DIGESTIBILITY AND PROXIMATE COMPOSITION OF *OREOCHROMIS NILOTICUS* FED DIETS CONTAINING PROCESSED *LEMNA PAUCICOSTATA*

Supported by tetfund
TERTIARY EDUCATION TRUST FUND

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Received: May 18, 2023 Accepted: July 10, 2023

Abstract:

This study evaluated the digestibility of processed duckweed based diets and its effect on proximate composition of *Oreochromis niloticus.* Blanching and sun-drying were employed as the processing methods to reduce antinutrients in the duckweed meal. The experiment was conducted in two outdoor concrete ponds with an area of 5m x 3.5m (l × b) and a depth of 1.5m each, using 27 Hapa nets measuring 1m^2 each. Nine iso-proteinous diets (D1-D9) were formulated using least-cost feed formulation software. Soybean meal was replaced by blanched and sun-dried duckweed meal at 25%, 50%, 75%, and 100% each. A total of 10 fingerlings of *O. niloticus* (7.46 ± 0.06g) were stocked per Hapa and fed three times a day at 5% biomass for 24 weeks. Indirect method using Chromic oxide was used to determine the digestibility while the fish carcass were analyzed following the standard method of Association of Official Analytical Chemistry. Highest apparent protein digestibility coefficient of 92.94% was recorded in the diet containing 75% blanched duckweed meal (D_4) while the least value of 86.86% was obtained in the diet with 100% blanched duckweed meal (D_5). The fish fed 75% blanched duckweed meal (D_4) gave significantly highest ($P \leq 0.05$) carcass protein of 60.80% followed by D_6 (25% sun-dried duckweed meal) and D_3 (50% blanched duckweed meal) which had similar values of 60.07% and 60.04%, respectively while significantly least value of 44.73% was recorded in the initial carcass protein. The dietary digestibility coefficients obtained in this study suggest that all the blanched and sun-dried duckweed meal can be used to replace soybean meal in *Oreochromis niloticus* diet without any reduction in protein digestibility and protein content of *O. niloticus.*

Keywords: Digestibility, proximate composition, duckweed, *Lemna paucicostata, Oreochromis niloticus,* Nile tilapia

Introduction

Oreochromis niloticus is commonly known as Nile tilapia, is the most preferred cultured fish in Nigeria but is the second most important cultured fish in the world after common carp (*Cyprinus carpio*) (Erick *et al*., 2014). The popularity of Nile tilapia is due to its fast growth rate, market acceptability, resistance to disease and ability to grow on a wide range of diets. It is also very tolerant to a wide range of environmental conditions, can reproduce in captivity and has a high prolific rate and good carcass taste (Francis *et al*., 2020).

Lemna paucicostata is commonly referred to as duckweed, is a monocotyledon, belonging to the Lemnaceae family, which consists of five genera (*Spirodela, Landoltia, Lemna, Wolffia, and Wolffiella*) and 37 species (Gui-Li *et al*., 2021). Duckweed (*Lemna paucicostata*) is a small, free-floating aquatic plant that grows well in static and nutrient-rich freshwater or a brackish aquatic medium (Abdullahi *et al*., 2023) The biomass of duckweed also doubles in 2 to 3 days under ideal conditions of nutrient availability, pH (6.5-7.5), sunlight and temperature (20°C to 30°C) (Christine et al., 2018). The plant is very rich in nutrients. The nutrient composition in each duckweed species varies depending on the condition of the water environment (Abdullahi *et al*., 2023). Recently duckweed has been accepted as protein-rich (25-45% of the dry weight) feed for fish, and it contains high protein content, about 400 g/kg, closely resembles soybean meal and it also has balanced amino acid profile, particularly lysine (6.9 g/100 g protein) which is a limiting amino acid in other plant proteins (Aslam and Zuberi, 2017). Compared with other plants such as soybean, duckweed leaves contain lower fibre (5% in dry matter) and are highly digestible. It can also easily grow abundantly with minimum cost and can be made available as much cheaper feed than other alternative plant protein sources (Alemayehu and Misganaw, 2021). Therefore, this study aimed to evaluated the digestibility of processed duckweed based diets and its effect on proximate composition of *Oreochromis niloticus.*

Materials and Methods

Collection, Culture and Preparation of Duckweed Meal

Fresh duckweed was collected during raining season from a burrow pit at Hanwa Low-cost, Kwangila, Kaduna State, with a hand net and transported in nylon bags. The fresh duckweed was cultured for two months in concrete ponds of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Ahmadu Bello University, Zaria. The cultured duckweed was used for the experiment. Blanching and sun-drying methods were employed to process the cultured duckweed samples.

Blanching of Duckweed Meal

Blanching was done by boiling duckweed samples in water for 5 minutes at 100°C as described by Akpodiete and Okagbare (1999).

Sun-drying of Duckweed Meal

The second treatment involved sun drying duckweed meal under hygienic conditions for three (3) days as described by Abdullahi *et al*, (2023).

Feed Formulation

Nine iso-proteinous diets (D1-D9) were formulated using least cost feed formulation software (Feed Solution Software version 2022) which took into consideration the cost and the nutritive value of each ingredients. Soybean meal which serves as the control in the diets was replaced by blanched duckweed meal and sun-dried duckweed meal at 25%, 50%, 75% and 100% each. All the feed ingredients were integrated into computing, at the required quantities to make up a 100-unit quantity of the feed (Table 1).

Feed Preparation

Feed was prepared by milling the grain ingredients separately, sieving, mixing all the ingredients, the addition of palm oil before adding water and mixing to form a dough. The mixture was pelleted using a 2mm diameter hand pelletizer. The pellets were sun-dried and packaged in a waterproof airtight container.

Experimental Design

The experiment was conducted outdoors, in concrete ponds of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Ahmadu Bello University, Zaria. A Completely Randomized Design (CRD) was employed in this research to avoid bias in the experimental set-up. The experiment consisted of one control (D1) and eight treatments (D2, D3, D4, D5, D6, D7, D8, D9) with three replications each. A group of 270 fingerlings of *Oreochromis niloticus* was acclimatized for 14 days. After the acclimatization, 10 fish were randomly assigned to a $1m^2$ Hapa net and nine formulated diets were fed to the experimental fish.

Results and Discussion

Digestibility Coefficients of Processed Duckweed Meal in the Diet of Oreochromis niloticus

The apparent digestibility coefficients of blanched and sun-dried *Lemna paucicostata* as a replacement for soybean meal in the diets of *Oreochromis niloticus* and faecal composition are presented in Table 2 and Table 3. There was significant difference ($P \leq 0.05$) in the apparent protein digestibility coefficient among all the treatments and the control. Highest apparent protein digestibility coefficient of 92.94% was recorded in the diet containing 75% blanched duckweed meal (D_4) while the least value of 86.86% was obtained in the diet with 100% blanched duckweed meal (D_5)

In this study, good values were obtained in all the experimental diets for the apparent digestibility coefficients. Variations in the quantity and quality of dietary nutrients influence the apparent digestibility in fish (Montoya-Mejía *et al.*, 2017). However, the apparent digestibility of nutrients and energy differs from one fish species to another and even within an individual fish depending on sex, age, species, diet composition and water temperature (NRC, 2011). Apparent Protein Digestibility (APD) is a key factor in the evaluation of the quality of a diet for fish and the potential of the diet to synthesize new tissues. All the experimental diets showed a high APD (>86.86%). A high apparent protein digestibility in Nile tilapia fed feed ingredients of varying origin has also been reported by Maina *et al.* (2002); Köprücü and Özdemir (2005). The range of the APD (86.86% - 92.94%) obtained in this study is higher than the range of APD (75.90% - 79.00%) and 46.30% - 92.40%) in *Oreochromis niloticus* reported by El-shafai *et al.* (2004) and Francis *et al.* (2020), respectively. The processed duckweed meal used in this study had a higher protein content and a lower fibre content than previously reported by El-shafai *et al.* (2004) and Francis *et al.* (2020), which might explain the higher apparent protein digestibility obtained in this study. The protein content of duckweed could vary widely depending on plant age, nutrient content of the aqueous environment and water temperature. In all the experimental diets, apparent ash digestibility (AAD) was in the range of 34.48-52.29%, which is lower than the range of 38.00-62.90% reported for Nile tilapia fishmeal-based diets, which included 20% and 40% of dry or fresh duckweed (El-shafai *et al.*, 2004). The lower values in this study could be attributed to the higher percentages of plant ingredients in the dietary treatments. The apparent lipid digestibility (ALD) showed a large variation among the experimental diets and it was below the range of values reported for apparent lipid digestibility coefficients of the treatment diets for *Oreochromis niloticus* by El-shafai *et al.* (2004). The variation could be explained by different lipid contents in the experimental diets used in this study and that of the previous authors. The highest apparent carbohydrate digestibility (ACD) value of 80.56% obtained in the treatment D_4 (75% duckweed meal) among all the treatments and the control diet could be attributed to the high amylase activity observed in the treatment (Abdullahi *et al*., 2023).

Proximate Composition of O. niloticus Fed Experimental Diets

The carcass proximate composition of *Oreochromis niloticus* before and after feeding trails is presented in Table 4. The fish fed 75% blanched duckweed meal (D_4) gave significantly highest ($P \leq 0.05$) carcass protein of 60.80% followed by D_6 (25% sun-dried duckweed meal) and D_3 (50% blanched duckweed meal) which had similar values of 60.07% and 60.04%, respectively while significantly least value of 44.73% was recorded in the initial carcass protein. The carcass composition of the experimental fish showed an increase in all the nutrients in the body of the fish when compared to the initial carcass composition. This reveals that the experimental fish *Oreochromis niloticus* was able to utilize all the diets however the difference in the values for carcass crude protein and ether extracts confirms the fact that there was a different level of utilization of the experimental diets. The fish carcass had more protein retained in the body at the end of the experiment. This suggests that the protein-to-energy ratio used in the feed was at an accurate level, hence there was no sparing of protein for energy. The carcass crude protein in this experiment increased significantly after the feeding trial. The mean initial crude protein (44.73%) was significantly lower than the values obtained after the feeding trial. The high value of 60.80% was recorded in fish fed D_4 (75% blanched duckweed meal). The high-value crude protein obtained in this study is similar to the high crude protein value (60.00%) of *Oreochromis niloticus* fed isocaloric diets containing animal and plant by-products reported by Montoya-Mejía *et al*. (2017). Ash ranged from 9.77% to 11.57% and moisture ranged from 6.00% to 10.35%. Soluble carbohydrates (nitrogen-free extract) differ significantly when compared with the initial carcass composition which ranged from 4.02% to 13.66%. Moisture content, ether extract and nitrogen-free extract are significantly higher in the initial carcass composition than after feeding with the experimental diets, while crude protein and ash content are significantly higher after the feeding trial.

Conclusion

The fish fed diet containing 75% inclusion level of blanched duckweed meal gave the best protein, lipid, ash and carbohydrate digestibility of 92.94%, 90.62%, 52.29% and 80.56%, respectively and also gave the best carcass protein of 60.80%. The dietary apparent digestibility coefficients obtained in this study suggest that all the blanched and sun-dried duckweed meal can be used to replace soybean meal in Nile tilapia diet without any reduction in protein digestibility and protein content of Nile tilapia.

Table 1: Feed formulation of the different experimental diets

Ingredients	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9
Soybean meal	23.74	17.81	11.87	5.93	0.00	17.72	11.81	5.91	0.00
BDM	0.00	5.93	11.87	17.81	23.74	-	-	-	-
SDM	-	-	-	-	-	5.91	11.81	17.72	23.62
Fish meal	11.87	11.87	11.87	11.87	11.87	11.87	11.87	11.87	11.81
Groundnut cake	35.61	35.61	35.61	35.61	35.61	35.61	35.61	35.61	35.61
Maize	9.39	9.39	9.39	9.39	9.39	9.39	9.39	9.39	9.39
Wheat bran	9.39	9.39	9.39	9.39	9.39	9.39	9.39	9.39	9.39
Palm oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pre-mix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Klinofeed	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition of diets (% DM basis)									
Crude protein	38.02	35.54	35.49	36.33	37.98	35.56	37.55	38.00	36.35
Ash	14.95	15.81	16.32	15.92	16.81	15.34	15.95	16.40	16.86
Moisture	11.45	10.12	10.50	10.46	11.52	10.54	9.36	10.03	9.21
Ether extract	12.59	10.06	11.54	10.83	10.49	10.11	11.55	10.53	10.02
Crude fibre	6.98	7.01	7.04	6.96	6.85	7.05	6.94	6.88	6.89
NFE	16.01	21.46	19.11	19.50	16.35	21.40	18.65	18.16	20.67

Legend-
D1 – 100% SBM (Control diet)
D2 - 75% SBM, 25% BDM D6 - 75% SBM, 25% SDM
D3 - 50% SBM, and BDM D7 - 50% SBM, and SDM
D4 - 25% SBM, 75% BDM D8 - 25% SBM, 75% SDM
D5 - 100% BDM D9 - 100% SLP
SBM – Soybean meal, BDM- Blanched duckweed meal, SDM– Sun-dried duckweed meal, NFE – Nitrogen free extract

Table 2: Apparent digestibility coefficients of the experimental diets for *Oreochromis niloticus*

Treatments	APD	ALD	ACD	AAD
D_1	$88.38{\pm}2.97^c$	$83.06{\pm}3.53^b$	$63.11{\pm}4.17^e$	$34.10{\pm}3.03^e$
D_2	$88.22{\pm}2.97^c$	$80.20{\pm}3.53^b$	$66.36{\pm}4.17^d$	$43.24{\pm}3.03^c$
D_3	$91.73{\pm}2.97^b$	$88.37{\pm}3.53^b$	$78.48{\pm}4.17^b$	$50.32{\pm}3.03^a$
D_4	$92.94{\pm}2.97^a$	$90.62{\pm}3.53^a$	$80.56{\pm}4.17^a$	$52.29{\pm}3.03^a$
D_5	$86.86{\pm}2.97^d$	$78.16{\pm}3.53^b$	$60.57{\pm}4.17^d$	$34.48{\pm}3.03^e$
D_6	$90.75{\pm}2.97^b$	$83.23{\pm}3.53^b$	$70.79{\pm}4.17^c$	$47.00{\pm}3.03^b$
D_7	$90.44{\pm}2.97^b$	$84.06{\pm}3.53^b$	$70.35{\pm}4.17^c$	$46.01{\pm}3.03^b$
D_8	$89.04{\pm}2.97^c$	$81.37{\pm}3.53^b$	$66.19{\pm}4.17^d$	$45.47{\pm}3.03^b$
D_9	$87.32{\pm}2.97^c$	$78.41{\pm}3.53^c$	$60.07{\pm}4.17^e$	$39.79{\pm}3.03^d$

Means with the same superscript along the same column were not significantly different (P>0.05)
Legend-
APD-Apparent protein digestibility, ALD-Apparent lipid digestibility, ACD-Apparent carbohydrate digestibility, AAD-Apparent ash digestibility.
D1 – 100% SBM (Control diet)
D2 - 75% SBM, 25% BDM D6 - 75% SBM, 25% SDM
D3 - 50% SBM, and BDM D7 - 50% SBM, and SDM
D4 - 25% SBM, 75% BDM D8 - 25% SBM, 75% SDM
D5 - 100% BDM D9 - 100% SLP
SBM – Soybean meal, BDM- Blanched duckweed meal, SDM– Sun-dried duckweed meal

Table 3: Faecal proximate composition (g/100g) of *Oreochromis niloticus* fed experimental diets

Treatments	Moisture	Ash	Ether extract	Crude protein	Crude fibre	Nitrogen free extract
D_1	54.33 ± 1.02^{ab}	9.74 ± 0.33^{ab}	2.11 ± 0.27^{a}	4.37 ± 0.38^{ab}	3.62 ± 0.27^{a}	25.83 ± 1.47^{cd}
D_2	46.00 ± 1.02^{c}	8.92 ± 0.33^{bc}	1.98 ± 0.27^{a}	4.16 ± 0.38^{ab}	3.45 ± 0.27^{a}	35.49 ± 1.47^{a}
D_3	51.03 ± 1.02^{b}	7.98 ± 0.33^{cd}	1.32 ± 0.27^{ab}	2.89 ± 0.38^{c}	3.21 ± 0.27^{ab}	33.57 ± 1.47^{ab}
D_4	51.12 ± 1.02^{b}	7.55 ± 0.33^{d}	1.01 ± 0.27^{b}	2.55 ± 0.38^{c}	2.77 ± 0.27^{b}	35.00 ± 1.47^{a}
D_5	55.29 ± 1.02^{a}	10.53 ± 0.33^{a}	2.19 ± 0.27^{a}	4.77 ± 0.38^{a}	2.12 ± 0.27^{c}	25.10 ± 1.47^{d}
D_6	47.50 ± 1.02^{c}	8.01 ± 0.33^{cd}	1.67 ± 0.27^{ab}	3.24 ± 0.38^{bc}	3.57 ± 0.27^{a}	36.01 ± 1.47^{a}
D_7	51.15 ± 1.02^{b}	8.56 ± 0.33^{cd}	1.83 ± 0.27^{ab}	3.57 ± 0.38^{b}	3.31 ± 0.27^{ab}	31.58 ± 1.47^{ab}
D_8	52.11 ± 1.02^{ab}	8.89 ± 0.33^{bc}	1.95 ± 0.27^{a}	4.14 ± 0.38^{ab}	2.91 ± 0.27^{b}	30.00 ± 1.47^{bc}
D_9	47.84 ± 1.02^{c}	10.09 ± 0.33^{a}	2.15 ± 0.27^{a}	4.58 ± 0.38^{a}	2.45 ± 0.27^{bc}	32.89 ± 1.47^{ab}

Means with the same superscript along the same column were not significantly different (P>0.05)

Legend-

D1 – 100% SBM (Control diet)

D2 - 75% SBM, 25% BDM   D6 - 75% SBM, 25% SDM

D3 - 50% SBM, and BDM   D7 - 50% SBM, and SDM

D4 - 25% SBM, 75% BDM   D8 - 25% SBM, 75% SDM

D5 - 100% BDM   D9 - 100% SLP

SBM – Soybean meal, BDM- Blanched duckweed meal, SDM– Sun-dried duckweed meal

Table 4: Carcass proximate composition of *Oreochromis niloticus* fed experimental diets (g/100g DM)

Treatments	Moisture	Ash	Crude protein	Ether extract	Nitrogen free extract
Initial	10.35 ± 0.60^{a}	10.21 ± 0.69^{b}	44.73 ± 1.26^{d}	21.05 ± 0.74^{a}	13.66 ± 0.73^{a}
D_1	7.11 ± 0.60^{d}	11.13 ± 0.69^{a}	58.02 ± 1.26^{c}	19.14 ± 0.74^{c}	4.60 ± 0.73^{b}
D_2	6.41 ± 0.60^{e}	10.40 ± 0.69^{b}	58.62 ± 1.26^{c}	20.44 ± 0.74^{b}	4.13 ± 0.73^{b}
D_3	6.77 ± 0.60^{e}	11.54 ± 0.69^{a}	60.04 ± 1.26^{b}	18.11 ± 0.74^{d}	3.54 ± 0.73^{b}
D_4	7.04 ± 0.60^{d}	11.55 ± 0.69^{a}	60.80 ± 1.26^{a}	17.23 ± 0.74^{e}	3.38 ± 0.73^{b}
D_5	8.25 ± 0.60^{c}	11.22 ± 0.69^{a}	57.51 ± 1.26^{c}	19.00 ± 0.74^{c}	4.02 ± 0.73^{b}
D_6	6.00 ± 0.60^{e}	9.77 ± 0.69^{c}	60.07 ± 1.26^{b}	19.57 ± 0.74^{c}	4.59 ± 0.73^{b}
D_7	8.71 ± 0.60^{b}	10.33 ± 0.69^{a}	59.47 ± 1.26^{c}	17.25 ± 0.74^{e}	4.24 ± 0.73^{b}
D_8	7.99 ± 0.60^{d}	11.15 ± 0.69^{a}	58.67 ± 1.26^{c}	18.17 ± 0.74^{d}	4.02 ± 0.73^{b}
D_9	6.23 ± 0.60^{e}	11.57 ± 0.69^{a}	57.08 ± 1.26^{c}	20.54 ± 0.74^{ab}	4.58 ± 0.73^{b}

Means with the same superscript along the same column are not significantly different (P>0.05)

Legend-

D1 – 100% SBM (Control diet)

D2 - 75% SBM, 25% BDM   D6 - 75% SBM, 25% SDM

D3 - 50% SBM, and BDM   D7 - 50% SBM, and SDM

D4 - 25% SBM, 75% BDM   D8 - 25% SBM, 75% SDM

D5 - 100% BDM   D9 - 100% SLP

SBM – Soybean meal, BDM- Blanched duckweed meal, SDM– Sun-dried duckweed meal

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