diets (M0, M10, M20, and M30, respectively). Proximate, mineral and vitamin composition of these diets, plus two commercial feeds (foreign - Copens™; and local - Vital™) were analyzed and compared using standard procedures. **Results:** The formulated feeds had significantly higher ($p<0.05$) contents of crude fat, fibre, and carbohydrate; but much lower in crude protein than the commercial feeds. The formulated feeds were also significantly richer in Cu, Fe, Zn and P; similar in Mn and Mg; and lower in Ca, Na and K. However, the vitamins profiles of the formulated feeds were significantly poor compared to that of the commercial feeds. Among the MLM supplemented feeds, there was a dose dependent increase in crude fat, crude fibre, protein, carbohydrate, Na, K, and vitamins. M10 was however richer in Cu, Fe, Zn, and P than M20 and M30. **Conclusion:** Although supplementation with MLM improved protein levels in formulated feeds, it is far from meeting the optimum protein requirement in fish feed. Further studies to reduce chitin and crude fibre content in the formulated feeds are recommended.

KEYWORDS: *Moringa oleifera* leaf meal Shrimp meal Fish feed Proximate composition Mineral nutrients Vitamins profile.

INTRODUCTION

Aquaculture is a rapidly growing business in Nigeria contributing almost 6% of all domestic fish production with annual turnover of 40,000 metric tons.[1] This, without doubt, is a major source of income for farmers who are presently being challenged to expand for foreign earnings.[1] Fish nutrition is a critical component of aquaculture amounting to about 60-70% of the total cost of production. Protein and energy still remain the most important components of fish feeds for maintenance, growth and reproduction. The adequate supply of both in quality and quantity allows fish to realize its potentials. It is recommended that a good diet for optimal growth and health of fish should contain the following nutrients requirement: carbohydrate, 15-20%; protein, 18-50%; fat, 10-20%, ash <8.5%, phosphorus <1.5%, water <10% and trace amounts of vitamins and minerals.[2] Protein remains the most expensive and challenging component of aquaculture diet. This is so because fish meal (the conventional feedstuff), makes up about 60% of the total protein source of the diet;[3] it is not only expensive, but it's quality, quantity, and availability fluctuate under the heavy competition between human and aquaculture.[4] This has led to the need to source for alternative protein sources for fish feeds. Non-conventional feed ingredients such as leaf meals and maggots are a very good example of such alternative protein sources.[5] They pose no competition for human consumption; are cheap and often times, free of cost.[5,6] One of such plants of great importance is *Moringa oleifera.* It is very rich in high quality protein and highly valued for its uncountable nutritional and pharmacological properties. Moringa dry leaf meal (MLM) is reported to contain about 10 times the vitamin A of carrots, 50% the vitamin C of oranges, 17 times the calcium of milk, 15 times the potassium of bananas, 25 times the iron of spinach and 9 times the protein of yoghurt.[7] The protein content ranges from 15 to 35%; it is rich in fiber, minerals (particularly Ca and Fe) and a wide range of vitamins (ß-carotene, ascorbic acid, vitamin B1, B6 and niacin)[8] as well as flavonoids.[9,10] An earlier study[11] suggested a not more than 10% substitution of fish meal with MLM in African catfish diet. In this study, we replaced fish meal totally with shrimp dust (a waste product from crayfish production/sales) which is considered as a cheap source of animal protein; and then replaced 10, 20, and 30% of maize and soya bean meal with MLM. We then evaluated the proximate, mineral and vitamin composition of the diets comparing same with two commercially available conventional fish feeds; one locally manufactured in Nigeria, and the other imported.

MATERIALS AND METHODS

Sourcing of materials

Fresh leaves of *M. oleifera* were obtained from a moringa farm located at Obudu Local Government Area of Cross River State. The *M. oleifera* leaves sample were identified and deposited in the herbarium of the Department of Plant and Ecological Studies in the University of Calabar, Calabar. All ingredients used for the formulation of the experimental diets and commercial feeds - C_1 and C_2 (Copens™ and Vital™ respectively)- were purchased from local markets in Calabar.

Preparation of Moringa leaf meal (MLM)

Five hundred (500) grams of freshly harvested leaves were washed and air-dried under shade until they were crispy to touch while still retaining their greenish coloration. The leaves were then threshed to strip off dry leaves from stalks to reduce the crude fiber content in the meal. The dried leaves were however, ground into a fine powder referred to as morning leaf meal using a hammer mill. The resulting moringa leaf meal (MLM) was sieved and then stored in plastic bags at room temperature until when needed.

Fish diets formulation

The ingredients and the proportions used for the formulation of the experimental diets are shown on Table 1. Four iso-nitrogenous experimental diets were formulated using the Pearson Square method[5,12] to provide 40% crude protein. *Moringa oleifera* leaf meal was included in the diets at 0, 10, 20 and 30% respectively, with shrimp meal as the other major source of protein. All the experimental diets were prepared by fine grinding of the dietary ingredients. The ingredients were thereafter hand mixed and produced into pellets (2mm in diameter) using a hand pelletizing machine. The experimental pellets were shade dried and packed in plastic bags and kept in a good storage condition prior to commencement of the experiment. The choice of the ingredients was intended to reflect locally used ingredients.

Table 1: Percentage (%) composition of the experimental diets.

Ingredients	M (0%)	M (10%)	M (20%)	M (30%)
MLM	-	10.00	20.00	30.00
Shrimp Meal	20.00	20.00	20.00	20.00
Maize	35.40	30.10	25.30	20.20
Soya Bean Meal	40.20	35.50	30.30	25.40
Bone Ash	0.50	0.50	0.50	0.50
Wheat Flour	1.50	1.50	1.50	1.50
Vitamin Premix	0.50	0.50	0.50	0.50
Palm Oil	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20

Proximate analyses of the diets

Proximate analysis (moisture, crude protein, crude lipid, crude fiber, ash and carbohydrate) was performed according to standard methods.[13] Moisture content was determined by drying samples to constant weight in an oven (Gallenkamp, UK) at 105^0C (12hrs). Crude protein was determined using the Kjeldahl method. Samples were digested in concentrated sulphuric acid using a digestor 2040 (Foss, Denmark) followed by distillation using Kjeltec 2300 auto-analyzer (Foss, Denmark) to determined nitrogen content which was converted to crude protein using a conversion factor of 6.25. Crude lipid was determined using a Soxhlet 2050 (Foss, Denmark) and was extracted using petroleum ether (40-60^0C). Crude fiber was determined using a moisture free defatted sample which was digested by a weak acid followed by a weak base using the fiber Tec system 20121 (Foss, Denmark). Ash was determined by overnight incineration of sample in a muffle furnace at 600^0C (size 2 Gallen Kamp, UK). Carbohydrate was determined by difference (subtracting the sum of moisture, crude protein, crude lipid, crude fiber and ash from 100).

Mineral analysis

Phosphorous was quantified using the method outlined by Allen.[14] Approximately 10mg of sample was extracted overnight in 250ml of concentrate nitric acid. This was followed by digestion in concentrated nitric acid and then per choric acid on a hot plate. Distilled water (20ml) was added to the sample and dried. until white fumes appeared. This was followed by addition of 5ml of ammonia solution and further boiling until crystals were formed. The crystals were dissolved using 20ml of acidified water and 80ml of distilled water followed by 20ml of mixed reagent for color development. The mixed reagent consisted of 250ml Sulphuric acid-antimony, 250ml Sodium Molybdate, 50ml distilled water and $2gl^{-1}$ ascorbic acid. The sample was left for 15 minutes for color to develop. The quantity of phosphorus was then determined using a spectrophotometer (Cecil Elegant Technology, UK).

Other minerals (copper, iron, magnesium, manganese, zinc, calcium sodium and potassium) were quantified using a thermo X series 2 inductively coupled plasma mass spectrophotometer, ICP MS (Thermo Scientific, USA). Samples weighing approximately 80mg were digested in nitric acid for 1hr in a mass Xpress microwave (CEM Corporation, USA) and then diluted to 10ml with distilled water ready for quantization.

Vitamins analysis

Antioxidant vitamins (Vitamins A, E, and C) were determined using the methods of Rutkowski & Grzegorczyk[15] while vitamins B_1, B_2, B_3, B_5, B_6, K and Folate were determined using standard methods.[16]

Statistical analysis

All data collected were analyzed using statistical analysis system software (SPSS, 1995 Version 9 for WINDOWS). One way analysis of variance and Duncan's multiple range tests was used to compare between parameters of the different feeding groups.

RESULTS

Proximate composition of experimental diets

The result of the proximate evaluation of the different experimental diets is represented on Table 2. The moisture content of the experimental diets (3.90- 4.29%) increased in a dose dependent manner but significantly lower (p<0.05) than that of the commercial feeds (7.19- 8.52%). Also the ash content (8.45 – 10.27%) was significantly higher in the formulated diets than the commercial feeds (6.73-7.21). The formulated diets had significantly higher (p<0.05) contents of crude fat (15.56 - 20.94%), crude fibre (27.74 – 32.23%) and carbohydrate (19.94 – 27.33%), than the commercial feeds which had 10.5 -12.46%; 5.31-6.98%; and 11.51 – 19.18%, for the respective parameters. Among the Moringa supplemented diets, crude fibre and carbohydrate contents increased with increase in dosage. The crude protein content of formulated diets (27.74 – 32.23%) on the other hand, was significantly lower than that of the commercial feeds (50.38 – 53.80).

Table 2: Proximate composition of MLM supplemented fish diets and commercial feeds.

	Moisture (%)	Ash (%)	Crude Fat(%)	Crude Fibre (%)	Crude Protein(%)	Carbohydrate (%)
C_1	8.52	6.73	12.46	6.98	53.80	11.51
(Copens™)	±0.29	±0.04	±0.04	±0.00	±16.08	±0.11
C_2	7.19	7.21	10.75	5.31	50.36	19.18
(Vital™)	±0.01*	±0.02*	±0.00*	±0.06*	±0.31	±0.25*
M_0	3.90	9.79	19.90	27.74	12.27	23.07
	±0.01*,§	±0.05*,§	±0.01*,§	±0.37*,§	±3.43*,§	±0.47*,§
M_{10}	4.01	8.45	17.14	29.55	14.37	26.47
	±0.02*,§	±0.33*,§,a	±0.00*,§,a	±0.35*,§,a	±0.04*,§	±0.62*,§,a
M_{20}	4.17	8.90	15.56	30.45	13.59	27.33
	±0.00*,§	±0.10*,§,a,b	±0.22*,§,a,b	±0.00*,§,a,b	±0.26*,§	±0.49*,§,a
M_{30}	4.29	10.27	20.94	32.23	12.34	19.94
	±0.15*,§,a	±0.20*,§,a,b,c	±0.00*,§,a,b,c	±0.53*,§,a,b,c	±0.12*,§	±0.33*,a,b,c

Values are expressed as mean ± SEM of 3 determinants
* = significantly different from C1 at p<0.05
§ = significantly different from C2 at p<0.05
a = significantly different from M0 at p<0.05
b= significantly different from M10 at p<0.05
c = significantly different from M20 at p<0.05

Mineral nutrient composition of formulated and commercial fish feeds

The results as presented on Table 3 show that the formulated feeds (M0, M10, M20 and M30) were significantly (p<0.05) richer in Fe, Mn, Zn and P; but poorer in Ca, Na, K, and Mg, than the commercial feeds. Among the MLM diets, Ca, Na, K and Mg increased in a dose dependent manner.

Vitamin composition of formulated and commercial fish feeds

Table 4 shows the result of analyses of selected vitamins of the formulated and commercial feeds. The mean levels of all the vitamins were significantly lower (p<0.05) in the formulated diets than the commercial feeds. M0 – M30 diets had 0.2 – 0.41mg/g of Vit. E; 532.2 – 733.74 IU/g of Vit. A; 0.52 – 0.87 mg/g of Vit. D; 0.35 – 1.01mg/g of Vit. B_1; 0.57 – 0.72mg/g of Vit. B_2; and 0.58 - .-97 mg/g of Vit. B_3. Similar values for the commercial feeds were 0.97 – 0.99 mg/g; 1010.38 – 1144.00 IU/g; 1.55 – 1.81 mg/g; 1.30 – 1.35 mg/g; 0.83 – 1.08 mg/g; and 1.03 – 1.25 mg/g for Vitamins E, A, D, B_1, B_2, and B_3, respectively. Among the MLM supplemented groups, there was a dose dependent increment in the levels of all the vitamins evaluated.

Table 3: Mineral composition (ppm) of the different experimental fish diets.

	Cu	Fe	Mn	Zn	Ca	Na	K	Mg	P
C_1	0.12	0.57	0.05	0.00	1.27	2.15	6.89	4.72	0.10
(Copens^{TM})	±0.01	±0.01	±0.01	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00
C_2	0.20	0.44	0.02	0.02	1.38	2.91	6.05	3.93	0.30
(Vital^{TM})	±0.01*	±0.01*	±0.00*	±0.01*	±0.03*	±0.01*	±0.01*	±0.01*	±0.00*
M_0	0.29	1.04	0.07	0.10	0.95	1.10	5.86	3.76	0.78
	±0.01*,§	±0.01*,§	±0.01*,§	±0.00*,§	±0.00*,§	±0.01*,§	±0.02*,§	±0.02*,§	±0.02*,§
M_{10}	0.34	1.20	0.09	0.18	0.74	1.09	4.10	3.23	0.85
	±0.01*^{,§,a}	±0.01*,§	±0.01*,§	±0.01*$^{,a, §}$	±0.01*^{,§,a}	±0.00*,§	±0.01*^{,§,a}	±0.04*^{,§,a}	±0.03*^{,§,a}
M_{20}	0.11	0.70	0.06	0.09	0.91	1.18	4.43	3.40	0.62
	±0.01^{§,a,b}	0.31^{a,b}	±0.01^{§,b}	±0.00*^{,§,a,b}	±0.01*^{,§,a,b}	±0.01^{b}	±0.02*^{,§,a,b}	±0.01*^{,§,a,b}	±0.01*^{,§,a,b}
M_{30}	0.09	1.04	0.07	±0.07	0.90	1.26	4.96	3.39	0.64
	±0.00*^{,§,a,b}	±0.01*,§	±0.01*,§	±0.01*^{,§,a,b}	±0.01*^{,§,a,b}	±0.01*^{,§,a,b,c}	±0.03*^{,§,a,b,c}	±0.07*^{,§,a,b}	±0.01*^{,§,a,b}

Values are expressed as mean ±SEM of 3 determinants
* = significantly different from C1 at $p<0.05$
§ = significantly different from C2 at $p<0.05$
a = significantly different from M0 at $p<0.05$
b = significantly different from M10 at $p<0.05$
c = significantly different from M20 at $p<0.05$

Table 4: Vitamin composition of the different experimental diet.

	Vit. E (mg/g)	Vit. A (IU/g)	Vit. D (mg/g)	Vit. B_1 (mg/g)	Vit. B_2 (mg/g)	Vit. B_3 (mg/g)
C_1	0.99	1144.10	1.81	1.30	1.08	1.25
(Copens^{TM})	±0.03	±22.67	±0.04	±0.04	±0.17	±0.01
C_2	0.97	1010.38	1.55	1.35	0.83	1.03
(Vital^{TM})	±0.03	±0.00*	±0.08*	±0.01*	±0.03*	±0.02*
M_0	0.20	532.20	0.52	0.35	0.57	0.58
	±0.01*,§	±4.22*,§	±0.01*,§	±0.00*,§	±0.00*,§	±0.00*,§
M_{10}	0.34	607.85	0.80	0.87	0.67	0.86
	±0.08*^{,§,a}	±0.62*^{,§,a}	±0.10*^{,§,a}	±0.14§	±0.06*	±0.11*^{,a}
M_{20}	0.36	643.77	0.79	0.89	0.65	0.94
	±0.01*^{,§,a}	±4.34*^{,§,a,b}	±0.01*^{,§,a}	±0.00§	±0.00*	±0.00*^{,a}
M_{30}	0.41	733.74	0.87	1.01	0.72	0.97
	±0.08*^{,§,a}	±10.60*^{,§,a,b,c}	±0.07*^{,§,a}	±0.13§	±0.05*	±0.11*^{,a}

Values are expressed as mean ±SEM of 3 determinants
* = significantly different from C1 at $p<0.05$
§ = significantly different from C2 at $p<0.05$
a = significantly different from M0 at $p<0.05$
b = significantly different from M10 at $p<0.05$
c = significantly different from M20 at $p<0.05$

DISCUSSION

The significantly lower levels of moisture in the formulated feeds in this study, coupled with antimicrobial potency of *Moringa oleifera*[17] portray better shelve life of the products as microbial growth is hindered by low water activity. The formulated MLM supplemented feeds were significantly richer in crude fibre, crude fat, and carbohydrate; but very poor in crude protein than the commercial feeds. The potential of a feedstuff in fish diets is rated particularly on the basis of its crude protein content[18] because of its nutritional relevance in meeting protein and energy requirements as well as boosting the immune system of the fish against diseases.[19,21] The highest crude protein content of MLM supplemented diets (14%) found in M10 (as against ≥ 50% found in the commercial feeds), is much lower than what is recommended for fish feed.[5] Previous studies have also reported relatively low protein content in fish feed formulations made with other plant materials as alternative protein source.[22,24] It is worthy of note that despite 20% of shrimp meal added in the formulation to boost protein content, the results still remained as poor as those obtained by previous workers.[18,25] Shrimp meal contains high levels of protein with comparatively excellent amino acid profile,[26,27] but its availability from the meal may have been limited by the presence of substantial quantity of chitin.[28] Cooking and bacterial fermentation are reported to improve the quality of shrimp meal.[29,30] The levels of crude fibre in the MLM supplemented diets were beyond the limits recommended for fish feed.[31] This finding is in line with other studies[4,18,25] which also recommended inclusion levels of not beyond 10% when using MLM. *M. oleifera* leaves are reportedly rich in essential fatty acids and polyunsaturated fatty acids.[21] This explains the high levels of crude fat in the MLM supplemented diets obtained in this study.

The levels of mineral nutrients and vitamins found in the MLM supplemented diets is in line with what is generally reported for *M. oleifera* leaves. Moringa leaf is said to be an excellent source of vitamins, minerals and proteins, perhaps more than any other vegetable.[18,32] High levels of minerals elements such as calcium, iron, copper, manganese, zinc, selenium and magnesium have been documented to be present in the leaves of the plant.[21] The mineral composition of *M. oleifera* plays a significant role in nutritional, medicinal and therapeutic values of the plant.[17]

The results of this study show appreciable levels of vitamins in the MLM supplemented diets.

These levels, though not as high as in the commercial feeds, were well within the FAO recommended range. *Moringa oleifera* leaves are rich in β-carotene, thiamine, riboflavin, niacin, pyridoxine, biotin, ascorbic acid, cholecalciferol, tocopherol and vitamin K.[33] Vitamin A is necessary for vision, bone growth, immunity and maintenance of epithelial tissues. In addition, it also maintains adequate levels of iron in plasma that supply the different body tissues, including the bone marrow.[34]

In conclusion, the results obtained from this study show that a combination of *Moringa oleifera* leaf meal and shrimp meal in the form in which they were added, cannot adequately replace fish meal in fish feed formulations. Further processing of the shrimp meal and MLM to remove chitin and fibre respectively, may enhance the protein quality and quantity, as well as the overall nutrient profile of MLM supplemented diets.

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Conflict of Interest
The authors declare that there are no conflicts of interest.

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The research was privately funded.

Authors' Contribution
David-Oku and Anani conceived and designed the study; Ntaji and Edide developed the methodology; Anani and Edide performed the bench work; Obiajunwa-Otteh analyzed and interpreted the data; David-Oku and Anani wrote and reviewed the manuscript; while Obiajunwa-Otteh revised the draft manuscript. Overall study supervision was by David-Oku.

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