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Nutritional assessment of fern (*Asplenium barteri*) in diets of *Clarias gariepinus* fingerlings

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ABSTRACT
A study was conducted for 56 days to test the effect of varying levels of fern (*Asplenium barteri*) at 0%, 5%, 10%, 15%, 20% and 25% on the growth, survival and nutrient utilization of *Clarias gariepinus* in plastic aquaria. Growth rate decreased as level of fern increased. Highest weight gain of 1.38±0.18 g and % weight gain, 184.16±71.89% was observed for fish fed 5% fern diet and was followed by 10% fern diet but no difference with 0% fern (P<0.05). Results were similar between 0%, 5% and 10% in feed conversion, protein efficiency ratio and fish condition. Fish survival was 48.22±3.56-72.92±3.56% for all diets. Diet containing 25% fern was most economical while 0% fern diet was uneconomical. By performance and economic considerations, authors suggest maximum dietary inclusion of fern to be 10%.

Keywords: Nutrition, fern plant, *Clarias gariepinus*, growth, survival.

1. Introduction
The plant soybean has been an important protein source used in aqua feeds either partially or complete to replace the costly fish meal in fish diets [16]. Its scarcity and high cost have made it necessary to evaluate the cheaper non-conventional plant ingredients for practical fish feeds. Plant parts such as leaves, flowers, stems, roots, seeds, fruits and bark of trees are materials utilized as non-conventional feed ingredients [18]. In the aquatic environment, there are some plants that are rich in protein and minerals and have high feeding values [12]. The nutrient composition of some aquatic macrophytes with potentials are *Azolla sp*. (fern), dry: crude protein, CP (25.3%), ether extract, EE (3.8%), crude fibre, CF (2.3%), ash (12.5%), NFE (49.1%) and Calcium (1.16%); *Pistia stratiotes* (water lettuce), dry: CP (15.9%), EE (4.2%), CF (20.8%), ash (23.0%), NFE (36.1%) and Calcium (2.35%), [14]. Okoye and Mbagwu [11] also reported that Duckweed was accepted by Tilapia, *Sarotherodon galilaeus* and that *Ceratophyllum demersum*, *Lemna sp*. and *Salvinia sp*. could be utilized in fish diet preparation.
Asplenium barteri, a fern (Pteridophyte) is a light floating aquatic plant with curly fiddle heads, lacy fan-like fronds and lance shaped spears. This plant possesses bright greenish colour when fresh and grows majorly in shady, woody marshy, moist and flood lands [8]. It has many purposes like being medicinal, fishermen use it to trap shrimps and herbivorous fishes, forms breeding grounds for mature catfishes in the wild [1], purifies and reduces the temperature of water. Recently, considerable attention has been given to its harvesting for use as an alternative plant protein source in livestock including fish [6, 17]. This plant is available in large quantity, lying waste in the wild, can be harvested and incorporated into feed as a contributor to the overall protein and mineral proportions in fish diets. Chemical analysis of *A. barteri* showed that it contained crude protein of 12.25±0.02% [17], very high fibrous or cell wall materials and very rich in amino acid profile [19]. However, due to the high fibre content of the whole fern plant meal, great limitation was put into its effective utilization by fish as feed ingredient despite its nutritive value. The objective of this study was to further evaluate the nutritional potentials of *Asplenium barteri* in feeds for *Clarias gariepinus* fingerlings.

2. Materials and methods
2.1 Production of experimental diets
This study was conducted at the Fisheries Unit of the Research Farm of Niger Delta University, Wilberforce Island in Bayelsa State, Nigeria between May and July, 2012. Six experimental

diets at varying inclusion levels of 0, 5,10,15,20 and 25% fern meal which contained 40% crude protein were prepared to substitute part of other dietary protein sources. Gross composition of the formulation is in Table 1. Various proportions of ground ingredients for the diets were weighed out with a triple beam balance (Model MB- 2610) into a clean basin and hand mixed thoroughly. A measured quantity of water (10%) and gelatinized starch were added and mixed again to form dough. The dough was made to pass through a manual meat mincer with 2mm die to produce strands of feed as pellets. The smaller and broken portions form crumbles and all of them were sun-dried for 48hours, cooled and packaged separately in clean plastic containers for later use. This process was repeated for each of the experimental diets according to the formulations.

2.2 Experimental fish
A total of 200 *Clarias gariepinus* fingerlings of mean weight 0.75±0.0 g and mean total length 4.5±0.0 cm were obtained from a private farmer in Yenagoa, Bayelsa State in Nigeria. The Fish were acclimatized and fed the control diet (0% fern) for 7 days.

2.3 Experimental system
Eighteen plastic aquaria of 25 litre capacity each were filled with clean bore-hole water to the 16.5 litre mark. Each aquarium was covered with a net mesh size of 3 mm to prevent the jumping out of fish. Each aquarium was stocked with 10 fish in triplicate per treatment and fed daily with 5% of their fresh body weight. The daily ration was divided into two halves and administered twice at 0730-0800 and 1600-1630 hours local time.
The quality of the water in the aquaria was maintained by aeration with Teca air pumps (Model AP 1500) fitted with air tubes and air stones, removal of wastes (urine and faeces) and uneaten feed with the aid of a hose, water changed every other day and aquaria washed and re-filled with clean aerated water to original level of 16.5 litres. Water samples were collected from the different aquaria fortnightly for dissolved oxygen, pH, ammonia and total hardness determination with Hach's Aquaculture test kit (Model FF-2). Water temperature was measured daily with a mercury-in-glass thermometer (0-100 ^{0}C).

2.4 Fish diet performance parameters
Total length and body weight measurement of fish was done at the start of experiment and on weekly basis in all the aquaria. Mean body weight gain (g) = difference between initial and final weights of fish, %weight gain= 100 x weight gain (g)/initial weight (g), specific growth rate (% day^{-1}) = $l_n w_2 - l_n w_1 \times 100/t_2 - t_1$ [5] where w_2 and w_1 are final and initial weight of fish, t_2 and t_1= end of growth period and beginning of same in days and l_n = natural logarithm. Condition factor (K) = $100w/l^3$ [3] where w and l are observed total weight (g) and length (cm) of fish, survival rate (%), S= $N_i \times 100/N_o$ [2] where N_i= number of fish at end of experiment and N_o= number of fish at beginning of experiment, Feed conversion ratio (FCR)= feed intake (g)/ weight gain (g), [15] Protein efficiency ratio (PER)= wet weight gain of fish (g)/ weight of protein fed (g), [20] and economy of weight gain (EWG)= cost of feed fed (₦) / body weight gain of fish (g), [13].

2.5 Data analysis
The results of the feeding trials were analyzed by one-way analysis of variance using the Special Package for Social Science (SPSS) version 16.0 computer software. Duncan's Multiple Range Test was used in separating the means at a confidence interval of P= 0.05 [10].

Table 1: Gross composition of experimental diets.

Ingredients	Diets					
	A (0%)	B (5%)	C (10%)	D (15%)	E (20%)	F (25%)
Fishmeal	42.0	41.05	40.60	43.15	47.21	52.65
Shrimp meal	5.0	5.0	5.0	3.5	3.5	2.5
Soybean meal	19.0	20.6	21.0	18.0	11.94	4.0
Fern meal	-	5.0	10.0	15.0	20.0	25.0
Corn	15.5	10.0	5.05	3.0	2.0	1.5
Wheat bran	6.0	6.0	6.0	5.0	3.0	2.0
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/Mineral Premix	0.50	0.50	0.50	0.50	0.50	0.50
Table salt	0.25	0.25	0.25	0.25	0.25	0.25
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Red palm oil	7.0	7.0	7.0	7.0	7.0	7.0
Starch (Binder)	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
% Crude Protein	40.07	40.03	40.02	40.04	40.04	40.01

3. Result
3.1 Water quality
Values of physico-chemical parameters monitored in this study are in Table 2. The water temperature ranged from 27.0-30.1 ºC and with the highest mean temperature of 27.42±0.02 ºC observed in Diet F (25% fern). Dissolved oxygen was 3.5-4.5 mg/l and mean pH, 6.44±0.03 (Diet B, 5% fern)-6.5±0.03 (Diet C, 10% fern) in the aquaria. Ammonia was 0.5 - 2.0 mg/l in the aquaria while total hardness ranged 24.0 - 30.0 mg/l for the diets.

3.2 Fish performance
Table 3 shows the growth response and nutrient utilization values for *Clarias gariepinus* fingerlings. The mean weight

gain 1.62±0.18 g and % weight gain (2.15.41±71.86%) were highest for Diets A (0% fern). This was closely followed by Diet B (5% fern), 1.38±0.18 g, 184.16±71.89% and C (10% fern), 1.18±0.18 g and 156.66±71.86% respectively. The specific growth rates of fish fed the different diets were similar and ranged 0.80±1.16-0.94±0.06% day^{-1}. Condition of fish was best with Diets A, 0.71±0.04 and B, 0.73±0.04. The FCR ranged 2.67±0.22 (Diet B) – 4.04±0.22 (Diet D) and best conversions with Diets A and B. Protein efficiency ratio was highest for Diet A (6.99±0.69) but not different from other diets (P<0.05). Fish survival ranged 48.22±3.56 (Diet A) - 72.92±3.56% (Diet E).The economic weight gain (EWG) was best for Diet F (N5.77/g weight gain), moderate for Diets C (N14.02/g. wt gain) and B (N16.84/ g. wt. gain) and least for Diet A (N 23.33/ g. wt. gain)

Table 2: Mean and range values of water quality parameters for *Clarias gariepinus* fingerlings fed diets containing varying fern levels.

	Diets					
Parameter	**A (0%)**	**B (5%)**	**C (10%)**	**D (15%)**	**E (20%)**	**F (25%)**
Temperature (ºC)	27.19±0.02^{b} (27.0-30.1)	27.11±0.02^{c} (27.0-30.0)	27.18±0.02^{b} (27.0-30.0)	27.36±0.02^{a} (27.0-30.0)	27.36±0.02^{a} (27.0-30.0)	27.42±0.02^{a} (27.0-30.0)
Dissolved Oxygen (mg/l)	4.03±0.02^{a} (4.0-4.5)	4.15±0.02^{a} (4.1-4.5)	3.94±0.02^{b} (3.5-4.0)	3.91±0.02^{b} (3.5-4.5)	4.03±0.02^{a} (4.0-4.5)	3.99±0.02^{ab} (3.5-4.0)
pH	6.51±0.03^{a} (6.5-7.0)	6.44±0.03^{b} (6.4-7.0)	6.54±0.03^{a} (6.5-7.0)	6.53±0.03^{a} (6.5-7.0)	6.50±0.03^{a} (6.3-7.0)	6.52±0.03^{a} (6.5-7.0)
Ammonia- nitrogen (mg/l)	0.71±0.02^{c} (0.5-2.0)	0.73±0.02^{b} (0.5-2.0)	0.79±0.02^{a} (0.5-2.0)	0.75±0.02^{b} (0.5-2.0)	0.75±0.02^{b} (0.5-2.0)	0.71±0.02^{c} (0.5-2.0)

Mean with same letter for a given parameter in same horizontal row are not significantly different (P>0.05).

Table 3: Growth, survival, nutrient utilization and economics of *Clarias gariepinus* fingerlings fed varying dietary levels of fern.

Growth	**Diets**					
Parameters	**A (0%)**	**B (5%)**	**C (10%)**	**D (15%)**	**E (20%)**	**F (25%)**
Initial mean weight (g)	0.75±0.0	0.75±0.0	0.75±0.0	0.75±0.0	0.75±0.0	0.75±0.0
Mean weight gain (g)	1.62±0.18^{a}	1.38±0.18^{a}	1.18±0.18^{a}	1.03±0.18^{a}	1.00±0.186^{a}	1.05±0.18^{a}
Mean weight gain (%)	215.41±71.86^{a}	184.16±71.89^{a}	156.66±71.86^{a}	140.91±71.86^{ab}	133.41±71.86	148.08±71.86^{ab}
Initial length (cm)	4.5±0.0	4.5±0.0	4.5±0.0	4.5±0.0	4.5±0.0	4.5±0.0
Increase in length (cm)	2.32±0.16^{a}	1.99±0.16^{a}	2.27±0.16^{a}	1.93±0.16^{ab}	2.00±0.16^{a}	1.47±0.16^{b}
Length increase (%)	51.06±3.58^{a}	44.62±3.58^{a}	49.53±3.59^{a}	42.23±3.58^{a}	43.89±3.58^{a}	33.05±3.58^{a}
Specific growth rate (%day^{-1})	0.92±0.06^{a}	0.88±0.06^{a}	0.94±0.06^{ab}	0.85±0.06^{a}	0.80±0.06^{a}	0.83±0.06^{a}
Condition factor (K)	0.71±0.04^{a}	0.73±0.04^{a}	0.59±0.04^{b}	0.69±0.04^{a}	0.60±0.04^{b}	0.59±0.04^{b}
Survival (%)	48.22±3.56^{a}	50.41±3.56^{b}	63.75±3.56^{ab}	63.75±3.56^{ab}	61.25±3.56^{ab}	72.92±3.56^{bc}
Feed conversion ratio	2.71±0.22^{a}	2.67±0.22^{a}	3.51±0.22^{ab}	3.90±0.22^{ab}	4.04±0.22^{ab}	3.43±0.22^{ab}
Protein efficiency ratio	6.99±0.69^{a}	5.93±0.69^{a}	5.03±0.69^{a}	5.33±0.69^{a}	4.22±0.69^{a}	6.11±0.69^{a}
Economy of weight gain (₦/g. wt. gain)	22.33	16.84	14.02	10.87	8.88	5.77

Means with save letter for a given parameter in same horizontal row are not significantly different (P>0.05); ₦= Nigerian Currency.

4. Discussion

The increase in weight observed with all the experimental diets indicated that they supported the growth of *C. gariepinus* irrespective of the amount of fern included in this study. This was shown in the weight gain, SGR and condition of fish. Feed conversion to flesh and nutrient utilization were fairly the same for diets with low levels of 5 and 10% fern inclusion when compared to the control Diet A without fern. Diet A (0%) showed its superiority in biological performance over other experimental diets. However, Diets B (5%) and C (10%) showed a fair and comparable performance to Diet A. Generally high dietary levels of fern meal were found to be deleterious to the performance of *C. gariepinus.* This could be due to possible presence of toxins as reported for some plants such as *Moringa oleifera* with potentials as a feed ingredient [19]. Increased level of cassava leaf meal which contained cyanogenic glycosides depressed fish growth and feed utilization efficiency [12] or caused nutrient imbalance associated with plant protein source [7]. The evaluation of cost per kilogramme of feed and fish weight gain showed that Diet

F (25% of fern) was more economical to utilize for feeding *C. gariepinus* than other diets in this study. Diet A costs most to produce but fish fed with it had the highest weight gain. The economic advantage to produce Diets B and C in addition to their slightly lower but comparable fish growth performance would probably limit the inclusion of the fern plant, *A. barteri* to *C. gariepinus* diet to a maximum of 10% in this study. The values of water quality parameters observed during this experiment were within acceptable ranges for fresh water fish culture practice [4]. The implication was that the diets did not reduce the quality of water below tolerable levels by *C. gariepinus* fingerlings. The good survival rate of fish observed could be attributed to the water quality and feed used.

5. Conclusion

The plant *Asplenium barteri* is abundant and can easily be harvested for use from the wild. The encouraging growth performance, survival and economic advantage observed showed *A. barteri* to be a potential protein supplement in practical feed for *C. gariepinus* fingerlings to not more than 10% inclusion level for acceptable performance.

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