



Product Quality Assessment of *Clarias gariepinus* Fed on Varying Dietary Levels of *Azadirachta indica* Leaf Meal

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Authors' contributions

This work was carried out in collaboration between all authors. Author JIO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author DCA reviewed the experimental design and all drafts of the manuscript. Authors JIO and UUN managed the analyses of the study. Author JIO identified the plants. Authors JIO and DCA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

This feeding trial was conducted to assess the body composition and product quality of *Clarias gariepinus* fed dietary levels of *Azadirachta Indica* leaf meal (AILM). Four isonitrogenous diets of 36% crude protein were formulated containing 0, 5, 10 and 15% AILM (Diets 1-4). The diet containing 0% leaf meal (Diet 1) served as the control. Each dietary treatment was tested in triplicate group of 15 fingerlings per aquarium. Samples from each treatment were analyzed bi-weekly to evaluate their carcass composition, and data subjected to a one way analysis of variance (ANOVA), while organoleptic assessment was carried out at the end of the experiment to determine the end product quality. The results for carcass composition show that fish fed on diets 3 and 4 (10% and 15% AILM respectively) were significantly ($P<0.05$) higher in crude protein but were significantly ($P<0.05$) poorer in moisture content. Crude fibre was significantly ($P<0.05$) highest for fish fed on diet 4. Fish on all dietary treatments were of good quality as shown in their mean scores

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both of fresh and cooked samples. Fish on diet 1 (0% AILM) dietary treatment were however significantly ($P < 0.05$) superior to fish on other dietary treatments both for fresh and cooked samples. The present study shows that *Azadirachta indica* leaf meal as feed ingredient in the diets of *Clarias gariepinus*, had no deteriorating effect on carcass composition and product quality of the fish.

Keywords: Product quality assessment; *Clarias gariepinus*; *Azadirachta indica* leaf meal.

1. INTRODUCTION

In Nigeria, the high cost of conventional feedstuffs, such as fish meal, soybean meal, groundnut cake meal etc., has resulted to an increased search for alternative feedstuff sourcing, especially of plant origin for example leaf meals to cope with feed and feeding challenges of the aquaculture industry. Alternative protein and energy sources using tropical browse plant leaf meals like *Azadirachta indica* and other terrestrial or fresh water aquatic plant leaf meals due to their easy availability, potentials for nutrients provision and low procurement cost, would seem to provide acceptable feeding regimes for species of fish like *Clarias gariepinus*, *Heterobranchus bidorsalis*, hybrids, other catfishes and farm animals generally [1-3]. Though, despite their content of certain anti-nutritional factors like limonoids, as in *Azadirachta indica* which are major problem to effective use and utilization of leaf meals by fish and other farm animals [4-6], browse plant leaf meals have remained valuable for their provision of proteins, vitamins, minerals etc. [7-9]. Besides, they grow luxuriously in the humid tropics, and much of these plants are underexploited. There is therefore the need to while considering the good potentials of *Azadirachta indica* leaf meal as feed ingredient in the diets of *Clarias gariepinus*, to determine its effect on carcass composition and product quality of the fish.

2. MATERIALS AND METHODS

The leaves of *Azadirachta Indica* used for this study were harvested from farms around Owerri, Imo State of Nigeria. The leaves were sundried and milled using hammer mill to produce the leaf meal. Samples of the leaf meal were subjected to proximate analysis according to AOAC12 (1995) methods. Other ingredients used for the feed were bought at fidelity feed mill in Owerri Imo State. Four isonitrogenous (36% CP) diets were formulated as shown in Table 1, using the trial and error method. The diets had 0%, 5%, 10% and 15% inclusion levels of the leaf meal

respectively. The feed stuffs were finely ground and thoroughly mixed in a plastic bowl using hot water. The mixture was then pelleted by passing it through a mincer of 2 mm diameter to produce 2mm diameter size of the pellets. The pellets were sundried for three days and stored in air tight nylon bags. Proximate compositions of the diets were also determined.

One hundred and eighty experimental fish post fingerlings were acclimatized in a concrete tank (2.5 m x 4 m) on control diet for 7 days. The fish were then completely randomized in 3 replicates of 15 each, and assigned to the 4 treatments – Diet1, Diet 2, Diet 3 and Diet4 at recorded initial weights. The fish were fed at 5% of their body weight twice daily, morning (08.00-09.00) and evening (17.00-18.00). The water in the aquaria was regularly monitored for the physico-chemical properties and renewed completely, every other day within the experimental period of 56 days. Proximate analysis of the test feedstuff (*Azadirachta indica* leaf meal), dietary feeds and biweekly fish samples were carried out to determine the moisture content, ash, lipid, crude protein, crude fibre and nitrogen free extract using the [10] methods; highlighted also in [11]. The organoleptic assessments of both fresh raw and cooked samples of fish were also carried out. Five well trained literate adults selected for their interest and sensorial capabilities of memorizing stimuli or discriminating intensities were used [8]. Thirteen (13) characteristics (skin pigmentation, skin mucus, eye tint, eye shape, gill tint, gill odour, rigidity of flesh, rigidity of abdominal wall, peritoneum, adherence of back bone, colour of surrounding flesh to back bone, odour and flavour) were assessed on 6 independent point assessment score chart [12]. Average score of less than or equal to 3.5 indicated freshness (of good quality), while mean scores above 3.5 indicated poor quality. Data collected were subjected to a one way analysis of variance (ANOVA) as described by Steel and [13]. Test of significance was by Duncan Multiple Range Test at 95 confidence level using Statistical Package for Social Sciences (SPSS) for windows (version 15).

Table 1. Percentage composition of experimental diets

Ingredients	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)
Maize	34.5	30.8	27.1	23.5
Fish meal	20.0	20.0	20.0	20.0
Soya bean meal	35.1	33.8	32.5	31.1
AILM	0.0	5.0	10.0	15.0
Cassava starch	2.0	2.0	2.0	2.0
Palm oil	1.0	1.0	1.0	1.0
Bone meal	1.0	1.0	1.0	1.0
Lysine	2.0	2.0	2.0	2.0
Methioine	2.0	2.0	2.0	2.0
Vitamin premise	0.5	0.5	0.5	0.5
Common slat	0.5	0.5	0.5	0.5
Blood meal	5.0	5.0	5.0	5.0
Total (%)	100.0	100.0	100.0	100.0
Proximate composition				
Crude protein (%)	36.00	36.00	36.00	36.00
Crude fibre (%)	13.00	13.60	14.20	14.72
Ether extract (%)	2.59	3.24	3.89	4.53
Ash (%)	6.98	7.29	7.60	7.90
ME (Kcal/kg)	3220.73	3043.80	2860.65	2660.09

3. RESULTS

The diets used for the feeding trial, the ingredients composition as well as the proximate composition are presented in Table 1. The diets were isonitrogenous (36% CP) The crude fiber, ether extract and ash content of the diets increased with increasing levels of AILM while energy content of the diets decreased with increasing levels of AILM. The proximate composition of the experimental leaf meal (AILM) is presented in Table 2.

Table 2. Chemical composition of *Azadirachta indica* leaf meal

Moisture content (%)	21.50±0.40
Crude protein (%)	20.37±0.30
Ash (%)	9.52 ±0.02
Crude fiber (%)	16.00±0.41
Ether extract (%)	2.48 ±0.02
Nitrogen free extract (%)	30.13±0.71

Crude protein content was found to be 20.37±0.30%. Ash, crude fibre, lipids and moisture contents were 9.52±0.02%, 16.00±0.41%, 2.48±0.02% and 21.95±0.40% respectively. Water temperature, pH and dissolved oxygen condition in the experimental aquaria showed very little variation throughout the feeding trial (Table 3).

Table 3. Water parameters

Treatments	Mean Temp. (°C)	Mean pH	Mean DO (mg/L)
Diet 1 (0%)	26.00±1.63	6.30±0.42	4.50±1.08
Diet 2 (5%)	25.00±2.16	6.40±0.35	4.60±1.13
Diet3 (10%)	26.20±1.65	6.50±0.28	4.20±1.36
Diet4 (15%)	26.00±1.63	6.40±0.35	4.40±1.02

The mortality rate of fish on the different dietary treatments is shown in Table 4. Mortality was as high as 52% in fish fed diets 2 and 3 containing 5% and 10% AILM respectively. Carcass compositions of the dietary treatments are also presented in Table 4.

Moisture content for diet I (control) and diet 2 (5% AILM) were significantly ($p<0.05$) higher than those of diets 3 and 4. Crude protein of fish on dietary treatments diets 3 and 4 (10% AILM and 15% AILM respectively) were significantly ($P<0.05$) higher than those on diets 1 and 2. Lipids was significantly ($P<0.05$) highest in fish on diet 2 while Ash content was significantly ($P<0.05$) superior for fish on diets 1 and 4.

Organoleptic assessment scores for both fresh and cooked fish samples of the treatments are presented in Tables 5 and 6 respectively.

Table 4. Carcass composition of *C. gariepinus* fed dietary levels of *Azadirachta indica* leaf meal

Parameters	*Initial carcass	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)	SEM
Moisture (%)	12.72	10.96 ^a	10.23 ^a	10.17 ^b	9.65 ^b	0.46
Crude protein (%)	60.23	66.12 ^c	66.56 ^b	67.33 ^a	67.86 ^a	0.67
Lipids (%)	6.24	5.24 ^c	7.63 ^a	4.10 ^d	6.43 ^b	1.31
Ash (%)	10.75	12.97 ^a	8.78 ^c	10.97 ^b	12.35 ^a	1.60
Crude fibre (%)	0.70	1.01 ^b	0.79 ^c	1.06 ^b	1.23 ^a	0.15
Nitrogen extract (%)	9.36	3.70 ^b	6.01 ^a	6.40 ^a	2.48 ^b	1.62
*Mortality (%)		14.00	52.00	52.00	43.00	

Means with same superscript are not significantly different. *Initial carcass= pre-experimental carcass composition of the fish sample

Table 5. Organoleptic assessment of fresh fish fed levels of AILM

Treatment	1	2	3	4	5	Means	SEM
Diet 1 (0%)	1.07	1.31	1.38	1.61	1.46	1.37 ^a	0.17
Diet 2 (5%)	1.54	2.00	1.54	1.62	1.62	1.66 ^b	0.17
Diet3 (10%)	1.62	1.92	1.38	1.53	1.38	1.57 ^b	0.19
Diet4 (15%)	1.92	1.77	1.38	1.46	1.23	1.55 ^b	0.28

Means with same superscript are not significantly different

Table 6. Organoleptic assessment of cooked fish fed levels of AILM

Treatment	1	2	3	4	5	Means	SEM
Diet 1 (0%)	2.15	2.30	2.46	2.85	2.77	2.51 ^a	0.26
Diet 2 (5%)	3.15	2.54	2.62	3.08	2.69	2.81 ^b	0.24
Diet3 (10%)	3.00	2.85	2.69	3.15	2.62	2.86 ^b	0.19
Diet4 (15%)	2.85	3.31	2.85	3.23	2.62	2.97 ^b	0.25

Means with same superscript are not significantly different

Fish on all dietary treatments were of good quality as shown in their mean scores both of fresh and cooked samples. Fish on 0%AILM dietary treatment were however significantly ($P<0.05$) superior to fish on other dietary treatments both for fresh and cooked samples.

4. DISCUSSION AND CONCLUSION

Azadirachta indica leaf meal with up to 20.37±0.30% crude protein and appreciable levels of other nutrients appears to be a promising ingredient for fish feed (Table 2). However, the high rate of mortality up to 52% recorded for fish fed diets 2 and 3 was so alarming. *Azadirachta indica* leaves are known to have moderate to good protein content (12-20% DM), moderate NDF and ADF content but a high level of lignin (10-15% DM). The leaves contain high amounts of calcium but low phosphorus, with high amounts of tannins (11%) and saponins (2.5%) and other antinutritional and toxic factors, especially the limonoids (azadirachtin, salanin, nimbin, nimbidiol, etc.), which are triperteroid

compounds that are both bitter and toxic to many animal species [6]. The good attributes of *Azadirachta indica* leaf meal is not in doubt [14,15], but the high mortality rate observed in the study may not be unassociated with the antinutritional and toxic factors inherent in the leaf meal. This would reasonably require further trial on other processing methods of the leaves. Besides, aquarium water may need to be changed daily. The water quality condition recorded throughout the experimental period (Table 3) however was within the optimum condition reported by [12]. Carcass compositions of fish were significantly different across treatments and in all parameters (Table 4). Crude protein interestingly increased with increase in leaf meal inclusion and was significantly higher in fish fed diets 3 and 4. This is an indication that AILM contains essential nutrients capable of producing fish of high crude protein content compared to those fed the control diet. This trend with regard to crude protein content is higher than the performance of fish fed Duck weed [16] and commercial trout feeds [17].

Crude fibre was markedly highest in fish fed diet 3 while moisture content was least in same diet. The high fibre content of fish fed diet 4 could be attributed to the high fibre content of the leaf meal, which is usually the case with most leaf meals. Lipids and ash contents of fish were significantly different across treatments. Lipids content was significantly highest in fish fed diet 2 while the ash content was significantly highest in fish fed diet 1 and 4. The result appears different from that obtained with the use of alfalfa meal, in which there were no significant differences in body moisture and lipids. The level of alfalfa meal in fish diets did not also affect body crude protein and ash content [18]. More so, the result obtained with the use of sweet potato leaf meal in fish diets, showed no significant differences in carcass composition [19]. It appears that *Azadirachta indica* leaf meal achieved better carcass composition and product quality than alfalfa and sweet potato leaf meals respectively. In rabbit does, a long term distribution of diets with 5 to 15% sun-dried *Azadirachta indica* leaves (112 days) significantly modified their blood parameters [20] without altering linear body growth and reproductive tract morphometry [21]. The quality of both fresh and cooked fish was slightly affected by leaf meal inclusion levels (Table 5 and 6). The organoleptic assessment shows significant superiority of fish fed on diet 1 (0%) to those fed other diets both for fresh and cooked fish. However, the mean scores of fish for the treatments were within the acceptable range of 3.5 [22,23] for very good quality fish. This varies slightly from the findings of [8] in which there were no significant differences in the quality of both fresh and cooked fish.

In conclusion, the finding of this study reveals that *Azadirachta indica* leaf meal as feed ingredient in the diets of *Clarias gariepinus*, had no deteriorating effect on carcass composition and product quality of the fish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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