Evaluation of the Nutritional Impact of Graded Oyster Mushroom (*Pleurotus ostreatus*) Meal on the Proximate Composition of Experimental Feed and Carcass Composition of *Clarias gariepinus*

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Abstract
This study evaluates the impact of graded levels of oyster mushroom (Pleurotus ostreatus) meal on the proximate composition of experimental fish feed and the carcass composition of Clarias gariepinus. Five experimental diets were formulated with oyster mushroom meal inclusion levels of 0%, 1%, 2%, 3%, and 4% (T_1, T_2, T_3, T_4, and T_5, respectively). The proximate analysis of the feeds revealed significant improvements in crude protein, ether extract, and ash content with increasing levels of mushroom meal. The highest crude protein content (47.79±0.17%) was observed in T5, while the highest ether extract (14.08±0.08%) and ash content (13.81±0.02%) were found in T_2 and T_5, respectively. Carcass composition analysis of Clarias gariepinus showed significant enhancements in crude protein and ether extract, with the highest crude protein (18.75±0.03%) observed in fish fed the T_5 diet. Moisture content decreased with increasing mushroom meal levels, indicating a higher dry matter content in the fish. The study demonstrates that oyster mushroom meal can be effectively incorporated into fish diets to enhance nutritional quality and suggests that a 4% inclusion level is optimal for maximizing growth and nutritional benefits in Clarias gariepinus.

Introduction

Fish, like all living beings, rely on nutrients for their growth, reproduction, flesh quality and maintenance (Obomunu and Aghoghovwia 2022). When formulating fish diets, factors like nutrient content cost, availability, and palatability to fish are considered (Aghoghovwia and Obomunu, 2022). Feed constitutes a significant portion of operational inputs in fish farming. This days research in animal nutrition focuses on identifying alternative, cost-effective energy sources to replace cereals and provide necessary nutrients in adequate amounts (Adejumo, 2005).

Nutrition is a critical aspect of aquaculture, significantly influencing the health, growth, and productivity of cultured species (Vishnu, 2023)). In Nigeria, as well as globally, proper nutritional strategies are essential for the sustainable development of aquaculture industries (Aghoghovwia and Obomunu, 2022). This exposition highlights the importance of nutrition in aquaculture, drawing on insights from both Nigerian and international research

Oyster mushrooms (*Pleurotus ostreatus*) have emerged as a promising alternative due to their high nutritional value, ease of cultivation, and low cost. Oyster mushrooms are rich in protein, vitamins, minerals, and essential amino acids, making them a suitable option for inclusion in fish diets (Chang and Miles, 2004). Studies have shown that incorporating oyster mushroom meal into animal feed can improve the nutritional profile of the feed, enhance growth performance, and boost immune responses in livestock (Mattila *et al.*, 2002).

Recent research has focused on evaluating the effects of incorporating mushroom meal into fish diets. For instance, investigations into the inclusion of mushroom meal in the diets of various fish species have demonstrated positive outcomes in terms of growth performance, feed utilization, and carcass composition (Mohapatra *et al*., 2014). However, there is limited information on the specific effects of graded levels of oyster mushroom meal on the proximate composition of fish feed and the resulting carcass composition of *Clarias gariepinus*, a commercially important species in aquaculture.

Clarias gariepinus, commonly known as African catfish, is widely farmed due to its fast growth rate, high feed conversion efficiency, and resilience to various environmental conditions (FAO202). Understanding how different inclusion levels of oyster mushroom meal affect the nutritional quality of the feed and the resulting fish carcass composition is crucial for optimizing feed formulations and improving the sustainability of catfish farming.

The present study is carried out to fill the knowledge gap by evaluating the proximate composition of experimental feeds containing graded levels of oyster mushroom meal and assessing their impact on the carcass composition of *Clarias gariepinus* fingerlings. This research provides valuable insights into the potential of oyster mushroom meal as a sustainable and cost-effective ingredient in aquafeed formulations.

MATERIALS AND METHODS

Experimental Site

The research was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Niger Delta University, at the fisheries unit.

Experimental Design/Setup

A Complete Randomized Design (CRD) was used for this study. Fifteen black round plastic bowls, each with a capacity of forty-five (45) litres of water were employed. Each bowl stocked ten *Clarias gariepinus* fingerlings each and feed with five different treatment diets, For a period of 84-days.

Preparation of Ingredients

Corn and soybean were sourced from Amassoma market, while wheat bran was purchased from Dio Agro Limited (Feed House) Akenpai branch, Yenagoa, Bayelsa State. Methionine and lysine were included to meet amino acid requirements. Ingredients were processed using a locally made hammer mill and grinding machine. The milled feed underwent sieving through a 0.2mm sieve to obtain uniformity and remove debris. Five diets were formulated, Oyster mushroom meal was incorporated as an additive at levels of 0%, 1%, 2%, 3%, and 4%, (Table 1) aiming for a crude protein content of approximately 40%, following the recommendation by Madu (2006). (Plate 1)

Plate 1 Experimental Feed (field work)

Plate 2 Experimental fish (field work)

Feeding Procedure for Experimental Fish

The fish were staved for a period of 12-hour before the experiment to ensure empty stomachs and stimulate appetite for the new diet. They were then fed the prepared diet at a rate of 5% biomass (Bello and Nzeh, 2013), twice daily: in the morning at 0800h and 0900h, and in the evening at 1700h and 1900h. Feed quantities were adjusted weekly to match the fish's new biomass. Tank waste and any leftover food were removed daily, with freshwater replenished from a storage tank every day.

Table 1: The gross composition of experimental diets

Ingredients	**T_1 (0%)**	**T_2 (1%)**	**T_3 (2%)**	**T_4 (3%)**	**T_5 (4%)**
Fish Meal *(Oreochromis niloticus)* (71.26% CP)	32	32	32	32	32
Soybean Meal (45.25%CP)	32.9	32.9	32.9	32.9	32.9
Corn Meal (10.85%CP)	21.3	21.3	21.3	21.3	21.3
Vitamin/mineral premix	0.5	0.5	0.5	0.5	0.5
Methionine	0.1	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1	0.1
Palm oil	7.0	7.0	7.0	7.0	7.0
Bone meal	2.0	2.0	2.0	2.0	2.0
Salt	0.1	0.1	0.1	0.1	0.1
Starch	4.0	4.0	4.0	4.0	4.0
Dietary Crude protein (%)	40.00	40.00	40.00	40.00	40.00
Pleurotus ostreatus	0	1	2	3	4

Proximate Analysis

Moisture Content

The moisture content was determined following the (AOAC, 2005) method. Initially, a clean, dry crucible was weighed. Two grams sample were then carefully measured and placed into the crucible. After weighing the samples using a precise balance, they were placed in an oven set at 105°C. Once heated, the crucible was transferred to a desiccator to cool before being reweighed. It was returned to the oven for further drying until a constant weight was achieved, enabling the calculation of moisture content using the provided formula.

MC (\%) = \frac{(W2-W1)-(W1-W3)}{(W2-W1)}MC(%)=

(W2−W1)

(W2−W1)−(W1−W3)

Where:

MC = Moisture content

W1 = Weight of empty crucible

W2 = Weight of crucible with sample

W3 = Weight of crucible with dry sample

Crude Protein Determination

The micro-Kjeldahl method, as described in (AOAC, 2005), was utilized. A small 250ml digestion flask was employed for the digestion process. Approximately two grams sample were added to the flask. Anhydrous sodium sulfate and copper sulfate were then introduced,

followed by 3.5ml of H_2SO_4. The mixture was heated for two hours until a blue-green coloration appeared, indicating completion. Subsequently, the tubes were extracted from the flask and allowed to cool.

In the distillation process, the sample was transferred to the distillation unit, and 15ml of 40% NaOH was added to facilitate the release of ammonia. The generated ammonia was collected in a 100ml conical flask containing 10ml of boric acid and 3-4 drops of methyl red indicator. During titration, the content was titrated against 0.02 NHCl, and the titration reading was recorded for further calculations.

CP (%)=(T-B) × N × 14 ×100 ×6.25/ WS × 1000

Where:

CP=Crude protein

T= Titration reading

B =Blank Titration

N=Normality of HCl

WS=Weight of sample

Lipid Determination

Using a Soxhlet apparatus, a dry round-bottom extraction flask was weighed (AOAC, 2005). Approximately two grams of sample were added to the flask, covered with cotton wool, and subjected to petroleum ether extraction for eight hours. The heat was regulated to ensure a sufficient number of siphoning cycles per hour. The ether residue was evaporated, and the remaining sample was dried in an oven at 105°C. After cooling and weighing, the lipid content was calculated using the provided formula.

$$LC=\frac{W_2-W_1}{W_3}x100$$

Where:

LC = Lipid content

W1 = Weight of extraction flask

W2= Weight of extraction flask with fat

W3= Weight of sample

Ash Content

A meticulously cleaned crucible, pre-weighed, was positioned in a muffle furnace. Two grams of sample were carefully placed onto the crucible, and the furnace was heated to 550°C for a duration of three hours or until achieving a light grey ash. Upon reaching the desired state, the crucible was removed from the furnace and allowed to cool in a desiccator before being reweighed (AOAC, 2005).

Calculation of Ash Content:

$$AC=\frac{W_2-W_1}{W_3}x\ 100$$

Where:

AC = Ash content

W1 = Weight of empty crucible

W2= Weight of crucible with ash sample

W3= Weight of sample

Nitrogen-Free Extract (NFE)

The NFE was determined by difference, calculated by subtracting the sum of percentages of moisture, crude protein, ether extract, crude fiber, and ash from 100, i.e., (100 – (\%M + \%CP + \%EE + \%CF + \%Ash)) (100−(%M+%CP+%EE+%CF+%Ash) (AOAC, 2005).

Statistical Examination

All collected data underwent analysis of variance (ANOVA). The Duncan Multiple Range Test was utilized to identify significant differences among the mean values of the diets at a significance level of 0.05. Statistical computations were performed using the Statistical Package for Social Science (SPSS) version 21.

Results

Proximate Composition of the Experimental Feed with graded Oyster Mushroom (*Pleurotus ostreatus*) Meal

Crude protein content of *P. ostreatus* exhibited an increase from a minimum of 37.14% at T_1 (0% inclusion) to a maximum of 47.79% at T_5 (4% inclusion) as shown in Table 2. Significant differences were observed across all treatments at a 0.05 alpha risk level. Ash content reached its peak at T_2 (1% inclusion), registering a maximum of 13.81%, and subsequently declined to a minimum of 10.10% at T_4 (3% inclusion). Ether extract (fat) content ranged from a minimum of 11.03% at T_1 to a maximum of 14.08% at T_5, with notable discrepancies among treatment means at a significant level of 0.05. Crude fiber content rises at T_3 (2% inclusion) with a maximum of 8.63%, then reduced to a minimum of 7.62% at T_5. Moisture content ranged from a minimum of 8.47% at T_2 to a maximum of 9.37% at T_4. Nitrogen-Free Extract (NFE) varied from a minimum of 9.9% at T_5 to a maximum of 26.27% at T_1. Significant differences were documented among the treatments at ($p<0.05$). Dry matter content ranged from a minimum of 90.08% at T_4 to a maximum of 91.53% at T_2. Calcium content ranged from 0.20% at T_1 to 0.22% at T_3 and T_5, while phosphorus content ranged from 0.32% at T_1 to 0.35% at T_5. Gross energy content varied from 2.68 at T_1 to 2.74 at T_5.

Table 2: Proximate Composition of Experimental Feed graded Oyster Mushroom (*Pleurotus ostreatus*) Meal

Parameter	T_1 (0%)	T_2 (1%)	T_3 (2%)	T_4 (3%)	T_5 (4%)
Crude Protein (%)	37.14 ± 0.04^{e}	40.64 ± 0.06^{d}	43.13 ± 0.07^{c}	45.85 ± 0.06^{b}	47.79 ± 0.17^{a}
Ash content (%)	10.46 ± 0.3^{d}	13.81 ± 0.02^{a}	12.51 ± 0.04^{b}	10.10 ± 0.04^{e}	11.64 ± 0.07^{c}
Ether Extract (%)	11.03 ± 0.06^{e}	12.68 ± 0.04^{c}	12.42 ± 0.04^{d}	13.6 ± 0.07^{b}	14.08 ± 0.08^{a}
Crude Fibre (%)	6.34±0.03e	8.02 ± 0.04^{b}	8.63 ± 0.06^{a}	7.7 ± 0.03^{d}	7.62 ± 0.04^{c}
Moisture (%)	8.76 ± 0.04^{c}	8.47 ± 0.07^{d}	9.02 ± 0.04^{b}	9.37 ± 0.09^{a}	8.97 ± 0.06^{b}
NFE (%)	26.27 ± 0.08^{a}	16.38 ± 0.24^{b}	14.29 ± 0.02^{c}	13.38 ± 0.15^{d}	9.9 ± 0.04^{e}
Dry matter (%)	91.26 ± 0.04^{b}	91.53 ± 0.07^{a}	90.97 ± 0.04^{c}	90.08 ± 0.09^{d}	91.02 ± 0.06^{c}
Calcium (%)	0.20 ± 0.00^{d}	0.21 ± 0.00^{c}	0.22 ± 0.00^{a}	0.21 ± 0.00^{b}	0.22 ± 0.00^{a}
Phosphorus (%)	0.32 ± 0.00^{d}	0.33 ± 0.00^{b}	0.34 ± 0.00^{b}	0.33 ± 0.00^{c}	0.35 ± 0.00^{a}
Gross energy (Kcal/g)	2.68 ± 0.00^{c}	2.70 ± 0.00^{b}	2.69 ± 0.00^{c}	2.73 ± 0.00^{a}	2.74 ± 0.00^{a}

Note: Means with the same letters for a given parameter within the same row are not significantly different ($p<0.05$)

Analysis of fresh *Clarias gariepinus* fingerlings Fed graded Mushroom Meal

The carcass analysis of *Clarias gariepinus* fed graded mushroom meal shown in (Table 3). The parameters measured include Crude Protein, Ether Extract (lipid), Crude Fibre, Ash, Moisture, and NFE (Nitrogen-Free Extract). Each parameter was measured before the experiment and for five different treatments (T_1 to T_5) with graded oyster mushroom meal. The letters (a, b, c) denote significant differences within the same row.

For Crude Protein, the minimum value of 12.64% was observed in T_1, while the maximum value of 18.75% was recorded in T_5, indicating an increase in protein level with the increased level of oyster mushroom. Conversely, for Ether Extract (lipid), the lowest value of 2.01% was found in T_2, while the highest (4.09%) was observed in T_3. Additionally, Crude Fibre value (0.21%) was recorded in T_4, while 0.45% was documented before the experiment. Moreover, Ash content of 1.00% and 2.40% were recorded in T5 and before the experiment, respectively. Moisture values recorded were 75.37% in T5 and 80.29% in T1 before the experiment. Furthermore, NFE was a minimum of 0.23% in T5 and a maximum of 0.64% in T2.

Table 3 Carcass Compositions of Experimental Fish (*Clarias gariepinus*) graded Mushroom meal (*Pleurotus ostreatus*)

Parameters (%)	Before Experiment	Wet sample				
		T_1 (0%)	T_2 (1%)	T_3(2%)	T_4 (3%)	T_5 (4%)
Crude Protein	12.64±0.03^{c}	15.26±0.03^{b}	15.87±0.03^{b}	16.79±0.03^{ab}	17.82±0.03^{a}	18.75±0.03^{a}
Ether Extract (lipid)	3.60±0.16^{ab}	2.01±0.16^{b}	4.09±0.16^{a}	2.52±0.16^{b}	4.00±0.16^{a}	3.00±0.16^{b}
Crude Fibre	0.45±0.01^{a}	0.28±0.01^{b}	0.28±0.01^{b}	0.21±0.01^{b}	0.30±0.01^{ab}	0.35±0.01^{ab}
Ash	2.40±0.12^{a}	2.20±0.12^{a}	2.29±0.12^{a}	2.18±0.12^{a}	1.00±0.12^{b}	2.30±0.12^{a}
Moisture	80.29±0.12^{a}	79.61±0.12^{a}	77.13±0.12^{ab}	76.83±0.12^{ab}	76.49±0.12^{b}	75.37±0.12^{b}
NFE	0.62±0.02^{a}	0.64±0.02^{a}	0.34±0.04^{bc}	0.50±0.02^{b}	0.39±0.04^{bc}	0.23±0.05^{c}

Note: Means with the same alphabets for a given parameter in the same horizontal row are not significantly different ($p < 0.05$)

Discussion

Proximate Composition of the Experimental Feed

The investigation reveals significant alterations in the proximate composition of the experimental feed upon incorporating Oyster Mushroom (*Pleurotus ostreatus*). Notably, the crude protein content increased progressively with higher levels of Oyster Mushroom inclusion, reaching 47.79% with 4% inclusion (T_5). This trend mirrors findings by Aghoghovwia *et al*. (2022), indicating a consistent rise in crude protein percentage with plantain peel supplementation, albeit with marginal increments compared to this study.

Additionally, the ash content initially peaked at 1% inclusion (T_2) before declining at higher levels, suggesting potential optimal ash content levels in feed formulations containing Oyster Mushroom, similar to observations by Khan and Hassain (2020) in studies on mushroom-based feeds. Concerning nutrient evaluation, the ether extract (fat) content exhibited an upward trend with higher levels of Oyster Mushroom inclusion, suggesting a contribution to the feed's fat content. This aligns with findings indicating the presence of lipids in mushrooms (Amporm and Suriyan, 2016). Moreover, the crude fiber content peaked at 2% inclusion (T_3) before slightly decreasing at higher levels, indicating potential saturation effects. Comparable trends in fiber content have been noted in studies on mushroom-based feed formulations (Olude *et al*., 2019). In contrast, the moisture content varied across treatments with no discernible trend, implying that the inclusion of Oyster Mushroom did not consistently affect the feed's moisture content, unlike studies reporting mushrooms' moisture retention properties in feed formulations

(Yusuf *et al*., 2022). Additionally, the nitrogen-free extract (NFE) content decreased as the inclusion level of Oyster Mushroom increased, suggesting a potential trade-off between protein and carbohydrate content in the feed. This underscores the importance of considering nutrient balance in feed formulations incorporating mushrooms (Liu *et al*., 2019).

Carcass analysis of *Clarias gariepinus* fingerlings fed graded mushroom meal

The proximate constituent of *Clarias gariepinus* carcass, particularly concerning varying levels of mushroom meal inclusion in their diet, has garnered attention in aquaculture due to its significance in nutrition and feed formulation (Smith, 2018). In the current study, it was noted that as the inclusion of oyster mushroom increased, so did the carcass yield of *Clarias gariepinus*, a finding supported by Obomunu and Aghoghovwia (2022), who reported similar trends in carcass composition and product quality of *Clarias gariepinus* fingerlings fed different levels of dietary inclusion of blood meal. These parallels extend to other parameters examined in this study, which align favorably with the observations of Lee and Lee (2019) regarding *Tilapia nilotica*, particularly in the increases in crude protein content with specific dietary treatments. Smith *et al*. (2020) also attributed the rise in protein content to the distinct amino acid profiles of the protein sources used, while Johnson *et al*. (2022) suggested that mushroom inclusion might directly influence protein synthesis in *Clarias gariepinus*. Conversely, Smith *et al*. (2020) found that different protein sources led to variations in lipid content, a contrast to this study which focus on the impact of mushroom inclusion levels on crude protein content. These disparate effects observed in various studies could stem from the unique nutritional profiles of the dietary components under investigation. However, mushrooms are rich in specific amino acids that may bolster protein synthesis in fish (Johnson *et al*., 2022), whereas the lipid content of different protein sources can affect lipid deposition in fish tissues (Smith *et al*., 2020). The findings of this study correspond to a general report that diets with high lipid content or low moisture content can reduce the overall moisture percentage in the fish flesh, often resulting in a moisture content slightly lower than that of normal-fed fish (Olaniyi and Salau, 2013). Diets with enhanced protein sources or amino acid supplementation can increase the protein content in the carcass, sometimes exceeding 20%. This improvement in protein content is indicative of better growth performance and muscle development (Olaniyi and Salau, 2013). The protein content of the diet is paramount as it directly affects the growth and muscle development of *Clarias gariepinus*. High-quality protein sources in the diet enhance muscle accretion, leading to a higher protein content in the fish's carcass. For instance, fish meal, a common protein source, has been shown to promote better growth performance and higher carcass protein content compared to plant-based proteins (Adewumi *et al*., 2014). Diets rich in essential amino acids improve nitrogen retention and protein synthesis, leading to a higher proportion of lean muscle mass in the fish (Adewumi *et al*., 2014). Furthermore, factors such as fish species, feeding regimen, and environmental conditions may also contribute to differences in carcass composition across studies (Lee and Lee, 2019).

Conclusion

In every increase in oyster mushroom meal, increases the crude protein level of the diet and the carcass of the fish fed with the diet which underscore the important of oyster mushroom meal in the diet and product qualities of *Clarias gariepinus* fingerlings, so therefore it is advisable that oyster mushroom should be included in the diet of *Clarias gariepinus* fingerlings.

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