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Impact of Avocado (*Persea Americana*) Seed Meal on the Liver and Kidney of *Oreochromis Mossambicus* at Different Diet Concentrations

Udo, O. O., Hart, A. I., Komi, G. W., Zitte, L. F., and Nwaiwu, J.

Department of Animal and Environmental Biology, Faculty Science, University of Port Harcourt, East-West Road Choba, PMB 5323, Port Harcourt.
Email: gentle.komi@uniport.edu.ng
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ABSTRACT

A study to investigate the impact of avocado *(Persea americana)* seed meal on the liver and kidney of 150 *Oreochromis mossambicus* at different diet concentrations was carried out. The fishes were randomly assigned to 5 treatment group, with each group having 3 replicate per group for 155 days. The objective of this study is to investigate the functionality, chemical composition of the seed and to established its suitability of use as food ingredient and for medicinal purposes. Proximate analysis of *P. americana* revealed mean dry weight as 4.39, CHO - 13.97, Moisture - 71.12 and Fibre - 6.93. AST in 10% treatment group (89 ± 0) was significantly higher than the control group 75 ± 14 ($P<0.05$) ALT in treatment groups 5%, 10%, 15%, and 20% were relatively consistent ranging from 32.67 ± 6.39 to 36.33 ± 3.33 UL. ALP had a downward consistent trend as the proportion of *P. americana* seed increased (29± 8.08ab, 28± 3.79ab, 17± 3.06b, 15± 1.53b) groups 5%, 10%, 15% and 20% respectively. There was a downward but non consistent trend in the Albumin value ranging from 77.67 ± 10.2^{a} for the control group to 27 ± 3.51^{b} with the 15% group having the least value. While TP significantly decrease from 67.67±127 in group 1 to 24±3.46 in group 5. Other liver enzyme parameters were not significantly affected by the treatment. Similarly, the chloride level ranged from 118.00 to 121.33 mmol/L, experiencing a downward consistent trend from the control group (121.33) to the 20% diet group (118.00). Potassium showed an upward but inconsistent trend ranging from 8.70 mmol/L for the control group to 9.57 mmol/L for the 20% diet group. It is therefore concluded that Avocado seed meal is a safe feed additive for fish feed.

Key words: Avocado, kidney, Liver, *Oreochromis mossambicus*

1. INTRODUCTION

1.1 Background of Study

Aquaculture is considered as the fastest growing food supply sector in the world, helping with global, regional and local food security. It is increasingly recognized as a potential solution to global challenges such as protein supply, water quality improvement, and climate change adaptation (Engle & Senten, 2022). Aquaculture is now globally accounting for more fish biomass than capture fisheries if non-edible amounts are included (Edwards, Zhang, Belton, & Little, 2019) and more total biomass than beef. Achieving sustainable aquaculture requires operational changes and new approaches that prioritize sustainability and address historical challenges (Boyd *et al.,* 2020). In 2020, the world's total aquaculture production reached an all-time high of 122.6 million tons in live weight (SOFIA 2022). With an anticipated 10 billion people expected to inhabit the planet by 2050, the demand for animal protein will increase by 52 percent (Global Seafood Alliance, 2019). Aquaculture has experienced rapid growth in recent years due to increasing demand for seafood and advancements in culture technology (Wang *et al.,* 2019).

Despite the fact that about 16 tilapia species have been involved in aquaculture production, out of which ten species are commercially farmed according to FAO, (2004), global tilapia's production is dominated by just three species: the Nile tilapia Oreochromis niloticus (L), the Mozambique tilapia *Oreochromis mossambicus* (Peters) and the blue tilapia *Oreochromis aureus* (Steindachner, 1864).

One of the major threats to aquaculture industry worldwide is bacterial infection. There is report of more than 6 billion USD per annum lost from the aquaculture industry due to diseases (Stentiford *et al*. 2017). The most effective method of treating and preventing bacterial diseases in aquaculture is the use of antibiotics as they are commonly used as therapeutic and/or prophylactic agents (Ee Lean *et. al,* 2021). Some of the commonly used antibiotics in aquaculture farming are tetracyclines, sulfonamides, oxolinic acid and erythromycin (ASEAN, 2013). These antibiotics are permitted for use in food producing animals based on the recommended Maximum Residue Level (MRL) set by joint Food and Agriculture Organization of the United Nations and the World Health organization (FAO/WHO), Codex Alimenterivs Commission, and European Union legislation (FAO and WHO 2020) En Lean *et. al,* (2021).

However, there are concerns of negative effects on human health arising from antibiotic resistance (Javadel *et. al*, 2008). There is considerable debate in veterinary medicine regarding the use of antibiotics in animals raised for human consumption. (Timothy *et al.* 2012). The potential threat to human

health as a result of inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these animals are bound to enter the food supply and could be widely disseminated in food products (Garofalo *et. al*, 2007). Other effects of the synthetic Chemotherapeutic drugs include accumulating in the fish flesh, and environmental pollution which can lead to immune suppression (Amstrong *et al.,* 2005; and Reverter *et al.,* 2014). These concerns and many more have left animal producers and aqua-culturists looking for alternatives to antibiotics that are relatively cheaper and ecofriendly with minimal residual effect. There is a variety of potentially useful feed materials of plant origin that could be added to animal feed in order to improve production as well as reduce the spread of diseases. Example of such feed material is the Avocado Seed meal (George*, et al* 2020).

The aim of this study is to determine the impact of avocado seed meal on the liver and kidney of *Oreochromis mossambicus* at different diet concentrations. What are the histological or functional effects of different concentrations of avocado seed meal on the liver and kidney of *O. mossambicus*? At what level, if any, does avocado seed meal begin to negatively impact liver and kidney health in tilapia? This research explores the potential of avocado seed (*Persea americana*) as a dietary supplement for Mozambique tilapia (*Oreochromis mossambicus*), evaluating its impact on organ health. The findings from this study imply that the assessment of the kidney, liver, and haematological parameters provides insights into the physiological impact of avocado seed inclusion. Understanding these effects will help determine safe inclusion levels that promote fish health and optimal growth, offering critical data for formulating balanced diets for aquaculture.

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted at African Regional Aquaculture Centre (ARAC), Aluu, Port Harcourt, Rivers State, Nigeria

(4°55'00.5" N 6°53' 50.1" E) in a 1500m^2 stagnant rain- set earthen pond. Situated on an 81-ha plot of land, ARAC is a research institute for freshwater fish species, with heavy vegetation on a plain land and diverse aquatic plants within and outside the pond areas providing food and shelter for the fish species cultured. Fifteen floatable cages were constructed and used in the culture of experimental fishes in a complete randomized set up.

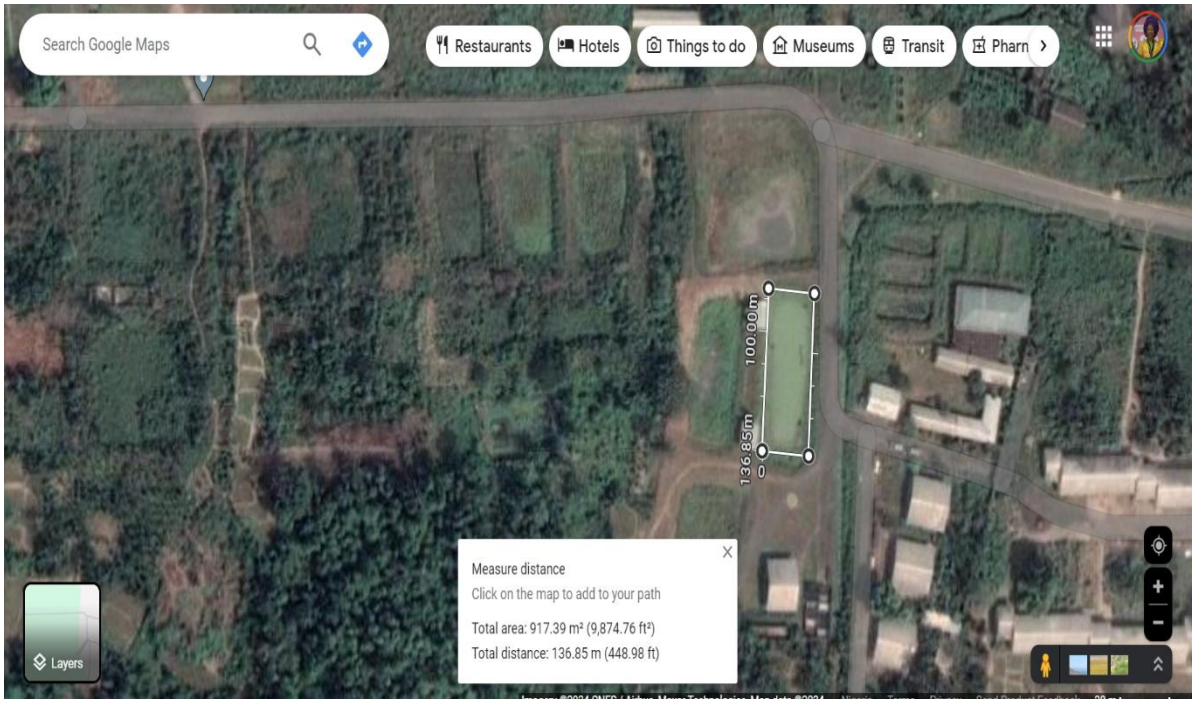

Figure 3.1 Map of the study area showing experimental pond.

Source: Google Map (2024) Ansa Ebinimi

3.2 Avocado Seed (Persea americana)

The avocado seeds were collected from Akpan –Andem market, a popular market in Uyo, Akwa Ibom State from fruit sellers and transported in bags to the house where they were washed and soaked in large rubber containers with cold water for 24 hours to reduce the anti-nutritional factors (tannin, saponin etc) present in the seed. They were then chopped into smaller parts with a kitchen knife and sun-dried for a maximum of 7 days (5-6 hours a day) depending on the weather condition of the day after the seeds were properly dried, they were ground into powdered form with the use of a grinding machine and the meal stored in an airtight container.

3.2.1 Proximate Analysis

Prior to the feed formulation, I carried out a proximal analysis of the avocado seed (wet and dried) to determine moisture, protein, fat, ash, fibre, carbohydrate content and energy value, as well as anti-nutrient contents (saponin, tannin, phytate etc). The proximate content was determined according to specified method of Association of Analytical Chemists (AOAC, 1990). Moisture content of the avocado seed powder was determined by the oven drying method. 2g of avocado seed powder sample was weighed into conditioned and preweighedmoisture dishes, and the samples were dried to a constant weight at 105^{o}C

$$\text{Moisture (\%)} = \frac{\text{weight loss}}{\text{weight of sample}} \text{X } 100.$$

Crude protein content of the avocado seed powder was determined using the Kjeldahl method (AOAC 988.05, 2012). The amount of protein was calculated from the nitrogen concentration in the sample using 6.25 as the conversion factor (Sáez-Plaza *et al.,* 2013).

To determine the Ash content, a 2-gram sample of the avocado seed powder was weighed onto conditioned and pre-weighed porcelain crucibles. The samples were then incinerated in the muffle furnace, set at 550^{o}C for 4 hours, cooled in the desiccator, and weighed.

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \text{X } 100$$

Crude fiber content of the avocado seed powder was analysed using the Henneberg – Stohmann method (Sowinski *et al.,* 2019). Fat was extracted from the 2g sample using the Soxhlet method and then transferred to a 500 ml flask

Plate 3.1 Sun drying of avocado seed (*Persea americana*)

where it was boiled under reflux in 200 ml 1.25% sodium hydroxide. The insoluble matter was washed with boiled distilled water and then 1% hydrochloric acid. The insoluble matter was then transferred to porcelain crucibles, dried in the oven and weighed (W1). The residue was then incinerated in the muffle furnace at 500^{o}C for 4 hours. The crucibles were then cooled, and the weight was recorded as W2.

$$\text{Crude fiber (\%)} = \frac{\text{W1}-\text{W2}}{\text{sample weight}} \text{X } 100$$

3.3 Preparation of plant material

The seed of *Persea americana* were manually chopped with the aid of a kitchen knife and properly washed to remove dirt and old skin. They were soaked in cold water for 24 hours to reduce the level of anti-nutritional factors present in it. The substances were occasionally stirred to ensure proper soaking of all seeds. The water was drained off and the seeds sun-dried for 7 days (5-6 hours, a day). When the seed were properly dried, they were ground into powdered form using a grinding machine. The fine powder Avocado seed meal was stored in an airtight container to avoid contamination by water or air prior to feed formula

3.4 Determination of Anti Nutrient

Phytate content was estimated by the titration method of Sudarmadji and Markakis (1977). Tannin content was determined by Folin–Dennis colorimetric method as described by Pearson (1976). Oxalate content was determined by a modified method of Libert and Franceshi (1987). 1 gram of the sample was weighed and put into a 250 ml conical flask, it was soaked in a 100 ml distilled water and left for a period of 3 hours. It was later filtered through a double layer filter paper. Absorbance of the particles was determined spectrophotometrically at 420 nm. Thereafter, the oxolate concentration was deduced from a standard curve. Saponin content was determined by the method of Makkar and Becker (1996).

3.5 Experimental Pond

The experiment was carried out in a large 1500m^2 earthen pond. In other to provide separate compartments for the fishes to be exposed to varying percentages of the Avocado seed meal, cages were constructed for the rearing of the fishes. Construction of cages was done locally with a netlon material of 5.0mm mesh size as the walls of the cage, and mop stick wood used as the frames. Empty rubber bottles were attached to the sides to provide floating ability. The cages were constructed with the diameter of 3x3x2.5 Ft. They were arranged in rows and columns to form a block of fifteen cages, 10 feet apart from each other and a minimum of 2 ft underneath between the pond bottom and the cage bottom to keep cage waste from fishes. Cages were fastened to stakes to keep them floating around their positions away from weed beds and from the dykes to avoid unnecessary disturbance from man or animals which could result to stress on the fishes.

Plate 3.2 Experimental Pond with hapas containing experimental fishes

3.6 Experimental diet

The avocado seed meal (ASM) was incorporated as an ingredient in the formulation of the fish's feed at varying inclusion levels of 0%, 5%, 10% 15% and 20%. Formulation was done using Pearson square method with 35% crude protein to meet the requirement for *Oreochromis mossambicus* (Juveniles) as given by Jauncey (1982) and as proposed by Bureau and Cho (1994). The ingredients were ground, mixed, pelleted and sundried for 5 days before being bagged and tagged respectively according to their percentage of ASM inclusion.

3.7 Experimental Fish

Oreochromis mossambicus fingerlings were used in this research work and were obtained from the hatchery of the African Regional Aquaculture Center, Aluu, Port Harcourt, where the research was carried out. They were graded and those of similar sizes were selected and used for the research

Plate 3.3 Experimental Fish *Oreochromis mossambicus*

3.8 Experimental set up and Management

A total of One Hundred and Fifty (150) *O. mossambicus* fingerlings were used for the experiment. The fishes were randomly assigned to five (5) treatment groups having 30 fish per group for 155 days. Each treatment group had 3 replicates of seven (10) fishes per replicate. The five (5) treatment groups were tagged as T_1, T_2, T_3, T_4 and T_5 with T_1 representing the control group with no addition of the Avocado seed meal.

The daily feed rations were divided into two portions and fed to the fish morning (0700-0800 hrs) and evening (1600-1700 hrs) at 5% fish body weight. Growth performance was monitored bi weekly as they were measured for total body weight and length, and quantity of feed was adjusted as growth was observed.

Plate 3.4: Formulation of experimental feed

Plate 3.5 Preparation of hapas for the culturing of the experimental fishes.

3.9 Data Collection and Analysis

Records of measurement of length (cm), weight (g) and condition factor of individual fish were taken. The total length (TL) of each fish was measured from the tip of the snout (mouth closed) to end of the caudal fin with the aid of a meter rule. Body weight was measured using electronic digital balance (Brand L.HPT). The relationship between length and weight of the fish was established by simple linear regression using WINKS software. The variations in the length –weight represented by 'b" were also recorded.

The parameter of length –weight relationship of the cultured fish species was evaluated using the equation.

$W= aL^b$ (Rickter, 1973)…… (eq 1)

Where, W is weight of fish (g), L is length of fish (cm), 'a' is initial growth coefficient, and 'b' is growth coefficient. The constant "a" and 'b' was estimated after logarithmic transformation of eq1 using the least square linear regression according to Zar (1984) to give.

$Log_{10} W=log10^a +blog_{10}L$ …….. (eq2)

The 95% confidence interval (C1) of "b" was computed using the equation (Montgomery *et al*., 2012):

CI= b ± (1.96x SE) ……. (eq.3)

Where SE is the standard error of "b" the condition factor was calculated by formula according to (Pauly, 1983).

Condition factor (K) = $100\ W/L^3$

Where W is weight (g) and L is total length (cm)

Specific Growth Rate = $100 (\ln W_2 – \ln W_1)/t$

Where; W1 and W2 are the initial and final fish weight respectively, and t represent the duration of the final feeding trial.

Percentage survival = $N_1/ N_0 \times 100$

Where: N_1= Total number of fish survival in pond at the end of experiments.

3.10 Determination of water quality

During the studies, bi weekly sampling was carried out from the month of July 2021 to December 2021. All the sample bottles were washed and rinsed with the same pond water. Water samples were usually collected 10cm below the surface of the water in the ponds into 100ml plastic water bottle. Temperature, dissolved oxygen, nitrite, ammonia and pH were all measured in-stitu.

3. 13 PLASMA ENZYME ACTIVITIES

3.13.1 Aspartate Amino Transaminase (AST)

The Reitman - Frankel (1987) method was used to analyzed the enzyme because it can be performed as a manual calorimetric end-point technique. Kit with ready-made reagents containing a pyruvate solution was used in the assay. The kit contains the following:

1. Reagent 1: AST pH 7.5 phosphate buffered substrate containing 200mmol/L asparate.
2. Reagent 2: 2mmol/L – ketoglutarate solution
3. Reagent 3: colour reagent containing 1mmol of 1, 2, 4-dinitrophenyl hydrazine (DNPH).
4. Reagent 4: Pyruvate standard
5. Reagent 5: 0.4mmol/L sodium hydroxide (NaoH)

Procedure

1. Six test tubes were labeled B (Blank) and 1-5 Reagents were pipetted into them.
2. 0.1ml distilled water was added to each test tube and mixed thoroughly.
3. All test tubes were left at room temperature for 20 minutes
4. 5 mls of 0.4mmol/L NaOH was added to each test tube and mixed thoroughly
5. All test tubes were left for 5 minutes at room temperature
6. Absorbance of the pyruvate standard solution in a colorimeter using a green fitter was read and confirmed in a spectrophotometer set at 505nm wavelength.
7. Absorbance of each standard against its equivalent AST was plotted.

3.13.2 Alanine Amino Transaminase (ALT)

The reagents used for the analysis were: lOOmmol/ L of Buffer-phosphate, at pH 4, 200mmol/L of L-alanine and 2.0mmol/L of a-ketoglutarate.

Procedure

The procedure involved two main stages: Measurement against Reagent blank and measurement against sample blank.

1. **Measurement against reagent mark**

i. 0.1ml of sample was pipetted, into test tube, none was put into reagent black test tube.

ii. 0.5ml of reagent 1 (buffer was put into sample and reagent blank test cubes

iii. 0.1ml of distilled water was later added to reagent blank test tube and none to sample tube

iv. Both test tubes were mixed and allowed to stand for 30 minutes at 37°c.

v. 0.5ml of solution 2/2,4, - dinitrophenyl hydrazine was added to both tubes.

vi. Both test tubes were mixed and allowed to stand for 20 minutes at 25°c

vii. 5ml NaOH was added to both test tubes.

viii. Both tubes were mixed and sample absorbance was read against reagent blank after 5 minutes.

2. **Measurement against Sample blank**

i. 0.1ml of sample was pipetted into test tube A and 0.5ml of solution 1 was pipetted into test tube B.

ii. Content of both tubes were mixed and incubated for 30 minutes at 37^{0c}.

iii. 0.5ml solution was pipetted into sample blank test tube and moved into sample test tube.

iv. Contents of both test tubes were, mixed and allowed to stand for 20 minutes at 25°c.

v. 5ml NaOH solution was added to both test tubes.

vi. Contents of both test tubes were mixed and absorbance of sample against sample was read after 5 minutes

3.14 Statistical Analysis

Data obtained from the experiments were subjected to ANOVA using Statistical Package for the Social Sciences, (SPSS) version 17.0 for windows. A one-way analysis of variance was used to determine if there were difference in the variables among treatments.

4.0 RESULTS AND DISCUSSION

The results of analysis for some anti-nutritional composition of the *P. americana* revealed that the soaking in water for 24 hours and sun drying of the seeds, helped in reducing the level of tannins, phytate, oxalate, HCN and saponin, as can be seen in Table 4.1. The tannin for this study was 11.80 ± 0.03 for the wet seed and reduced to 3.67 ± 0.01 after processing. The fresh seed had 9.88 ± 0.02 of phytate as against 5.06 ± 0.01 for the dry seed. The Oxalate level in this study equally reduced from 358.39 ± 0.02 for the fresh sample to 248.92 ± 0.05 for the dry sample after been soaked for 24hours in water. Also, Saponin value in this study for fresh avocado seed.

Table 4.1 Phytochemical composition of the Avocado seed (wet & Dry)

Phytochemicals	Fresh avocado seed	Dry avocado seed
Tannin	11.80 ± 0.03	3.67 ± 0.01
Phytate	9.88 ± 0.02	5.06 ± 0.01
HCN	2.48 ± 0.03	0.91 ± 0.01
Oxalate	358.39 ± 0.02	248.92 ± 0.05
Saponin	2.06 ± 0.03	0.50 ± 0.01

The p-values are all less than 0.001, indicating that there are statistically significant differences between the mean concentrations of each compound in fresh and dry avocado seeds. Saponin was 2.06 ± 0.03 and reduced to 0.50 ± 0.01 after being processed.

The results of the proximate composition of *P. americana* seed as presented in Table 4.2 shows that protein in the seed was 4.39 g/100g. Table 4.3 indicated that protein content showed an increasing trend as the concentration of the compound increased. The control group has the lowest protein content (18.79%), while the 20% concentration group has the higher (24.51%). The standard deviation suggests consistent protein levels within each concentration group.

Table 4.2 Proximate properties of *P. americana* seed

Nutrients	Mean concentration	Standard deviation
Moisture	13.97	0.62
Lipid	1.05	0.07
Ash	2.54	0.00
CHO	71.12	0.00
Protein	4.39	0.00
Fibre	6.93	0.62

The Carbohydrate content decreases as the proportion of avocado seed added increases as can be seen in Table 4.3. The values indicate a consistent downward trend, with lower carbohydrate percentages in sample containing higher amounts of avocado seed.

The ash content which represents the minerals content present in the plant was 2.54 ± 0.00. The moisture content reported in this study indicated that the seed is rich in moisture.

Table 4.3 Ingredient Composition (%) and proximate composition (%) of experimental diets

Ingredients	T1(0%)	T2 (5%)	T 3 (10%)	T 4 (15%)	T 5 (20%)
Fisl Meal	25.00	25.00	25.00	25.00	25.00
SoyBean M.	25.00	25.00	25.00	25.00	25.00
Wheat Bran	37.50	37.50	37.50	37.50	37.50
Bone Meal	1.50	1.50	1.50	1.50	1.50
Palm Oil	5.00	5.00	5.00	5.00	5.00
Garri	5.00	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25

Methaorine	0.15	0.15	0.15	0.15	0.15
Vit C	0.10	0.10	0.10	0.10	0.10
ASM	0.00	.075	1.50	2.25	3.00

Proximate composition (%)

Parameter					
Moisture	4.30 ± 0.02	4.39 ± 0.02	4.43 ± 0.02	4.42 ± 0.02	4.46 ± 0.03
Protein	18.79 ± 0.02	22.87 ± 0.06	23.28 ± 0.02	23.88 ± 0.03	24.51 ± 0.03
Fat	5.26 ± 0.02	5.75 ± 0.03	6.09 ± 0.01	6.20 ± 0.01	6.64 ± 0.03
Ash	7.71 ± 0.01	8.10 ± 0.01	8.24 ± 0.02	8.51 ± 0.01	8.60 ± 0.02
Fiber	5.44 ± 0.03	5.80 ± 0.01	6.27 ± 0.03	6.67 ± 0.01	6.79 ± 0.08
CHO	58.50 ± 0.06	52.80 ± 0.21	51.69 ± 0.43	50.32 ± 0.10	49.43 ± 0.31
Energy K_{cal}	356.51 ± 0.20	356.22 ± 0.67	354.67 ± 0.55	352.96 ± 0.16	353.19 ± 0.24

According to table 4.3, the moisture content of the samples remains relatively consistent across the different proportions of avocado seed added in the formulated feed. The values show slight variations within the specified range but overall do not exhibit a significant change.

Similar to the protein, the fat percentage also increases as the proportion of avocado seed added to the formulated feed increases. The values indicate a gradual rise in fat content with increasing *P. amaricana* seed proportions, Table 4.3. The fibre which is a type of dietary material found in the cell walls of plant-based foods. Fibre is composed mainly of polysaccharides such as cellulose and pectins. The value for fibre shows a consistent upward trend indicating higher fiber percentages in sample with greater *P. americana* seed according to Table 4.3.

The proximate composition of the experimental feed prior to feeding as seen in Table4. 3, reveals that moisture, protein, fat, ash and fiber all experienced an increase value with increase in percentage of addition of *P. amaericana* seeds with 20% having the highest values for all against the control samples. The significant increase that is observed in crude protein and liquid contents of the fish feed containing *P. americana* seeds in this study, suggests improvements in protein synthesis attributed to the presence of *P. americana* seed in the feed.

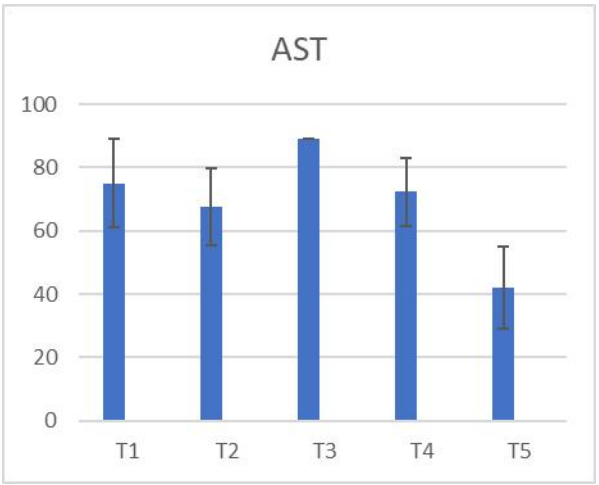

Figure 4.1 Concentration of Aspartate Aminotransferase (AST) in the different treatment groups

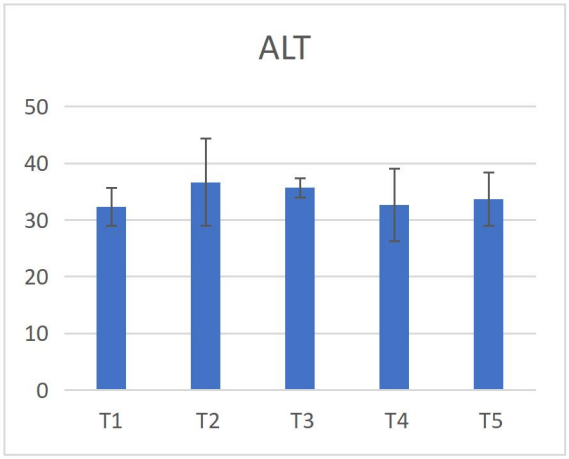

Figure 4.2 Concentration of Alanine Aminotransferase in the different treatment groups.

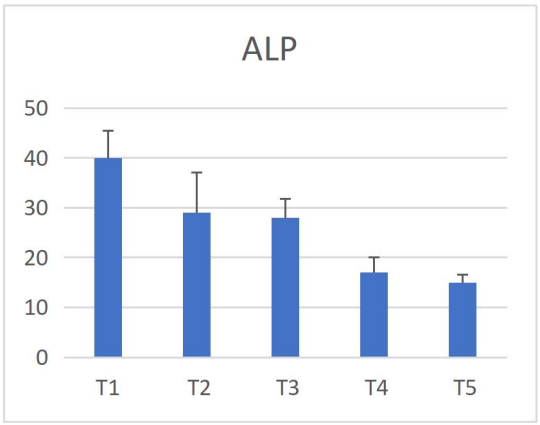

Figure 4.3 Concentration of Alkaline phosphate in the different treatment groups.

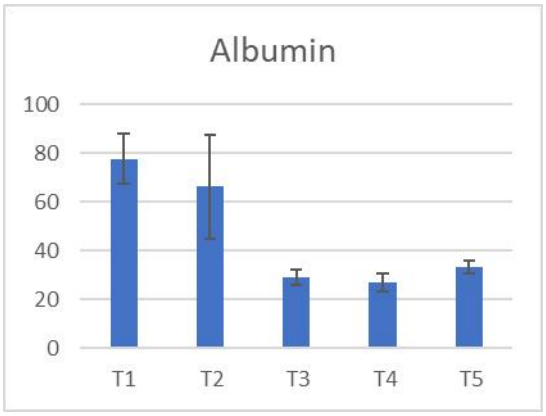

Figure 4.4 Concentration of Albumin in the different treatment groups.

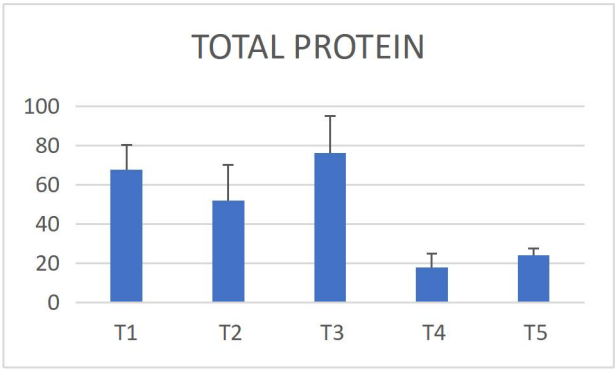

Figure 4.5 Concentration of Total Protein in the different treatment groups.

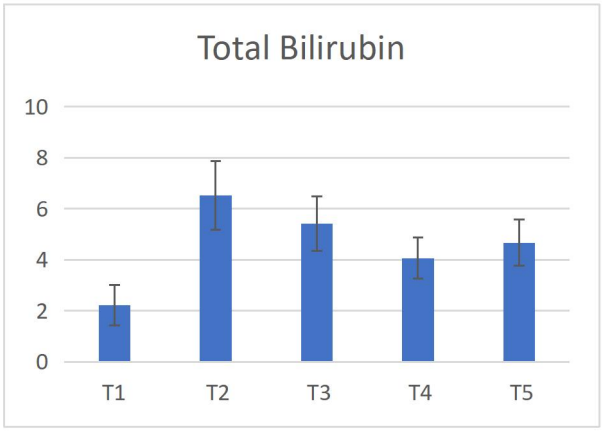

Figure 4.6 Concentration of Total Bilirubin in the different treatment groups

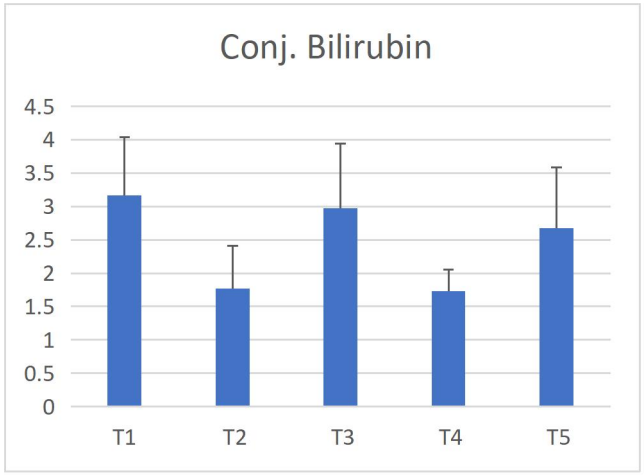

Figure 4.7 Concentration of Conjugate Bilirubin in the different treatment groups

Table 4.1 Phytochemical composition of the Avocado seed (wet & Dry)

Phytochemicals	Fresh avocado seed	Dry avocado seed
Tannin	11.80 ± 0.03	3.67 ± 0.01
Phytate	9.88 ± 0.02	5.06 ± 0.01
HCN	2.48 ± 0.03	0.91 ± 0.01
Oxalate	358.39 ± 0.02	248.92 ± 0.05
Saponin	2.06 ± 0.03	0.50 ± 0.01

4.1 Liver

Enzymes assays such as AST, ALT, and ALP are parts of standard laboratory test to detect abnormalities in animals (Ayalagu *et al.,* 2001; Saka *et al.,* 2011). Changes in these enzymes activities resulting from exposure to therapeutic application in plasma of fish have been reported (Ribas *et al.,* 2007; Shalaby *et al.,* 2006). AST levels in the 5%, 15%and 20% treatment groups were67.67 ± 12.1, 72.33 ± 10.8, and 42 ± 13.1 respectively. Notably, the AST level in the 10% treatment group 89 ± 0 is significantly higher than in the control group 75 ± 14 ($P<0.05$). The AST result indicates that there were associated disease processes affecting hepatocytes such as viral hepatitis, metabolic liver diseases, because high level of Aspartate aminotransferase (AST) is a pointer to hepatitis. A reduced AST in the 20% inclusion of avocados seed meal suggest the therapeutic potential of the treatment against hepatotoxicity.

The ALT levels in all treatment group (5%, 10%, 15% and 20%) are relatively consistent, ranging from 32.67± 6.39 to 36.33±3.33UL. There are no significant differences in ALT levels among the treatment groups compared to the control group. However, ALT within the normal range of 15/42 IU/L also suggest that the avocado seed meal is not toxic to the liver of fish. Hence a healthy food supplement.

Alkaline phosphatase (ALP) indicates the levels of liver disease or some bone disorders. In the present study, ALP showed a downward consistent trend as the proportion of *P. americana* seed diet increased. This could be as a result of the therapeutic properties of the avocado seeds rich in antioxidants

and antimicrobial compounds. The control group has an ALP level of 40±5.51^{a}Ul. ALP levels in the 5%, 10%, 15% and 20% treatment group are 29±8.08^{ab}, 28±3.79^{ab}, 17±3.06^{b} and 15±1.53^{b}, respectively (P<0.05).

The control group has a mean albumin level of 77.67±10.2^{a}g/l. The albumin level in the 15% treatment group is significantly lower than the control (P<0.05) Table 7. Total protein levels in the treatment groups range from 17.67±7.17^{b} to 76.33±18.8^{a}g/l. The control group has a mean total protein level of 67.67±12.7^{a} g/l. The total protein level in the 15% treatment group is significantly lower than in the control group (P<0.05), while the 10% treatment group has a significantly higher total protein level (P<0.05). The total bilirubin levels range from 2.23±0.79 to 6.53±1.35µmol/L. There was no significant difference in total bilirubin levels among the treatment group compared to the control group. Bilirubin is the product of haemoglobin breakdown. When there is a raised bilirubin level, it is primarily as a result of haemolysis. This produces lipophilic unconjugated bilirubin in the blood, which is further conjugated (made hydrophilic) in the liver cell and later excreted through the biliary tree (excreted in the bile). The total bilirubin is a sum of the conjugated and unconjugated bilirubin. The bilirubin can be a useful marker of liver function, as bilirubin rises with increasing severity of liver disease. Bilirubin is an indication of the detoxification/excretory function of the liver

The levels of conjugated bilirubin range from 1.73±0.32to 3.17±0.87µmol/L. There was no significant difference in conjugated bilirubin levels among the treatment groups compared to the control group.

Table 4.6: Water quality parameters

Months	Turbidity	BOD	COD	Total Hardness	Alkalinity	pH	Temperature	DO	Nitrite	Ammonia
July	10.71±0.6	6.85±1.15^{c}	32.5±1.5^{bc}	9.61±0.59	13.5±2.5	6.15±0.65	27.55±0.45	4.5±1.5	0.01±0	0.2±0
August	41.23±36	6.9±2.1^{c}	34±12^{bc}	11.02±5	14±0	6.8±0	26±1	4.5±2.5	0.01±0	0.2±0
September	32.46±10	8.16±1.44^{bc}	69±1^{a}	7.01±1	15±1	6.8±0	28±0	3±0	0.01±0	0.2±0
October	31.58±9.47	8.8±0.8^{bc}	26±6^{c}	15.06±3.06	18±2	6.8±0	28.5±0.5	3±0	0.01±0	0.2±0
November	11.93±0	15±1^{a}	52±0^{ab}	11.01±1	12.8±1.2	6.8±0	29±0	4±0	0.01±0	0.2±0
December	12.28±0	14±0^{ab}	44±0^{abc}	12.01±0	16±0	6.8±0	27±0	6±0	0.01±0	0.2±0
p-value	0.736	0.036	0.028	0.523	0.370	0.584	0.083	0.749	–	–

Means in the same column followed by different superscripts differ significantly (p<0.05)

4.2 Kidney

Sodium:

In this current study, the control group has a mean Sodium level of 102.67mmol/L. Sodium levels in the 5%, 10%, 15% and 20% treatment groups are 146.33, 155.33,153.00 and 140.33mmol/L, respectively as seen in Figure 4.1. The Sodium levels in the treatment groups are significantly higher than in the control group (P<0.05). Additionally, the Sodium level is highest in the 10% treatment group.

Potassium:

The potassium levels range from 8.57 to 9.57mnol/L across all groups (Figure 4.5). There are no significant differences in the Potassium levels among the treatment groups compared to the control group (P>0.05).

Bicarbonate:

The control group has a bicarbonate level of 31.00mmol/L. Bicarbonate levels in the treatment groups ranged from 31.00 to 32.67mmol/L (Figure 4.6). The bicarbonate levels in the treatment groups are slightly higher but no significantly different from the control group(P>0.05).

Chloride:

The chloride levels in all groups range from 118.00 to 121.33mmol/L. There are no significant differences the chloride levels among the treatment groups compared to the control group. There was a downward gradient of the values of chloride from the control group to the 20% dietary group. Figure 4.7

Urea:

The Urea levels range from 22.23 to 35.33 mmol/L. The Urea level in the control group is significantly higher than in the 5% treatment group (P<0.05).

Creatinine:

The control group had the list value of creatinine with 1.00 mmol/L (Figure 4.9). There is no significant difference in creatinine levels among the treatment groups (P>0.05).

Table 4.7: Liver function parameters for *O. mossambicus* fed graded level of *P. americana*

Parameters	**Control**	**5%**	**10%**	**15%**	**20%**	**p-value**
AST (UI)	75±14	67.67±12.1	89 ± 0	72.33±10.8	42±13.1	0.126
ALT (UI)	32.33±3.33	36.67±7.67	35.67±1.67	32.67±6.39	33.67±4.67	0.967
ALP (UI)	38.00±8.09^{a}	29±8.08^{ab}	28±3.79^{ab}	17±3.06^{b}	15±1.53^{b}	0.029
Albumin (g/l)	77.67±10.2^{a}	66.33±21.4^{ab}	29±3^{ab}	27±3.51^{b}	33.33±2.6^{ab}	0.021
Total protein (g/l	67.67±12.7^{a}	52±18^{ab}	76.33±18.8^{a}	17.67±7.17^{b}	24±3.46^{b}	0.041
Total bilirubin (Mmol/L)	2.23±0.79	6.53±1.35	5.43±1.07	4.07±0.8	4.67±0.9	0.105
Conjugated bili. (Mmol/L)	3.17±0.87	1.77±0.64	2.97±0.97	1.73±0.32	2.67±0.91	0.584

Means in the same column followed by different superscripts differ significantly (p<0.05)

Key: ALT -Alanine transminase; AST - Aspartate transaminase; ALP -Alkaline phosphatase.

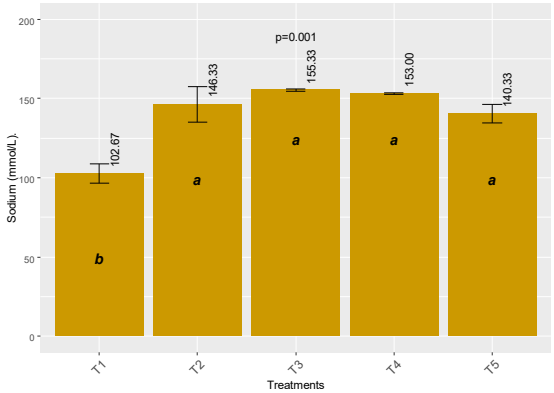

Figure 4. 11.Sodium levels in kidney test, Bars representing treatments with different letters within, differ significantly (p<0.05)

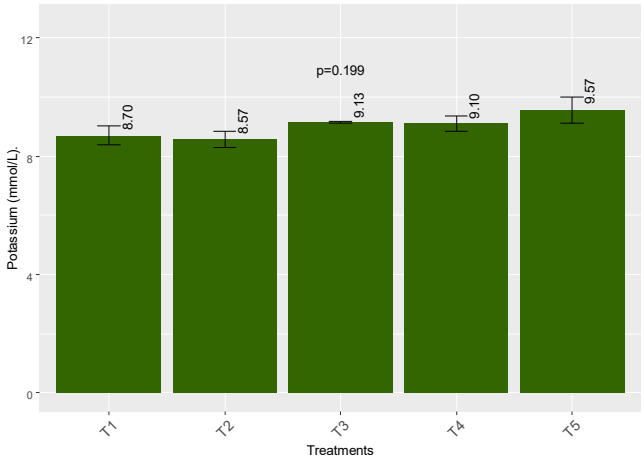

Figure 4.12. Potassium levels in kidney test, no significant difference between treatments (P>0.05)

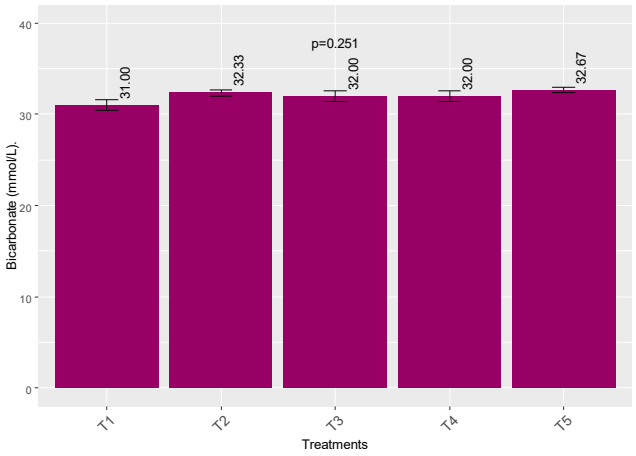

Figure 4.13. Bicarbonate levels in kidney test, no significant difference between treatments (p>0.05)

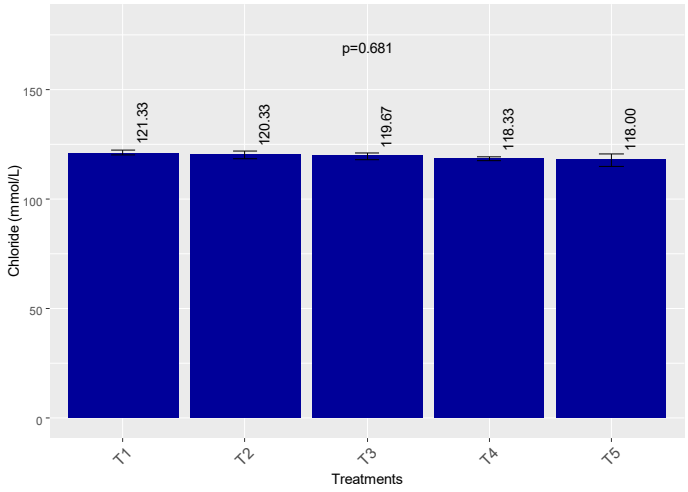

Figure 4.14. Chloride levels in kidney test, no significant difference between treatments (p>0.05)

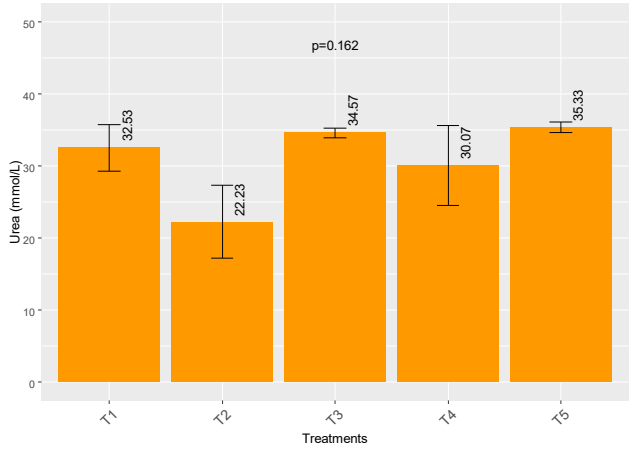

Figure 4.15. Urea levels in kidney test, no significant difference between treatments (p>0.05)

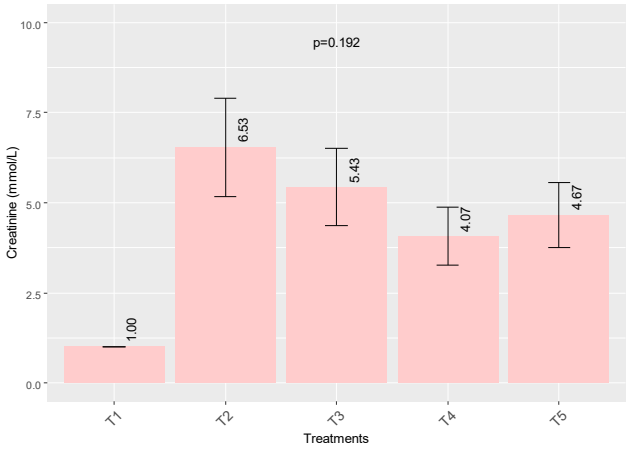

Figure 4.16. Creatinine levels in kidney test, no significant difference between treatments (p>0.05)

The liver enzymes are in two main groups – Alkaline phosphatase (ALP) and ɣ–glutamyl transpeptidase (GGT) are produced predominantly by the bile ducts; whereas Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST), are produced predominantly by hepatocytes. Hence these two groups of liver enzymes indicate cholestasis and liver cell integrity, respectively

When ALP is raised more than its upper limit of normal ULNit suggests a cholestatic pattern indicating biliary pathology. A more chronic effects may be due to pancreatic, liver or bile duct tumours, (Giannini *et al* 2005)

ALT and AST are produced mostly by hepatocytes, hence, abnormalities in ALT and AST are fairly specific to the liver. However, when ALT or AST are raised more than their ULNIt reflects a disease process affecting hepatocytes such as viral hepatitis, metabolic liver diseases, drug or alcohol toxicity and autoimmune hepatitis

In this study, the activities of enzymes assays such as AST, ALT, and ALP decreased as the proportion of the *P. americana* seed meal increased. This agrees with the report of Ukwe and Etire, (2021) where the leaves of *P. americana* was administered on *C. gariepinus* after being infected with *Pseudomonas aeruginosa.* Also, Fadi *et al.,* (2013) observed reduction in these enzymes when Nile tilapia fed cyanobacteria was infected with *A. hydrophila.* There was a consistent reduction of ALT level in all treatment groups (5% - 20%) but without clear significant difference amongst the treatment groups compared to the control group. This reduction suggests that the *P. americana* does not express any hepatotoxicity to the fish. Similarly, there were no clear trend or consistency in variation for ALP, Total Protein, Total Bilirubin and Albumin. These results of relatively normal concentrations of liver enzymes in this study strongly substantiate that *P. americana* can pass for a good food supplement for fish, having being feed supplementation of as much as 20% in feed. The ALP levels reported in this study disagrees with the report by Ukwe and Oladapo-Akinfolarin, (2019) where the ALP increased in the infected fish groups. The increase in ALP in this case must have been as a result of the infections in the studied groups.

The AST and ALT plays an important role in the utilization of amino acid for the oxidation activities and gluconeogenesis, hence the possible reason for the increased Total Protein of 76.33 ± 18.8^{a} recorded for the 10% group as well. According to Daniel, (2009) the function of AST and ALT are links between carbohydrate and protein metabolism under altered physiological, pathological and stress induced environmental conditions. The increase in the ALT as the proportion of the *P. americana* increases agrees with the findings by Khalil *et al.,* (2011) who postulated that increase in AST and ALT levels in plasma was associated with hematopoietic organs damage when *Anguilla anguilla* was experimentally infected with *Vibrio anguillarum.* Similarly, the decrease of AST and ALT levels as the percentage of *P. americana* seedmeal increased, agrees with an earlier study by Bardhan *et al.,* (2022), who reported that ALT and AST levels were negatively influenced by dietary FFC in a dose-dependent manner, in an experiment to check the Biological Responses of Nile tilapia *Oreochromis niloticus* as Influenced by Dietary Florfenicol. Though the ALT and AST had a slight increase above the value of the control, the increase were not significant and were within the normal range of 15-42IU/L for a healthy fish. Hence were not seen to suggest any hepatotoxicity.

Albumin is a protein synthesized exclusively in the liver. As such it is a marker of liver synthetic function and liver health. Albumin levels can be reduced by many kinds of illnesses and so has relatively low specificity as a marker of liver function

The study revealed that albumin (g/L) was highest in the control group (77.67 ± 10.2^{a}) than the 20% group (33.33 ± 2.6^{ab}) in a consistently decreasing pattern. There was an observed increasing trend of the concentration of compound as the level of avocado seed meal was added, with the group having the highest concentration of 20% recording 24.51% against the control group with 18.79%. However, the carbohydrate content as revealed in the proximate analysis of the experimental diet, indicated a downward decrease as the proportion of the avocado increased from 5% to 20%, and could be attributed to the soaking in water for 24hours as reported by Justina *et al.,* (2016), who recorded reduced carbohydrate (29.66 ± 0.11) of avocado soaked for 24hours, against (52.75 ± 0.09) for seeds boiled at 100^{o}C for 20min.

There was also a positive response of *O. mossambicus* to the feeding trials in all the fish groups fed *P. americana* seed diet of different proportions. The SGR increased as the level of *P. americana* seed meal inclusion increased in the diet, with its highest (2.96 ± 0.06) recorded for the 20% group, and the least value (2.58 ± 0.04) for the control group (Table 4.5). This indicate that *P. americana* seed powder improved the fish growth positively. Also, the

downward trend of FCR values from the control to the group with the highest proportion of *P. americana* seed, indicated that there were higher FCR efficiency as the proportion of the avocado seed meal increased. The survival rate was seen to be highest amongst the control group and 10% group.

CONCLUSION

The feeding trial of *O. mossambicus* with diet of *P. americana* that lasted for 155 days revealed that *P. americana* seed is safe for incorporation into animal feed. Although haematological parameters in this study revealed no significant difference (P>0.05) in the values of PCV, Hb, RBC, WBC, MCH, MCV and MCHC of the fish fed the experimental diets compared to the control at the end of 22 weeks, there were some variations of PCV values across the different diet groups. The 15% diet group had the least value for Red Blood Cells, Haemoglobin, Packed Cell Volume and Neutrophils. Alkaline phosphatase (ALP) indicates the levels of liver disease or some bone disorders. Assessing organ health, particularly the liver and kidney, can be complex. Observing and interpreting histopathological changes might be limited by the sample size, precision of the techniques, or potential variability in fish health unrelated to diet.

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