

Blood Chemistry and Enzyme Activity of *Oreochromis niloticus* (Linnaeus, 1758) Fed Dietary Processed *Lemna paucicostata* (Hegelm) as a Replacement for Soybean Meal

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Abstract

This study evaluated the effect of replacing *Glycine max* with processed *Lemna paucicostata* on haematological parameters and enzyme activity of *Oreochromis niloticus*. The processing methods employed were blanching and sun-drying. Nine diets (D1-D9) were formulated using the least cost feed formulation software. Soybean meal was replaced by blanched and sun-dried *L. paucicostata* at 25%, 50%, 75% and 100% each. 27 nets measuring 1m² each were used in outdoor concrete ponds of 5m x 3.5m (l x b) and depth of 1.5m each. Ten fingerlings of *O. niloticus* were stocked per Hapa and fed three times daily at 5% body weight. The fish fed 75% blanched *L. paucicostata* obtained the best concentration of the packed cell volume (32.01%) in the blood while a poor concentration of 27.01% was observed in the fish fed 100% blanched *L. paucicostata*. The fish fed 75% blanched *L. paucicostata* and 50% blanched *L. paucicostata* gave higher total enzyme activities of 4,675.13(TU) and 4,648.06(TU), respectively. While the fish fed 100% blanched *L. paucicostata* gave the lowest value of 3,558.33(TU). The digestive enzyme activities were observed to be the main factors that helped the experimental fish to digest the blanched and sun-dried *Lemna paucicostata* efficiently.

Introduction

Duckweed (*Lemna paucicostata*) is a small, fragile, free-floating aquatic plant that grows well in static and nutrient-rich freshwater or a brackish aquatic environment. The biomass of duckweed also doubles in 2 to 3 days under ideal conditions of nutrient availability, sunlight, pH (6.5-7.5), and temperature (20°C to 30°C) (Christine *et al.*, 2018). There are about 40 duckweed plant species worldwide the major ones are of the four genera; *Lemna*, *Spirodela*, *Wolfilla*, and *Wolffiella* (Dorothy *et al.*, 2018). The plant is very rich in nutrients. Different authors reported varying amounts of nutrients in duckweed (Mohapatra and Patra 2013; Dorothy *et al.*,

2018). The plant is rich in both macro and micro-elements such as calcium and chlorine. Generally, duckweed contains 6.8 to 45% crude protein, 1.8 to 9.2% crude lipid, 5.7 to 16.2% crude fibre, 12 to 27.6% ash, and the carbohydrate content is in the range of 14.1-43.6% on a dry matter basis (Christine *et al.*, 2018). The nutrient composition in each duckweed species varies depending on the condition of the water environment. Duckweed is suitable for animal consumption and is rich in invaluable nutrients (Mwale and Gwaze, 2013). Fresh duckweed has been successfully used as feedstuffs for common carp, silver carp and tilapia (Dorothy *et al.*, 2018).

Fish haematology is gaining increasing importance in fish culture because of its importance in ascertaining the health status of the fish (Hrubec *et al.*, 2000). Haematological characteristics of most fish have been studied to establish a normal value range for an excellent physiological process. Environmental and physiological factors are known to influence fish haematology, these include stress due to capturing, transportation, high stocking density and sampling. Cells naturally contain enzymes for their functions such that damage to cellular membrane lead to their escape into the blood where their presence or activities can be measured as an index of cell integrity (Coppo *et al.*, 2002).

The inclusion of plant protein sources in the ration of fish requires investigation of proper processing for effective utilization (Francis *et al.*, 2001). The presence of certain limiting factors in plant ingredients such as antinutritional factors has been demonstrated (Alegbeleye *et al.*, 2001; Nwana *et al.*, 2008), although processing, such as sun-drying and blanching can reduce the anti-nutritional content in the feed ingredients. Hence the present study is aimed to determine changes that occur in the blood parameters, like haemoglobin concentration, red blood cells, mean corpuscular haemoglobin, packed cell volume and enzyme activities such as protease, lipase amylase, trypsin and chymotrypsin of Nile tilapia (*Oreochromis niloticus*) fed processed duckweed (*Lemna paucicostata*) as a replacement for soybean meal (*Glycine max*) in the diet.

Materials and Methods

Experimental Site

The experiment was conducted outdoors, in concrete ponds of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria which is within latitude 11° 17' North and longitude 7° 63' East in the northern guinea savannah zone of Nigeria.

Procurement of Experimental Fish

Three hundred Nile tilapia (*Oreochromis niloticus*) were procured from Kuka Farm, Gabasawa, Kano State, Nigeria and transported in an air-tied oxygenated nylon polythene bag placed in 50 litres of "Jerry-cans".

Collection, Culture and Preparation of Duckweed (*Lemna paucicostata*)

Fresh duckweed (*Lemna paucicostata*) was collected during raining season from a burrow pit at Hanwa Low-cost, Kwangila, Zaria, Kaduna State, with the help of a hand net and transported in nylon bags. The fresh *Lemna paucicostata* was cultured in concrete ponds at Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. The cultured *Lemna paucicostata* was used for the experiment. Blanching

Table 1. Proximate Composition of Ingredients used in Feed Formulation

Ingredients	Moisture%	CP%	Ash%	CF%	EE%	NFE%
Maize	9.16	8.75	3.19	2.4	2.4	74.1
Wheat bran	11.0	14.8	6.4	10.0	4.0	53.8
Blanched <i>L. paucicostata</i>	8.52	37.13	21.90	3.34	6.07	23.04
Sun-dried <i>L. paucicostata</i>	9.10	36.75	20.48	3.62	5.9	24.15
Soybean meal	10.0	38.0	18.0	5.0	6.6	22.4
Fishmeal	10.2	60.0	14.7	0.8	7.8	6.5

CP – Crude protein; EE – Ether Extract; CF – Crude fibre; NFE – Nitrogen-free extract

Table 2. Ingredients Composition of formulated diets (% Dry weight)

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
Blanched <i>L. paucicostata</i>	0.00	5.93	11.87	17.81	23.74	-	-	-	-
Sun-dried <i>L. paucicostata</i>	-	-	-	-	-	5.91	11.81	17.72	23.62
Fish meal	11.87	11.87	11.87	11.87	11.87	11.81	11.81	11.81	11.81
Groundnut cake	35.61	35.61	35.61	35.61	35.61	35.43	35.43	35.43	35.43
Maize	9.39	9.39	9.39	9.39	9.39	9.57	9.57	9.57	9.57
Wheat bran	9.39	9.39	9.39	9.39	9.39	9.57	9.57	9.57	9.57
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	2.0
Klinofeed	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

D₁–100% SBM (Control diet); D₂-75% SBM, 25% BLP; D₃-50% SBM and BLP; D₄ - 25% SBM, 75% BLP; D₅ - 100% BLP

D₆ - 75% SBM, 25% SLP; D₇ - 50% SBM, and SLP; D₈ - 25% SBM, 75% SLP; D₉ - 100% SLP

SBM – Soybean meal, BLP- Blanched *L. paucicostata*, SLP– Sun-dried *L. paucicostata*

and sun-drying methods were used to process the cultured *Lemna paucicostata* samples.

Blanching of *Lemna paucicostata*

The cultured *Lemna paucicostata* was boiled in water for 5 minutes at 100°C. This process is referred to as blanching following the methods of Sogbesan and Ugwumba (2008). The blanched *L. paucicostata* were milled into a fine powder and sieved through a 0.5 mm mesh screen.

Sun-drying of *Lemna paucicostata*

The other treatment was formed by sun drying the cultured *Lemna paucicostata* under hygienic conditions for three (3) days. After which the sun-dried *L. paucicostata* were then milled into a fine powder and then sieved through a 0.5 mm mesh screen.

Determination of Proximate Composition

The proximate composition (moisture, crude protein, crude lipid crude fibre, ash and nitrogen-free extracts) of the raw and processed *L. paucicostata*, soybean meal and experimental diets were determined using the methods of the Association of Official Analytical Chemists (A.O.A.C., 2019). All chemical analyses were triplicated.

Feed Composition

The test ingredient (*L. paucicostata*) served as one of the main sources of protein in the fish feed, this was used to substitute the soybean meal at different levels of inclusion. Fish meal and groundnut cake were also used as supplementary protein sources (Table 1). The energy sources for formulating the *Lemna paucicostata*-based feed included maize (*Zea mays* 8.75% CP) obtained locally from the Sabon Gari market in Zaria. This ingredient was milled using a hammer mill grinder. Wheat bran (14.8% CP) was also used to supplement the energy base. The proximate analysis of the ingredients was carried out and the composition is shown in Table 1. The fixed ingredients consisted of Klinofeed (binder) obtained from the market and used as the binder for the ingredients for easy pelleting. Other ingredients included vitamins and minerals pre-mix, palm oil, and table salt. DL-Methionine and L-Lysine were also used to balance the amino acid contents of the feed.

Feed Formulation

Nine diets (D1-D9) were formulated using least cost feed formulation software (Feed Solution Software) at 35% crude protein which took into consideration the cost and the nutritive content of the ingredients. The soybean meal which serves as the control in the diets

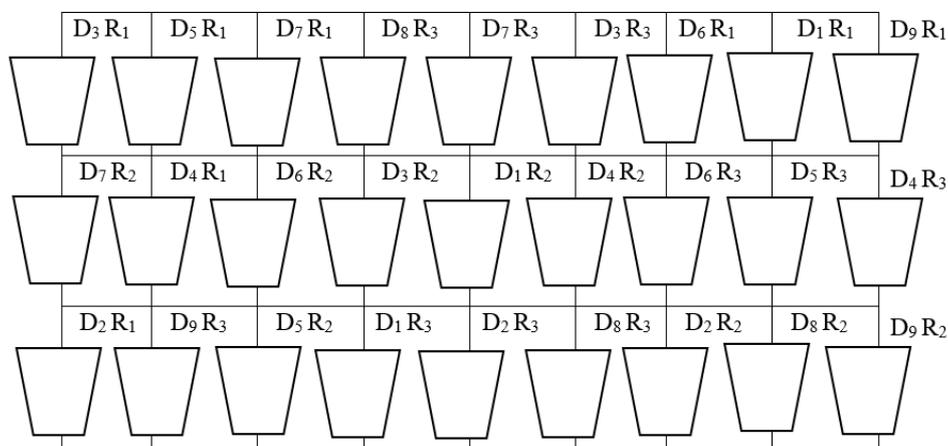


Figure 1. Experimental set-up in a completely randomized design

Table 3: Proximate composition of raw, blanched and sun-dried *Lemna paucicostata*

Parameters (g/100g)	Treatments			P values
	RLP	BLP	SLP	
Moisture	10.23±0.63 ^a	8.52±0.63 ^a	9.10±0.63 ^a	0.23
Crude protein	35.08±0.62 ^a	37.13±0.62 ^a	36.75±0.62 ^a	0.12
Crude fibre	4.72±0.62 ^a	3.34±0.62 ^a	3.62±0.62 ^a	0.32
Ether extract	6.20±0.59 ^a	6.07±0.59 ^a	5.90±0.59 ^a	0.93
Ash	18.18±0.60 ^b	21.90±0.60 ^a	20.48±0.60 ^a	0.01
Nitrogen free extract	25.60±0.63 ^a	23.04±0.62 ^a	24.15±0.62 ^a	0.07

Means with the same superscript within the same row were not significantly different (P>0.05)
 RLP - Raw *Lemna paucicostata*; BLP - Blanched *Lemna paucicostata*; SLP - Sun-dried *Lemna paucicostata*

was replaced by blanched *Lemna paucicostata* and sun-dried *Lemna paucicostata* 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 2).

Feed Preparation

Feed preparation involved milling the grain ingredients separately, sieving, mixing all dry 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 airtied container.

Experimental Design

A completely randomized design was employed in this research (Figure 1). The experiment consisted of eight treatments (D2, D3, D4, D5, D6, D7, D8, D9) and one control (D1) with three replications each. A group of 270 fingerlings of *Oreochromis niloticus* (6.48g-8.48g) was acclimatized for 14 days and fed with Coppens (an imported feed from the Netherlands). After the period of acclimatization, 10 fish were randomly assigned to a 1m² Hapa net (Massgold). A total of 27 Hapa nets were used in outdoor concrete ponds of 5m x 3.5m (l x b) and depth of 1.5m each and nine formulated diets (Table 2) were fed to the experimental fish.

Feeding of Experimental Fish

The experimental fish were starved for 12 hours before the onset of the experiment, to empty their gut and, increase their appetite and reception to the new diet. They were thereafter fed with the assigned test diets at 5% biomass. Feeding was done three times daily morning (08:00am - 09:00am), afternoon (1:00pm - 2:00pm) and evening (5:00pm – 6.00pm). The feed quantity was adjusted after new biomass was

obtained. The pond water was monitored and the experiment lasted for 24 weeks.

Monitoring of Water Quality Parameters of Experimental Ponds

Water quality parameters were taken weekly, before feeding the fish. Water temperature was measured using a mercury-in-glass thermometer calibrated in degree centigrade. Oxygen was determined using a digital oxygen meter while pH was determined with a pH meter (pH-009) model.

Determination of Haematological Parameters (Blood Chemistry)

At the end of the feeding experiment, 2 fish were taken per Hapa for haematological analysis. Blood samples were taken using 0.5 ml syringes from the heart regions. The blood was emptied into EDTA (Ethylene Diamine Tetra Acetate) treated bottles to prevent coagulation. After that, the blood was analyzed according to standard haematological procedures as described by Dacie and Lewis (2001).

Haemoglobin Count (Hb)

Haemoglobin count was determined using the cyanmethemoglobin method. Blood was mixed with Drabkin’s reagent, which contains Ferricyanide and cyanide. Ferricyanide oxidizes the iron in the haemoglobin. The methemoglobin united with the cyanmethemoglobin which produced a colour that was measured in the colorimeter. 5ml of Drabkin’s solution was measured into a clean, dry test tube. The blood was mixed thoroughly and exactly 0.02ml was transferred into the reagent. The tube was allowed to stand for 10 minutes after the reading was done in the spectrophotometer at 540nm, using Drabkin’s reagent as blank. The haemoglobin value was read in grams per decilitre (g/dl) from the standard table.

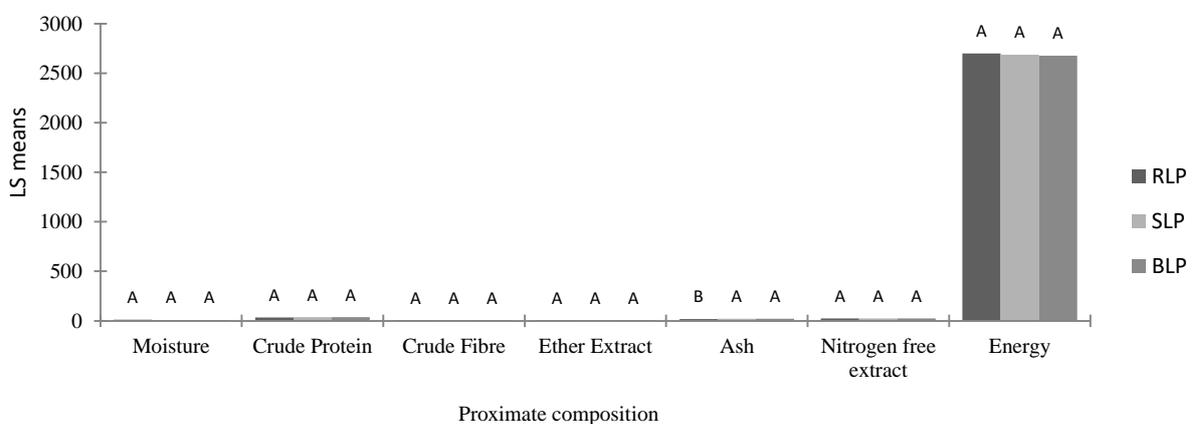


Figure 2. LS means for the proximate composition of raw, blanched and sun-dried *Lemna paucicostata*

Haematocrit Determination (PCV)

The haematocrit which is known as the packed cell volume (PCV) of each fish was determined by centrifugation of blood using a haematocrit centrifuge. By definition, haematocrit is the percentage volume of packed red cells following centrifugation. The purpose of centrifugation was to obtain a maximum packing of the red blood cell so that little or no plasma remains trapped between the cells. The packed cell volume is a function of red blood cell size and the number of cells per unit volume. This was done by centrifuging the blood sample for 5 minutes at 10,000 (rpm) as described by Stoskopf (1993). The force obtained by centrifugation depends upon the radius at which a particle is distant from the centre of the revolution. The radius is the distance in centimetre from the centre of the shafts to the bottom of the haematocrit tube.

Red blood Cell Count (RBCC)

The red blood cell was determined using the manual haematocytometer method as described by Dacie and Lewis (2001). A small amount of whole blood was accurately diluted with a fluid, which is isotonic with blood. The diluted blood was mounted in a

haematocytometer and the number of cells in a circumscribed volume was counted. Blood was drawn to the 0.5 mark in a clean dry red cell pipette then wiped and the level of blood was carefully adjusted to the 0.5 mark. The pipette was plunged quickly into the red cell-diluting mix of the blood and diluents. The content of the pipette was well-shaken before carrying out the count. Immediately after shaking 6-8 drops were expelled from the pipette and the tip of the pipette was wiped. The central square millimetre in the counting chamber was used for counting the erythrocytes. The cells contained in 80 of the 400 small squares were counted.

White Blood Cell Count (WBCC)

A small amount of blood was accurately diluted with 2% acetic acid, which destroys the non-nucleated erythrocyte and makes visible the nuclei of the leukocytes. Nucleated erythrocytes remain intact. Blood was drawn to the 0.5 mark in a clean, dry WBC pipette, the outside of the pipette was wiped and the level of the blood was carefully adjusted to the 0.5 mark. The pipette was plunged quickly into the 2% acetic acid, and the fluid was drawn to the 11 mark (1:20 dilution). The pipette was twirled to mix the blood and diluents. The

Table 4. Proximate composition of experimental diets (g/100g DM)

Diets	Moisture	Ash	Ether extract	Crude protein	Crude fibre	Nitrogen free extract
D ₁	11.45±1.04 ^a	14.95±0.91 ^a	12.59±0.59 ^a	38.02±0.99 ^a	6.98±0.66 ^a	16.01±0.97 ^c
D ₂	10.12±1.04 ^a	15.81±0.91 ^a	10.06±0.59 ^b	35.54±0.99 ^a	7.01±0.66 ^a	21.46±0.97 ^a
D ₃	10.50±1.04 ^a	16.32±0.91 ^a	11.54±0.59 ^{ab}	35.49±0.99 ^a	7.04±0.66 ^a	19.11±0.97 ^{bc}
D ₄	10.46±1.04 ^a	15.92±0.91 ^a	10.83±0.59 ^{ab}	36.33±0.99 ^a	6.96±0.66 ^a	19.50±0.97 ^{bc}
D ₅	11.52±1.04 ^a	16.81±0.91 ^a	10.49±0.59 ^b	37.98±0.99 ^a	6.85±0.66 ^a	16.35±0.97 ^c
D ₆	10.54±1.04 ^a	15.34±0.91 ^a	10.11±0.59 ^b	35.56±0.99 ^a	7.05±0.66 ^a	21.40±0.97 ^a
D ₇	9.36±1.04 ^a	15.95±0.91 ^a	11.55±0.59 ^{ab}	37.55±0.99 ^a	6.94±0.66 ^a	18.65±0.97 ^{bc}
D ₈	10.03±1.04 ^a	16.40±0.91 ^a	10.53±0.59 ^b	38.00±0.99 ^a	6.88±0.66 ^a	18.16±0.97 ^{bc}
D ₉	9.21±1.04 ^a	16.86±0.91 ^a	10.02±0.59 ^b	36.35±0.99 ^a	6.89±0.66 ^a	20.67±0.97 ^{ab}
P values	0.78	0.85	0.08	<0.0001	1.00	0.005

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

D₁ - 100% SBM (Control diet); D₂ - 75% SBM, 25% BLP; D₃ - 50% SBM, and BLP; D₄ - 25% SBM, 75% BLP; D₅ - 100% BLP; D₆ - 75% SBM, 25% SLP; D₇ - 50% SBM, and SLP; D₈ - 25% SBM, 75% SLP; D₉ - 100% SLP

Table 5. Mean weekly water quality parameters of the experimental ponds

Treatments	DO (mg/l)	pH	T (°C)
D ₁	5.20±0.01	6.52±0.02	28.04±0.93
D ₂	5.20±0.01	6.51±0.02	28.10±0.93
D ₃	5.22±0.01	6.52±0.02	28.00±0.93
D ₄	5.21±0.01	6.51±0.02	28.16±0.93
D ₅	5.20±0.01	6.51±0.02	28.30±0.93
D ₆	5.22±0.01	6.51±0.02	28.12±0.93
D ₇	5.21±0.01	6.51±0.02	28.05±0.93
D ₈	5.21±0.01	6.50±0.02	28.40±0.93
D ₉	5.23±0.01	6.52±0.02	28.30±0.93
P values	0.24	0.95	1.00

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

DO-Dissolved oxygen, T-Temperature

D₁ - 100% SBM (Control diet); D₂ - 75% SBM, 25% BLP; D₃ - 50% SBM, and BLP; D₄ - 25% SBM, 75% BLP; D₅ - 100% BLP; D₆ - 75% SBM, 25% SLP; D₇ - 50% SBM, and SLP; D₈ - 25% SBM, 75% SLP; D₉ - 100% SLP

pipette was removed from the diluents then the rubber tubing was well mixed by shaking before an accurate count was done. The leukocytes were counted under low power in the four large squares. As in the red blood cell touching the left and upper margins were included: those on the right and lower margins were not checked within 10 cells, and the central square was counted.

The mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Dacie and Lewis (2001).

$$MCV (FL) = \frac{\text{Packed cell volume}}{\text{RBC (106 } \mu\text{L}^{-1})} \times 10$$

$$MCH (pg) = \frac{\text{Hb (gdL-1)}}{\text{RBC (106 } \mu\text{L}^{-1})} \times 10$$

$$MCHC (\%) = \frac{\text{Hb (gdL-1)}}{\text{Packed cell volume}} \times 100$$

Determination of Digestive Enzyme Activities

Three fish from each Hapa were randomly chosen for the determination of digestive enzyme activities. The fish were starved for approximately 24h to ensure an empty digestive tract. The entire digestive tract (from

the oesophagus to the anus) was removed and adipose tissue was carefully cleaned. Where necessary, the remaining feed residues were gently squeezed out. Samples were then rinsed, weighed and homogenized (using a ceramic mortar and pestle) with 5ml of ice-cold phosphate buffer solution pH 8 (Furné *et al.*, 2008). The homogenate was centrifuged at 4°C at 2,000 rpm for 20 minutes; the supernatant was collected and kept in ice-cold condition in a plastic container for further analysis.

Trypsin (EC 3.4.21.4) Activity

Trypsin (EC 3.4.21.4) activity was determined using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate according to Erlanger *et al.* (1961). One unit of enzyme activity was defined as 1 μmol nitroanilide released per min using a molar extinction coefficient of 8800 M⁻¹ cm⁻¹.

Protease (EC 3.4.21.63) Activity

Alkaline protease (EC 3.4.21.63) activity of the samples was assayed according to García-Carreño (1992), using azocasein (2.0% Tris-HCl 100 mM, pH 9) as substrate. One unit of alkaline protease (EC 3.4.21.63) activity was defined as the amount of enzyme required to increase 0.01unit absorbance min⁻¹.

Table 6. Haematological indices of *Oreochromis niloticus* fed experimental diets

Treatments	PCV(%)	RBC×10 ⁶ m ³ /l	WBC×10 ³ m ³ /l	Hb(g/100ml)	MCH(pg)	MCHC(%)	MCV(fl)	LYMPH(%)
D ₁	27.34±0.71 ^c	2.35±0.65 ^a	7.70±0.60 ^a	8.79±0.67 ^{cd}	37.40±2.23 ^{ab}	32.15±0.81 ^b	116.34±3.78 ^{ab}	90.68±5.23 ^a
D ₂	28.95±0.71 ^{bc}	2.61±0.65 ^a	7.52±0.60 ^a	9.42±0.67 ^{cd}	36.09±2.23 ^{ab}	32.54±0.81 ^b	110.92±3.78 ^{bc}	90.44±5.23 ^a
D ₃	32.00±0.71 ^a	3.37±0.65 ^a	7.28±0.60 ^a	11.98±0.67 ^a	35.55±2.23 ^{ab}	37.44±0.81 ^a	94.96±3.78 ^d	88.31±5.23 ^a
D ₄	32.01±0.71 ^a	3.39±0.65 ^a	7.25±0.60 ^a	12.00±0.67 ^a	35.39±2.23 ^{ab}	37.49±0.81 ^a	94.42±3.78 ^d	88.71±5.23 ^a
D ₅	27.01±0.71 ^c	1.85±0.65 ^a	7.85±0.60 ^a	7.93±0.67 ^d	42.86±2.23 ^a	29.36±0.81 ^c	146.00±3.78 ^a	90.62±5.23 ^a
D ₆	31.57±0.71 ^a	3.27±0.65 ^a	7.30±0.60 ^a	10.87±0.67 ^{ab}	33.24±2.23 ^b	34.43±0.81 ^b	96.54±3.78 ^d	88.34±5.23 ^a
D ₇	30.92±0.71 ^{ab}	3.06±0.65 ^a	7.50±0.60 ^a	10.11±0.67 ^{bc}	33.04±2.23 ^b	32.69±0.81 ^b	101.05±3.78 ^{cd}	89.01±5.23 ^a
D ₈	28.97±0.71 ^{bc}	2.58±0.65 ^a	7.80±0.60 ^a	9.45±0.67 ^{cd}	36.63±2.23 ^{ab}	32.62±0.81 ^b	112.29±3.78 ^{bc}	90.33±5.23 ^a
D ₉	27.32±0.71 ^c	2.37±0.65 ^a	7.72±0.60 ^a	8.76±0.67 ^{cd}	36.96±2.23 ^{ab}	32.06±0.81 ^b	115.27±3.78 ^{ab}	90.71±5.23 ^a
P values	<0.0001	0.711	0.995	0.004	0.049	<0.0001	<0.0001	1.000

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

PVC-Packed cell volume, RBC-Red blood cell, WBC-White blood cell, Hb-Haemoglobin, MCH-Mean corpuscular haemoglobin, MCHC-Mean corpuscular haemoglobin concentration, LYMPH-Lymphocyte, pg-picograms, fl-femtoliters.

D₁ - 100% SBM (Control diet), D₂ - 75% SBM, 25% BLP; D₃ - 50% SBM and BLP; D₄ - 25% SBM, 75% BLP; D₅ - 100% BLP; D₆ - 75% SBM, 25% SLP; D₇ - 50% SBM, and SLP; D₈ - 25% SBM, 75% SLP; D₉ - 100% SLP

Table 7. Digestive enzymes activities (TU) of *Oreochromis niloticus* fed experimental diets

Treatments	Trypsin	Protease	Chymotrypsin	Amylase	Lipase
D ₁	857.45±37.29 ^e	120.10±6.53 ^b	2079.47±60.17 ^{cd}	90.43±5.96 ^{de}	691.75±3.61 ^a
D ₂	973.53±37.29 ^d	120.91±6.53 ^b	2125.37±60.17 ^{cd}	95.37±5.96 ^{cd}	657.13±3.61 ^b
D ₃	1256.77±37.29 ^{ab}	147.98±6.53 ^a	2451.09±60.17 ^a	124.57±5.96 ^{ab}	611.50±3.61 ^{de}
D ₄	1327.53±37.29 ^a	149.53±6.53 ^a	2507.24±60.17 ^a	137.21±5.96 ^a	609.77±3.61 ^e
D ₅	832.81±37.29 ^e	115.72±6.53 ^b	1822.05±60.17 ^e	85.67±5.96 ^e	702.08±3.61 ^a
D ₆	1256.93±37.29 ^{ab}	135.32±6.53 ^{ab}	2448.93±60.17 ^a	112.43±5.96 ^b	621.39±3.61 ^d
D ₇	1154.74±37.29 ^b	124.19±6.53 ^b	2342.03±60.17 ^{ab}	110.52±5.96 ^b	642.66±3.61 ^c
D ₈	1092.51±37.29 ^c	122.78±6.53 ^b	2255.41±60.17 ^{bc}	106.03±5.96 ^{bc}	643.51±3.61 ^c
D ₉	854.98±37.29 ^e	117.97±6.53 ^b	1971.02±60.17 ^{de}	88.91±5.96 ^{de}	693.02±3.61 ^a
P values	<0.0001	0.008	<0.0001	<0.0001	<0.0001

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

D₁ - 100% SBM (Control diet); D₂ - 75% SBM, 25% BLP; D₃ - 50% SBM, and BLP; D₄ - 25% SBM, 75% BLP; D₅ - 100% BLP; D₆ - 75% SBM, 25% SLP; D₇ - 50% SBM, and SLP; D₈ - 25% SBM, 75% SLP; D₉ - 100% SLP

Chymotrypsin (EC 3.4.21.1) Activity

Determination of chymotrypsin (EC 3.4.21.1) activity was carried out using the method of DelMar *et al.* (1979), with slight modifications as previously described for the trypsin method, using N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (SAAPNA) as a substrate and Tris-HCl 60 mM pH 8 and CaCl₂ pH 8. One unit of chymotrypsin activity was defined as the enzyme required to release 1 μ Mol of 4-nitroanilide per minute.

Amylase (EC 3.2.1.1) Activity

Amylase (EC 3.2.1.1) activity was determined according to the method of Vega-Villasante *et al.* (1995), using starch solution (1% Tris HCl, 100 mM, pH 8) as substrate. One unit of amylase activity was defined as the amount of enzyme that produced 1 μ mol of maltose per min

Lipase (EC 3.1.1.3) Activity

Triacylglycerol lipase (EC 3.1.1.3) activity was determined according to Versaw *et al.* (2006), with β -naphthyl caprylate as substrate (100 mM in DMSO). One unit of lipase activity was defined as the amount of enzyme that produced 1 μ Mol of β -naftol per minute. The activity of trypsin, proteases, chymotrypsin, amylases, and lipases was expressed as the number of units of enzyme per organism (total units, TU).

Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) to test for significant differences among treatment means using XLSTAT version 2022, followed by Duncan pairwise comparisons which were used to separate significantly different means at a confidence interval of 95%. The level of significance set for treatments was $P \leq 0.05$. The enzyme activity was subjected to stacked analysis to determine the total activities in all the treatments. Principal component analysis (PCA) was carried out to establish the relationship between the haematological parameters and enzyme activities.

Results

Proximate Composition of Raw, Blanched and Sun-dried *Lemna paucicostata*

The result of the proximate composition of raw, blanched and sun-dried *L. paucicostata* is presented in Table 3. The crude protein in the blanched treatment was higher than all the other treatments with 37.13% followed by 36.75% in the sun-dried *L. paucicostata* and 35.08% in the raw *L. paucicostata*. The crude protein value in the blanched *L. paucicostata* was not significantly different ($P > 0.05$) from the treatment that

was sun-dried and the raw *L. paucicostata*. The raw *L. paucicostata* had the highest (4.72%) crude fibre content while the blanched treatment gave the lowest (3.34%) crude fibre content. The sun-dried treatment had crude fibre content of 3.64%. The ether extract was highest (6.20%) in the raw *L. paucicostata* followed by the blanched with (6.07%) and the sun-dried treatment gave the lowest ether extract content (5.90%). The blanched *L. paucicostata* had the highest (21.90%) ash content than the sun-dried *L. paucicostata* which had 20.48%. The raw *L. paucicostata* had the lowest (18.18%) ash content. The raw *L. paucicostata* had the highest (25.60%) nitrogen-free extract content which was not significantly different ($P > 0.05$) among all the other treatments. The sun-dried treatment had 24.15% while the blanched treatment had 23.04% nitrogen-free extract content. The energy value of the sun-dried *L. paucicostata* was not significantly different ($P > 0.05$) from the raw and blanched treatments. The LS means for the proximate composition of raw and processed *Lemna paucicostata* is presented in Figure 2.

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets is shown in Table 4. The diets were iso-nitrogenous as there was no significant difference ($P > 0.05$) in the protein composition of the diets at 35% crude protein and they all met the dietary requirement for the experiment. The soluble carbohydrate which is the Nitrogen Free Extract ranged from 16.01% to 21.46%. The oil contents in the feeds ranged from 10.02% to 12.59%. The crude fibre ranged from 6.88% to 7.05%. The ash contents ranged from 14.95% to 16.86%.

Water Quality Parameters of the Experimental Ponds

The mean dissolved oxygen means hydrogen ion concentration and means the temperature in the experimental ponds is presented in Table 5. There was no significant difference in the water quality parameters among all the treatments and the control ($P > 0.05$). All the parameters recorded were within the recommended range. The DO ranged from 5.20 to 5.23, the pH ranged from 6.50 to 6.52 and the temperature ranged from 28.00°C to 28.40°C.

Haematological Indices of *Oreochromis niloticus* Fed Experimental Diets

The haematological indices of *Oreochromis niloticus* fed experimental diets are shown in Table 6. Treatments D₄ (75% blanched *Lemna paucicostata*), D₃ (50% blanched *L. paucicostata*) and D₆ (25% sun-dried *L. paucicostata*) had higher packed cell volume values of 32.01%, 32.00% and 31.57%, respectively. Similarly, treatments D₈ (75% sun-dried *L. paucicostata*) and D₂ (25% blanched *L. paucicostata*) had similar moderate values of 28.97% and 28.95, respectively. The control D₁

(100% soybean meal), treatment D₉ (100% sun-dried *L. paucicostata*) and D₅ (100% blanched *L. paucicostata*) had lower values of 27.34%, 27.32% and 27.01%, respectively. There was a significant difference ($P \leq 0.05$) in the packed cell volume between the treatments and the control. The red blood cell ranged from $1.85 \times 10^6 \text{mm}^3$ to $3.39 \times 10^6 \text{mm}^3$. There was a significant difference ($P \leq 0.05$) in the mean corpuscular haemoglobin concentration between the treatments and the control which ranged from 29.36% to 37.44%. The highest (37.44%) value was obtained in treatment D₃ (50% blanched *Lemna paucicostata*) while the lowest value (29.36%) was recorded in treatment D₅ (100% blanched *L. paucicostata*). Treatment D₉ (100% sun-dried *L. paucicostata*) had the highest lymphocyte value of 90.71% followed by the control D₁ (100% soybean meal) with a value of 90.68%. The least value of 88.31% was obtained in treatment D₃ (50% blanched *Lemna paucicostata*). However, there was no significant difference ($P > 0.05$) between all other treatments and the control.

Digestive Enzymes Activities (TU) of *Oreochromis niloticus* fed Experimental Diets

The digestive enzyme activities of *Oreochromis niloticus* fed experimental diets are presented in Table 7. The trypsin activity was highest in treatment D₄ (75% blanched *Lemna paucicostata*) with a value of 1327.53 followed by treatment D₇ (50% sun-dried *L. paucicostata*) which gave 1154.74. The least trypsin activity value of 832.81 was obtained in treatment D₅ (100% blanched *L. paucicostata*) which is not significantly different ($P > 0.05$) from the control D₁ (100% soybean meal) and treatment D₉ (100% sun-dried

L. paucicostata) which had 857.45 and 854.98, respectively. There was a significant difference ($P \leq 0.05$) in the trypsin activity between the treatments and the control. The protease activity value ranged from 115.72 to 149.53. Treatment D₄ (75% blanched *Lemna paucicostata*) had the highest chymotrypsin activity with a value of 2507.24 while treatment D₅ (100% blanched *Lemna paucicostata*) had the lowest which was 1822.05. The control D₁ (100% soybean meal) recorded 2079.47 which was similar to 2125.37 obtained in treatments D₂ (25% blanched *L. paucicostata*). There was a significant difference ($P \leq 0.05$) in the chymotrypsin activity between the treatments and the control. The amylase activity value ranged from 85.67 to 137.21. Treatments D₄ (75% blanched *Lemna paucicostata*) recorded the highest (34.21) while treatment D₅ (100% blanched *Lemna paucicostata*) had the lowest value (85.67). Treatment D₆ (25% sun-dried *L. paucicostata*) had a moderate value of 112.43 which was not significantly different ($P > 0.05$) from treatment D₇ (50% sun-dried *L. paucicostata*) which had 110.52. However, there was a significant difference ($P \leq 0.05$) between the treatments and the control. Treatment D₅ (100% blanched *L. paucicostata*) had the highest lipase activity value of 702.08 which was not significantly different from treatment D₉ (100% sun-dried *L. paucicostata*) and the control D₁ (100% soybean meal) which had 693.02 and 691.73, respectively. The lowest value of lipase activity of 609.77 was obtained in treatment D₄ (75% blanched *Lemna paucicostata*). There was a significant difference ($P \leq 0.05$) in the lipase activity between the treatments and the control. The result of stacked analysis for the total enzyme activity of *Oreochromis niloticus* fed experimental diets is presented in Figure 3.

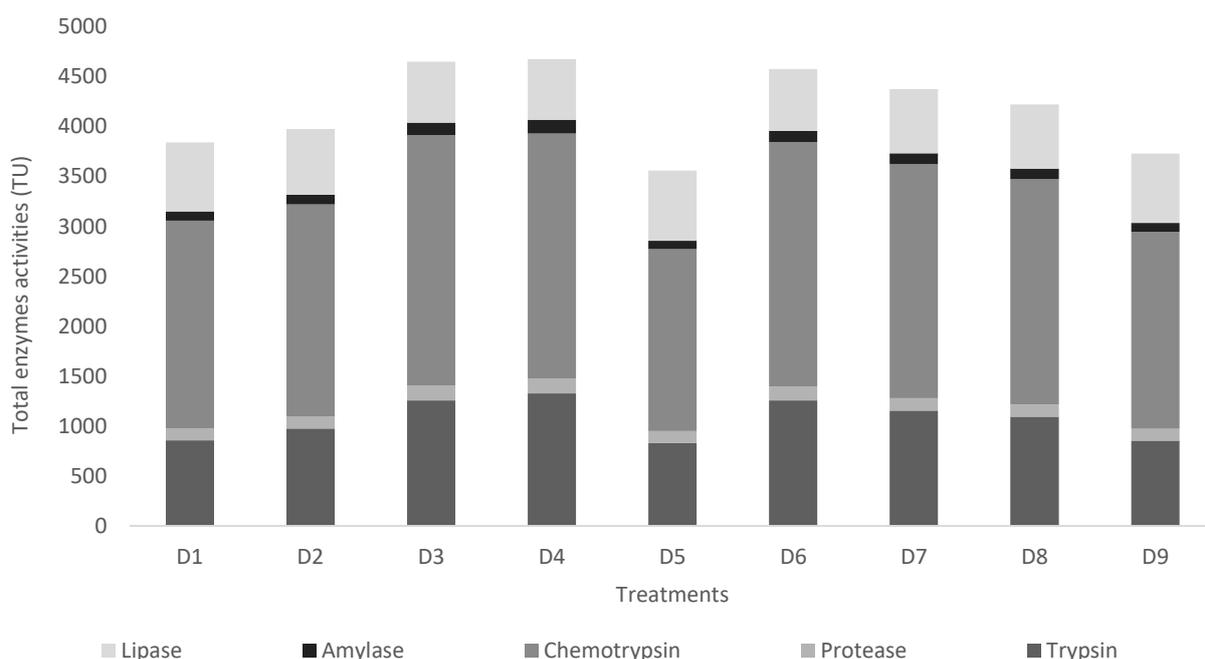


Figure 3. Stacked column chart for total enzymes activity of *Oreochromis niloticus* fed experimental diets

Discussion

The result of this study showed that blanching and sun-drying methods affected the proximate composition of *Lemna paucicostata*. The high value of crude protein in the blanched *L. paucicostata* showed that blanching was superior to the sun-drying method employed in this study. Blanching *Lemna paucicostata* led to the stabilization of the greenish part of the plant thereby retaining the chlorophyll a molecule needed for protein synthesis and phosphorylation. The sun-dried *L. paucicostata* with lower crude protein could have been a result of solar radiation which would have caused the yellowing of the greenish part of the plant, consequently affecting the chlorophyll. Yellowing of the greenish part of *L. paucicostata* as a result of the solar radiation which affected the crude protein content was reported by Sogbesan *et al.* (2015). These authors observed that the sun-dried *L. paucicostata* had lower crude protein content when compared to the blanched *L. paucicostata*. The crude protein content of the raw, blanched and sun-dried *L. paucicostata* obtained in this study is higher than 29.28%, 30.04% and 28.62% reported by Sogbesan *et al.* (2015) for raw, blanched and sun-dried duckweed (*L. paucicostata*), respectively. However, the crude protein content obtained in this study is lower than the 40.20% reported by Khanum *et al.* (2012). The variation in the crude protein contents of the duckweed (*L. paucicostata*) in this study and that of previous studies could be attributed to differences in levels of organic matter in the culture medium or water environment. The crude protein of the blanched *L. paucicostata* obtained in this study is similar to 38.00% and 37.70% reported by Tavares *et al.* (2010) and Du *et al.* (2012) for duckweed (*L. paucicostata*), respectively. The crude fibre content in this study revealed that *L. paucicostata* has a very low amount of fibre, therefore even monogastric animals can digest it and many fishes, especially *Oreochromis niloticus*. The low crude fibre content of duckweed (*L. paucicostata*) is attributed to the fact that the cell wall has low lignin as reported by Panahabadi *et al.*, (2021) who stated that cellulose and lignin are the two main components of secondary plant cell walls. Thus, enhanced digestibility and is considered an ideal protein source of fish feed (Tao *et al.*, 2013). The crude fibre content obtained in this study is lower than the 5.7% reported by Christine *et al.* (2018). A fibre content above 8-12% in a fish diet is not enticing because it may cause a reduction in the number of usable nutrients in the diet. Furthermore, high fibre content can result in decreased nutrient digestibility which results in poor fish performance and increased faecal waste consequently, affect the water quality. The ash content of the raw, blanched and sun-dried duckweed (*L. paucicostata*) obtained in this study was higher than 12.3%, 14.00%, 15.34% and 15.90% reported by Hlophe and Moyo (2011), Khanum *et al.* (2012), Sogbesan *et al.* (2015) and Heuzé and Tran (2015) for duckweed (*L. paucicostata*), respectively. The

ash content values recorded in this study are within the range of 12-27.6% reported by Christine *et al.* (2018). The differences between the results of this study and those of the above-referenced authors could be due to different conditions of the water environment. The hydrolyzable carbohydrate (nitrogen-free extract) values in this study showed that *L. paucicostata* in all the treatments have the potential to contribute energy so that protein will be used for growth. Prabu *et al.* (2017) stated that carbohydrates improve growth and provide precursors for some amino acids and nucleic acids. According to Azaza *et al.* (2015), an increased dietary carbohydrate content improves metabolism and growth in tilapia. When there is limited energy in the fish diet other nutrients will be broken down and used for the maintenance of the body. The hydrolyzable carbohydrate content was higher in the sundried *L. paucicostata* than in the blanched *L. paucicostata*. This observation may be due to the lower levels of other proximate components (crude protein, ash, crude fibre and ether extract) since nitrogen-free extract values are obtained by subtracting other components from 100. Sogbesan *et al.* (2015) also reported higher hydrolyzable carbohydrate content for sun-dried *L. paucicostata* when compared with the blanched duckweed (*L. paucicostata*). Energy levels in all treatments indicate that *L. paucicostata* can be a good source of energy in the *Oreochromis niloticus* diet.

The basic nutrient that cannot be altered in the choice of ingredients for feed formulation is protein, and the crude protein observed among the experimental diets was per optimum dietary protein, 35-40% required by *Oreochromis niloticus* fingerlings for optimal growth and development as reported by FAO (2022). There was an indication of adequate utilization of the experimental diets since all of the diets supplied the optimum amount of protein required by the fish. All the experimental diets were proteinous as recommended by Maundu (2020). The crude protein content of the experimental diets ranged from 35.49% to 38.02% which was adequate for Nile tilapia fingerling's feeds. The value is however higher than the value obtained by Olaniyi and Oladunjoye (2012) who replaced duckweed with a fish meal at various inclusion levels in the diet of Nile tilapia *Oreochromis niloticus*. The value of crude protein in the experimental diets obtained in this study is also higher than the value obtained by Oyas *et al.* (2018) who replaced the fish meal with duckweed meal at 0%, 15%, 30% and 45% inclusion levels in the diet of common carp (*Cyprinus carpio*) fingerlings. While the ash content of the experimental diets obtained is considerably similar to that reported by the same authors. The range value of crude protein content of the experimental diets obtained in this study is lower than the range of 41.87% to 45.06% reported by Effiong *et al.* (2009) who partially replaced the fish meal with duckweed (*L. paucicostata*) in the diet of *Heterobranchus longifilis* fingerlings. The differences observed in this study from that of the previous authors could be a result of different

Anyanwu *et al.* (2011) stated that there are usually wide fluctuations associated with white blood cell values which may be due to endogenous factors such as experimental conditions, diet and anti-nutrients. The fish fed diet containing 100% blanched *Lemna paucicostata* recorded the lowest value (7.93g/100ml) of haemoglobin (Hb) among the control and other treatments. The reduction in the Hb concentration could imply that the diet contained low-quality protein. Therefore, resulting in poor transportation of oxygen from the respiratory organs to the peripheral tissue (Robert *et al.*, 2000). The mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) observed in this study recorded their highest values in the fish fed D₅ (100% blanched *Lemna paucicostata*) which are comparable with value ranges reported by previous workers (Adedeji and Adebile 2011; Anyanwu *et al.* 2011). The haematological parameters in this study were within the range reported for *Oreochromis niloticus* as documented by Magouz *et al.* (2020) which indicates the experimental fish were not stressed and cultured in good health condition.

The activities of digestive enzymes (trypsin, protease, chymotrypsin, amylase and lipase) observed in this study were the main factors that helped the experimental fish digest duckweed (*Lemna paucicostata*) efficiently, without reducing the growth performance and nutrient utilization. Thus, the best growth performance and nutrient utilization might be associated with the improved enzyme activities observed in this study. Pérez-Jiménez (2009) stated that digestive enzymes play a significant role in the utilization of diets, with the activities of enzymes affecting the efficiency of nutrient absorption, and their performance provides key information on the digestive ability of fish to hydrolyze protein, lipid, and carbohydrate in diets. In this study, amylase activity was reduced in *Oreochromis niloticus* fed the diet containing 100% *Lemna paucicostata* when compared to the fish fed the control diet and other treatments. Similarly, lower amylase activity was observed in Nile tilapia fed diets formulated with 15% extruded chickpea meal compared to fish fed other plant by-products (Montoya-Mejía *et al.*, 2017). However, protein digestion in Nile tilapia (*Oreochromis niloticus*) observed in this study was influenced by the processed duckweed-based diets with total protease, trypsin and chymotrypsin activities all significantly higher in fish fed the diets containing 75% blanched *Lemna paucicostata* when compared among other treatments and the control. Similar results were reported in *Labeo rohita* fed a pelleted diet containing *Lemna minor* (Goswami *et al.*, 2020). Higher activities of these enzymes showed enhanced protein digestion in *Oreochromis niloticus* fed the processed duckweed-based diets, which indicated that the consumed diet was used more efficiently. Lower protease, trypsin, and chymotrypsin activities were recorded in *Oreochromis niloticus* fed the control diet containing 100% soybean meal (D₁), the diet containing 100% blanched *L.*

paucicostata (D₅) and 100% sun-dried *L. paucicostata* (D₉). Lower enzyme activities were also observed in Atlantic salmon (Krogdahl, 2003), Nile tilapia (Lin and Luo, 2011) and Japanese seabass (Zhang, 2018) fed soybean meal-based diets. However, higher levels of trypsin activity in tilapia (*Oreochromis mossambicus*) and chinook salmon (*Oncorhynchus tshawytscha*) have also been reported (Kurtovic *et al.*, 2006). Abolfathi *et al.* (2012) reported that trypsin activity is higher in carnivorous and omnivorous fish compared to herbivorous species. These authors also reported that trypsin activities were generally higher compared to chymotrypsin for carnivorous fishes while in omnivorous and herbivorous species, activities of chymotrypsin were higher. So, as was expected, chymotrypsin activity was observed to be higher in this study when compared with trypsin activity. However, both types of proteases are believed to play a collaborative role in protein digestion in the intestinal tract. Also, according to the observed enzymatic profile and considering the amylase activity in this study, the results suggest that the digestive tract of *Oreochromis niloticus* is well adapted to carbohydrate digestion. This confirms that the digestive physiology of *Oreochromis niloticus* is proximate to that of omnivorous species. Various studies have demonstrated that amylase activity is greater in herbivorous and omnivorous fish than in carnivorous fish, this has been related to the digestion of diets rich in carbohydrates and proteins, respectively (Chan *et al.*, 2004; Abolfathi *et al.*, 2012).

The stacked column chart showed higher total enzymes activities of *Oreochromis niloticus* in the fish fed D₄ (75% blanched *Lemna paucicostata*) and D₃ (50% blanched *Lemna paucicostata*) with similar values of 4,675.13 and 4,648.06, respectively. While the lowest value of 3,558.33 was observed in the fish fed D₅ (100% blanched *L. paucicostata*).

The principal component analysis of the haematological indices and enzyme activity as shown in Figure 4 revealed that a strong correlation exists between PROT, MCHC, CHEMO, RBC and PCV. Principal Component Analysis (PCA) F1 (88.80%) and F2 (6.03%) combined to give the biplot axes of 94.83%.

Conclusions

The haematological indices in this study indicated that all the parameters observed were within the range required for the good health condition of *Oreochromis niloticus*, showing the non-harmful effect of the blanched and sun-dried *Lemna paucicostata* in the diets. The fish fed diet containing a 75% inclusion level of blanched *L. paucicostata* obtained the best concentration of the packed cell volume (32.01%) in the blood while a poor concentration of 27.01% was observed in the fish fed 100% blanched *L. paucicostata*. The fish fed 75% blanched *Lemna paucicostata* and 50% blanched *Lemna paucicostata* gave higher total enzyme activities of 4,675.13(TU) and 4,648.06(TU),

respectively. While the fish fed 100% blanched *L. paucicostata* gave the lowest value of 3,558.33(TU). The digestive enzyme activities were observed to be the main factors that helped the experimental fish to digest the blanched and sun-dried *Lemna paucicostata* efficiently.

Ethical Statement

Not applicable.

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Author Contribution

Abdurrazzaq Ibrahim Abdullahi conceived the work, designed the experiment and carried out the experiment. Prof. J. Auta, Prof. S. A. Abdullahi, Prof. P. I. Bolorunduro and Associate Prof. H. U. Onimisi supervised the work and also read and approved the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest in this paper.

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