WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Effects of Avocado (*Persea Americana*) Seed Meal on Hematological Parameters and Liver Enzymes of Mozambique Tilapia *(Oreochromis Mossambicus)*

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DOI : https://doi.org/10.55248/gengpi.5.1224.250132

ABSTRACT

Effects of avocado (*P. americana*) seed meal on hematological parameters and lipid profile of Mozambique tilapia was studied.150 Mozambique Tilapia were used for the experiment. They were divided into five groups of 30 fish per group. Each group was treated with different levels of Avocado seed meal inclusion in their diet. Groups1,2,3,4, and 5 were included as 0% 5% 10%, 15%, and 20% respectively for 155 days. Result indicate that the treatment did not cause any significant difference ($p>0.05$) in the hematological parameters. However, the red blood cell (RBC) and white blood cell (WBC) and monocytes had substantial increase. while the platelet, mean Corpuscular hemoglobin concentration (MCH) mean corpuscular volume eosinophils were reduced though not statistically significant. leucocytes and. The liver enzyme test result showed that ALP was significantly lowered by the treatment from a value of 38.00±8.09 to a value of 15±1.53 in the group 5. 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. It is therefore concluded that Avocado seed meal is a safe feed additive for fish feed.

Key word: Avocado, blood parameters, liver enzyme fish feed.

INTRODUCTION

Oreochromis mossambicus, commonly known as the Mozambique tilapia, is a species of tilapia that is widely cultured in aquaculture systems. Several studies have investigated different aspects of *O. mossambicus* culture, including its biology and genetic characteristics. They are endemic to southeastern Africa and are most frequently found in Mozambique's Zambezi River basin and other bodies of water. It belongs to the Cichlidae family, which also includes numerous other well-known farm fish species.

There is wide dispersal of *O. mossambicus* due to human introduction for fish farming across many tropical and subtropical habitats around the world, and it can become an invasive species due to its hardy nature. In South Africa, they are known *as* blue kurper and black tilapia in Colombia. Mossambicus tilapia possesses remarkable adaptive traits that enable it to tolerate large and rapid salinity fluctuations such as trout and Nile tilapia, exhibits opportunistic omnivorous characteristics feeding on algae, plant matter, organic particles, small invertebrates and other fish (Akunga. G. N. (2019). It is easy for Mozambique tilapia to colonize different environments due to their broad diets.

Also, several studies have investigated the effects of different dietary components on the growth and health of tilapia. One study by Figueiredo-Silva *et al.,* (2014) examined the effect of DL-methionine supplementation on the success of replacing fish meal with soybean meal in diets for hybrid tilapia. The study found that dietary fat above a certain level reduced the growth of Nile tilapia, but other studies indicated a protein-sparing effect of dietary fat in hybrid tilapia. This suggests that the dietary requirements of tilapia may vary depending on the specific species or hybrid. Another study by Olvera-Novoa *et al.,* (2002) investigated the utilization of torula yeast as a protein source in diets for tilapia fry. The study observed growth depression when more than 10% of the fish meal protein was replaced with the bacterium Micrococcus glutamicus in diets for tilapia. This highlights the importance of considering the composition and source of protein in tilapia diets.

Rapeseed meal has also been studied as an alternative protein source for tilapia diets. Davies *et al.,* (1990) explored the potential of rapeseed meal as a protein source in complete diets for tilapia. The study found that rapeseed meal could be a viable alternative to fish meal in tilapia diets, suggesting the possibility of diversifying the protein sources in tilapia feed.

In terms of health and disease resistance, Chia, *et al.,* (2011). investigated the effects of herbs and spices as feed additives on the health status of tilapia challenged with *Streptococcus iniae*. The study found that thyme, rosemary, and fenugreek as feed additives had positive effects on haematology, innate immune response, and disease resistance of tilapia. This suggests that dietary interventions, such as the inclusion of specific herbs and spices, can

enhance the health and disease resistance of tilapia. Waiyamitra *et al.,* (2021) in a recent study, inoculated Mozambique tilapia with a virulent strain of Tilapia tilapinevirus (TiLV) to investigate the infection of TiLV in the fish species. The study examined the effects of different concentrations of the virus on the tilapia.

In terms of genetic differentiation, Mossambicus tilapia has been found to exhibit relatively low genetic variability compared to other tilapia species (Mather & Arthington, 1991). This is important for understanding the genetic diversity and population structure of Mossambicus tilapia populations. Muhtadi *et al.,* (2021) in a study conducted in Siombak Tropical Coastal Lake in North Sumatra, Indonesia aimed to determine the population dynamics of *O. mossambicus*, including aspects of growth, reproduction, recruitment, mortality, feeding habits, and the rate of exploitation. The research provided insights into the ecological characteristics and population dynamics of Mossambicus tilapia in specific environments.

The avocado (*Percea americana*) is a tree classified as a member of the flowering plant family Lauraceae and originating from Mexico and Central South America (Tassew *et. al,* 2019). It is now grown in a variety of subtropical and tropical places around the world. Mexico is still the leading avocado grower. It is a fruit known colloquially as an "avocado pear" because of its form and texture. It is crucial to clarify, however, that avocados are not botanically classified as pears; rather, they are a sort of berry. The fruit of the plant, also called an avocado, is a large berry containing a single seed (Storey, 1973). It weighs 50 grams to 1 kilogram with 50-80% and the seed, 10 – 20% of its total weight (Nwaokobia *et. al*, 2018).

According to Maitera *et al.,* (2014), the edible part (pulp) is fleshy and contains 65-80% water; 1-4% protein; 1-2% sugar and 3-30% oil. The avocado is naturally enriched with plenty of B vitamins, and moderate amount of vitamins A, D and E. The presence of digestible oil makes it to have the highest energy than any other fruits. Biale & Young, (1971) stated that the oil content of the fruit depends upon its ecological origin and on the cultivar, as for example, in Guatemalan and Mexican cultivars, the oil content varies from 10 to 13% and from 15 to 25%, respectively while in the fruits from Caribbean, a low fat (2.5 to 5%) has been reported (Fagbenro, and Adeparusi, (2003).

It is however difficult to detect adulteration of olive oil with avocado oil to a level of 35% on the basis of flavour as the avocado oil possesses similar characteristics to that of olive oil (Gutfinger & Letan 1974;). Fats and oils play important roles in human nutrition and their sources, composition and extraction process determine their end use. Reports by Ihekoronye, (1999) states that oils from major oilseeds like groundnuts, palm fruits, sunflower seed, safflower seed and soybean have been utilized in the manufacture of margarine with success. Maitera *et al.,* (2014) reported that ripe avocado pear deteriorates rapidly due to softening and discoloration of fruit pulp that is attributable to microbial attack and oxidative changes (Okaka, 2005).

According to Maitera *et al.,* (2014), the avocado also contains the following: thiamine (Vitamin B1); riboflavin (vitaminB2); niacin (vitamin B3); pantothenic acid (vitamin B5); vitamin B6, folate (vitamin B9); and vitamins C, E and K. Also, recorded mineral elements include calcium 12mg; iron 0.55mg; magnesium 29mg; phosphorus 52mg; zinc 0.64mg and potassium 485mg. avocado contain 35% more potassium than banana which has 358mg per 100g. 75% of the high fibre content is insoluble while 25% is soluble (Naveh *et al.,* 2002).

Avocados are typically pear-shaped with thick, tough skin that ranges in color from green to black depending on type and ripeness. The skin is inedible and must be removed before eating. Inside, the flesh is creamy and varies in hue from pale green to yellow. It is high in monounsaturated fats, which are good for the heart. Avocados are also high in vitamins and minerals such as vitamin E, vitamin K, vitamin C, potassium, and folate. Avocado eating has been linked to a variety of health advantages due to its nutritional content. Improved cardiovascular health, better digestion, weight management, and possibly anti-inflammatory properties are among them. According to Monge *et al.,* (2022) Avocado is rich in dietary fiber, potassium, magnesium, mono and polyunsaturated fatty acids, and bioactive phytochemicals, which are nutritional components associated with cardiovascular health

Fish haematology studies are essential for evaluating the health and well-being of fish. These studies provide valuable information about the physiological and pathological changes in fish (Karapanagiotidis *et al.,* 2018; Satheeshkumar *et al.,* 2011; Seibel *et al.,* 2021; Maqbool & Ahmed, 2014; Fazio *et al.,* 2020; Tang *et al.,* 2015). Haematological parameters, such as red blood cell counts and biochemical assessments, serve as important diagnostic tools in monitoring the health status of fish species in aquaculture (Maqbool & Ahmed, 2014; Fazio *et al.,* 2020).

They can also detect and diagnose metabolic disturbances and diseases in fish (Restiannasab *et al.,* 2014). Hematological studies of fish can be traced back to 1943 (Field *et al.,* 1943), and since then, several studies have investigated various factors that can influence the haematological parameters of tilapia. It can be used in monitoring fish health (De-Pedro *et al*, 2005). Haematological and biochemical changes in blood are important indicators used in monitoring physiological and pathological changes in fish (Satheeskumar *et al,* 2011 Gabriel *et al.,* 2011 Parrino *et al.,* 2018 Fazio *et al.,* 2013a),

Weymann *et al.,* (2017) focused on the prediction of new-onset and recurrent atrial fibrillation (AF) using complete blood count tests. Fish haematology studies have shown that fish consumption, particularly lean fish, is associated with beneficial changes in metabolic syndrome components and decreased blood pressure (Tørris *et al.,* 2017). On the other hand, exposure to toxic chemicals and pesticides can impact fish haematology (Sinha *et al.,* 2022). Additionally, the presence of parasites in fish can cause changes in haematological indices, providing insights into fish health (Restiannasab *et al.,* 2014; Úngari *et al.,* 2022). In aquaculture, haematological parameters are used as diagnostic tools to assess the overall health, nutritional status, and water balance of various fish species (Hassan *et al.,* 2022; Lopes *et al.,* 2022). These parameters can also be used as physiological indicators of stress and energy expenditures in fish (Lopes *et al.,* 2022). Furthermore, haematological studies have been used in ecological monitoring of reservoirs to assess water pollution (Serpunin & Likhatchyova, 1998).

Another study by Khawaja *et al.,* (2013) compared the growth performance, meat quality, and haematological parameters of different crossbred chickens in a subtropical environment. The study found that haematological parameters can be influenced by diurnal fluctuations and changes in daily physical and metabolic activities. This highlights the importance of considering environmental factors when interpreting haematological parameters. In the field of clinical laboratory testing, Triplett *et al.,* (2019) conducted a study comparing the results obtained from blood gas analyzers and laboratory

auto-analyzers for haematological and biochemical parameters. The study found controversy over the agreement between the two methods, suggesting the need for careful interpretation of haematological parameters obtained from different testing platforms.

Gitaka *et al.,* (2017) conducted a cross-sectional observational study to establish clinical laboratory reference values for haematological parameters in children aged 4 weeks to 17 months. The study examined parameters such as haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, mean corpuscular volume, platelets, and white blood cell differentials. This study provides valuable reference values for haematological parameters in this specific age group. Furthermore, Chen & Luo (2022) discussed the application of haematology parameters for health management in fish farms. The study emphasized the importance of haematological parameters as indicators of fish health and the need for regular monitoring to detect potential diseases or stressors in fish populations.

The diagnosis of disease using hematological analysis is especially important because it can provide a reliable evaluation via non-lethal means (Satheeshkumar *et al.,* 2012). Haematological analysis in fish can be conducted using automated haematology analyzers, which provide accurate and efficient results (Parrino *et al.,* 2018). This method is particularly useful for determining total white blood cell counts and differential counts in fish species (Hrubec *et al.,* 2000). Haematological parameters act as physiological indicators to changing external environments (Caruso *et al.,* 2005) as a result of their relationship with energetic (metabolic levels), respiration (haemoglobin) and defense mechanisms (leukocyte levels). It has been widely used in freshwater fish pathology to assess the health status of fish (Parrino *et al.,* 2018). Evaluation of hematologic analysis is beneficial for fish cultivation as it facilitates early detection of situations of stress and/or diseases that could affect production performance (Rehulka *et al.,* 2004). Several studies have investigated the haematological parameters of tilapia, specifically focusing on factors such as diet, plant extracts, and environmental conditions. Jerônimo *et al.,* (2011) investigated the influence of seasonality and fish farm handling characteristics on haematological parameters in Nile tilapia. The study found that certain parameters, such as thrombocytosis and lymphocytosis, varied with the seasons and differed among fish farms. This suggests that seasonal variations and farm-specific factors can impact the haematology of tilapia.

Malathi *et al.,* (2012) studied and compared the baseline values of the hematological parameters for spotted snakehead (*Channa punctatus*) and stripped snakehead (*Channa striatus*) from freshwater bodies of Cauvery delta, Thanjavur, India. They reported that there is a slight difference between the two species. The RBC count, hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) showed higher value in *C. punctatus* and lower value in *C. striatus* (Fazio, F. 2019). Yakubu *et al.,* (2022) evaluated the acute toxicity of *Terminalia catappa* leaf extract on the blood profile and histopathology of vital organs in tilapia. The study used liquid chromatography tandem mass spectrometry to profile the metabolites present in the leaf extract. The results showed that the leaf extract had an impact on the complete blood counts and plasma biochemical analysis of tilapia

Sirimanapong *et al.,* (2018) examined the effect of a *Francisella noatunensis* (Fno) infection on hybrid red tilapia subsequently infected with *Streptococcus agalactiae*. The study measured various haemato-immunological parameters and found that the presence of an Fno infection affected the haematological profile of the fish. This highlights the importance of considering the potential interactions between different pathogens and their impact on haematological parameters in tilapia. Smith *et al.,* (2018) examined the hematological profile of Nile tilapia (*Oreochromis niloticus)* under different water temperatures.

Another study by Ng *et al.,* (2006) investigated the effects of substituting marine fish oil with palm oil-laden spent bleaching clay in the diets of Nile tilapia. The study found that the dietary treatments did not affect the haematocrit of tilapia, indicating that the substitution did not have a significant impact on the blood parameters of the fish. Afrose *et al.,* (2022) examined the effects of dietary synbiotics on the growth performance and haematological properties of tilapia. The study concluded that diets containing synbiotics had constructive effects on the growth and haematological parameters of tilapia compared to the control treatment. Diets with synbiotics, specifically a combination of sugar and probiotics, were found to be the most effective in promoting growth and maintaining haematological health in tilapia.

Study by Jones *et al.,* (2019) investigated the effect of dietary supplementation with probiotics on the hematological parameters of rainbow trout (*Oncorhynchus mykiss*). The results showed that probiotic supplementation significantly increased WBC count and enhanced immune response in the fish.

Feist *et al*, (2000) reported that the composition of blood can be changed by dietary treatment, malnutrition and disease condition, while Ferreira *et al.,* (2007) reported that biochemical parameters provide early warning of potentially harmful changes in stressed organisms. Oriakpono and Hart (2012) reported a significant reduction in red blood cells and haemoglobin of *Sarotherodon melanotheron* exposed to various levels of crude oil. Conversely, *George et al.,* (2020) reported a progressive increment in the platelet level with increase ratio of Avocado Seed Meal (ASM) inclusion, on broiler chickens.

Akinduro *et al.,* (2021) reported significant difference in the haematological parameters of broilers fed with dried Avocado Seed meal. Jimo *et al.,* (2015) also reported increase in haematological parameters of the Nile tilapia fed with diets containing C*itrullus lanatus* seed meal. According to Togun and Oseni (2005), haemoglobin indices such as RBC, WBC, PCV and Hb have been found useful for disease prognosis and for therapeutic as well as feed stress monitoring. Garcia-Lopez *et al.,* (2020) studied the hematological characteristics of European sea bass (*Dicentrarchus labrax*) during a parasitic infection with *Sparicotyle chrysophrii*. The study revealed alterations in RBC count, Hb concentration, and WBC count as a result of the infection, indicating a potential impact on fish health.

However, the standardization of haematological parameters is difficult in fish because these parameters can be influenced by deficient diets, diseases and environmental stress situations (Silveira and Rigores, 1989). Nevertheless, the analysis of these parameters may improve the diagnosis of fish health (Chen, and Luo, (2022).

Overall, fish haematology studies play a crucial role in understanding the health and well-being of fish. They provide valuable information about physiological and pathological changes, serve as diagnostic tools in aquaculture and ecological monitoring, and shed light on the effects of various factors on fish health. The hematological parameters are an important tool to express the conditions of fish health (Datta *et al.,* 2018, Olapade and Lombi, 2015).

Materials and Method

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). Fifteen floatable cages were constructed and used in the culture of experimental fishes in a complete randomized set up.

The avocado seeds were purchased from Akpan –Andem market, a popular market in Uyo, Akwa Ibom State. They were washed and soaked in large rubber containers with cold water for 24 hours They were then sliced and sun-dried for 7 days. The dry seeds were ground and stored in an airtight container.

Experimental Pond

The experiment was carried out in cages of 3x3x2.5 Ft diameter in a large 1500m^2 earthen pond. The cages 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.

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 groups 1 (0%), 2 (5%), 3 (10%) 4 (15%) and 5 (20%). Formulation was done using Pearson square method with 35% crude protein to meet the requirement for *Oreochromis mossambicus* (Juveniles) as given by Muhtadi *et al.,* (2021) 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.

Experimental set up and Management

A total of one hundred and five (150) *O. mossambicus* fingerlings divided in to five treatment groups of 30 fishes per group were kept for 155 days period. The five (5) treatment groups were tagged as group1,2,3,4, and 5. Group1 representing the control group with no addition of the Avocado seed meal. While groups 2,3,4 and 5 were having Avocado seed meal inclusion of 5%,10%,15% and 20% respectively

The daily feed rations of 5% fish body weight were divided into two portions and fed to the fish in the morning (0700-0800 hours) and in the evening (1600-1700 hours)

After the experimental period, blood was collected from fifteen (15) fishes per treatment. The collected blood samples were used for the determination of haematological parameters as follows: packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), platelet count (PLT) and differential count.

Packed cell volume (PCV) was determined using microhematocrit method. In the laboratory the capillary tube was filled with two –third of the well mixed venous blood and one end sealed with plasticine. The filled tubes were placed in the microhematocrit centrifuge and spun at 12,000 revolution per minute (RPM) for 5 minutes. The spun tube was placed into a specially designed scale and the PCV read as a percentage.

Haemoglobin (HB). The haemogblin was determined by dividing the value of PCV by 3, White blood cell count: 0.02ml of blood was added to 0.38ml of diluting fluid. The improved neubauer counting chamber was charged with the well mixed diluted blood. It was allowed to settle in a moist chamber for 3-5 minutes using x10 objective of the microscope, the four large corners square area 1, 2, 3, and 4 were located. The area of the squares was 4mm^2. The cells were checked to ensure they were evenly distributed and then the total number of white cells in the four large squares was counted and recorded.

Red blood Cell:

Whole blood was diluted appropriately using an isotomic dilution to avoid lysis of red cells. The number of red cells all in a known volume and of known dilution is counted using counting chamber. 0.02ml of blood was added to 3.98ml of diluting fluid. The improved neubauer counting chamber was charged carefully with the well mixed diluted blood. The sample was allowed to settle in a moist chamber for 3-5 minutes. The ruled area of counting chamber was located under x10 objective of the microscope. The cells with x 40 were checked to be evenly distributed using x40. The total number of the red cells were counted in five groups of ten small squares.

Platelet Count:

To determine the platelet, 0.02ml of blood was added to 3.98ml of diluting fluid. The improved neubauer counting chamber was filled with the well mixed diluted blood and allowed to settle in a moist chamber for 3 to 5 minutes. The ruled area of the counting chamber was located under 10 x objectives of the microscope. The illumination was reduced by closing the iris diaphragm; the platelet appeared as highly refractile particles.

Total of platelets was counted using a high power (40x) objective in the four large corner squares ($4mm^2$) Calculation Total number of platelet or µl is

Number of platelets counted x $\frac{1}{area\ counted\ (mm^{2})}$ x $\frac{1}{depth}$

Differentiate count: The differentiate count is expressed as percentage of the total number of cells counted. The differential counts (neutrophils, lymphocytes, eosinophils and monocytes) were evaluated by dropping thoroughly mixed blood film on clean microscope slides and allowed to dry. The slides were then fixed in methanol and stained with leishman stain. The counting was done based on different cell types and recorded. The values of haematological indices were calculated using the method of (Russia, & Sood, 1992) The white blood cell (WBC) was done using improved Neubauer counter. The values of thrombocytes were determined using the Rees and Beeker method (Seiverd. 1964).

Biochemical Analysis The following biochemical parameters were analyzed in extracted organs of fish, at Divic Specialist Medical Laboratory, Rumuosi, Port Harcourt.

Creatinine the reagents were reconstructed in the required proportion of four parts picric acid to one part sodium hydroxide (NaOH). The samples were prepared for determination by deproteinizing them. This was achieved by pipetting 1.0ml of sample into the test tubes and adding 0.5ml of distilled water and 0.5ml of trichloroacetic acid (TCA), this was spurned for 10 minutes at 300 revolutions per minutes to obtain the supernatant. The test tubes were now labeled as; blank, standard and samples, 1.0ml of distilled water, standard and supernatants were pipetted and dispensed into the labeled test tubes accordingly. 1.0ml of the prepared reagent was added. The mixture was shaken and allowed to stand for l0 minutes after which the absorbances of the sample and standard were read against the reagent blank at 490nm on the spectrophotometer (Woo and Chiu, 2005).

Albumin The reagent compositions were bromocresol reagent (R_1), Succinate buffer, 75mmol/L (pH 4.2), BCG, 1.10mmol/ L and albumin standard (Borine serum albumin, 50.0g/L). The reagents were arranged, and 2.0ml were pipetted into 24 test tubes at room temperature 10µ of sample, standard and blank were each pipetted into their respective test tubes. The test tubes with the mixtures were allowed to stand for 10 minutes. The absorbance of samples and standard were read 630nm against reagent blank, on the spectrophotometer (Woo and Chiu, 2005).

Total Bilurubin

Two test tubes were set up for each test. Test tube 1 (sample blank), test tube 2 (sample) 0.2ml of sulphanilic acid was dispensed into the test tubes. 0.5ml of sodium nitrate was dispensed into the sample test tube, mixed and allowed to stand for 10 minutes at 20 - 25°c. 2.0ml of titrate was dispensed into the tubes, mixed and allowed to stand for 5 minutes at 20 - 25°c and the absorbance of the test sample against the sample blank was read spectrophotometrically at a wave length of 600nm (Woo arid Chiu, 2005).

Total Protein

In the biuret reaction, a chelate is formed between the copper (cu^{2+}) ion and the peptide bonds of the proteins in alkaline solutions to form a violet-colored complex where absorbance is measured spectrophotometrically. The intensity of the colour produced mixture proportional to the concentration of protein in the sample $cu^{2+;'}$ + serum protein copper - protein complex. Three boiling test tubes were labeled, blank, standard and sample. 'The standard reagent was dispensed into the standard. Distilled water was dispensed into blank while the sample |was dispensed into sample test tube. The contents were well mixed and left to stand at room temperature for 10 minutes. Absorbance of standard test tube against blank were read at 540nm wave length in a spectrophotometer. (Woo and Chiu, 2005).

For Total Urea test, the test tubes were respectively labeled as blank, standard, sample and control. 10ml of urease was dispensed into all the test tubes. The test tubes were incubated at 37°C for 10 minutes. 2.5ml of diluted phenol was added to all the test tubes. 2.5ml of diluted sodium hypochlorite was also added. The content of each test tube was mixed and incubated at 37°C for 15 minutes. These contents were read against the content of the blank at the wavelength of 540nm in a spectrophotometer.

Aspartate Amino Transaminase (AST)

The Reitman - Frankel (1987) method was used to analyzed the enzyme. Kit with ready-made reagents containing a pyruvate solution was used in the assay. The kit contains the following: Reagent 1: AST, pH 7.5, phosphate buffered substrate containing 200mmol/L asparate. Reagent 2: 2mmol/L - ketoglutarate solution. Reagent 3: colour reagent containing 1mmol of 1, 2, 4-dinitrophenyl hydrazine (DNPH). Reagent 4: Pyruvate standard. Reagent 5: 0.4mmol/L sodium hydroxide (NaOH).

Six test tubes were labeled B (Blank) and 1-5 Reagents were pipetted into them. 0.1ml distilled water was added to each test tube and mixed thoroughly. All test tubes were left at room temperature for twenty minutes. 5mls of 0.4mmol/L NaOH was added to each test tube and mixed thoroughly. All test tubes were left for five minutes at room temperature.

Absorbance of the pyruvate standard solution in a colorimeter using a green fitter was read and confirmed in a spectrophotometer set at 505nm wavelength. Absorbance of each standard against its equivalent AST was recorded.

Alanine Amino Transaminase (ALT)**:** The reagents used for the analysis were: l00 mmol/L of Buffer-phosphate, at pH 4, 200mmol/L of L-alanine and 2.0mmol/L of a-ketoglutarate.

Procedure stages were: Measurement against Reagent blank and measurement against sample blank.

Measurement against reagent mark: 0.1ml of sample was pipetted, into test tube, none was put into reagent black test tube. 0.5ml of reagent 1 (buffer was measured into sample and reagent blank test tubes. 0.1ml of distilled water was later added to reagent blank test tube and none to sample test tube. Both test tubes were mixed and allowed to stand for 30 minutes at 37°C. 0.5ml of solution 2/2,4, - dinitrophenyl hydrazine was added to both tubes. Both test tubes were mixed and allowed to stand for 20 minutes at 25°C. 5ml NaOH was added to both test tubes.

Both tubes were mixed and sample absorbance was read against reagent blank after five minutes. Measurement against Sample blank: 0.1ml of sample was pipetted into test tube A and 0.5ml of solution 1 was pipetted into test tube B. Content of both tubes were mixed and incubated for 30 minutes at 37^0C. 0.5ml solution was pipetted into sample blank test tube and moved into sample test tube. Contents of both test tubes were, mixed and allowed to stand for 20 minutes at 25°C.

5ml NaOH solution was added to both test tubes. Contents of both test tubes were mixed and absorbance of sample against sample was read after 5 minutes.

RESULTS

Haematological parameters

This present study revealed that there was 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 (Table 4). The values of PCV in group3 of the present studies were significantly (P≤ 0.05) higher (24.67 ± 5.17) than group 4 (17.67 ± 1.45).

Hb value exhibit slight variations, but again there was no consistent trend or significant changes observed. The values of hemoglobin were highest in the 5% diet group (7.47 ± 0.39) and lowest in group 4 (5.9 ± 0.49) respectively.

The values of WBC were highest in group 3 and lowest in the group 4 (14.2 ± 2.5, and 12.2±1.22) respectively. The Neutrophils (%) was higher in the group1 (34.67 ± 3.38) than the treatment groups with avocado seed inclusion, with the group 4 (30.33 ± 3.28) having the lowest.

Table 1. Haematological parameters in plasma of *O. mossambicus* fed graded level of avocado pear seed supplemented diets

	Diets				
Parameters	**Group1 (0%)**	**Group 2 (5%)**	**Group3 (10%)**	**Group4 (15%)**	**Group5 (20%)**
Packed Cell Volume (%)	21.57 ± 0.88	22.33 ± 1.2	24.67 ± 5.17	17.67 ± 1.45	21 ± 2.08
Haemoglobin (g/dl)	7.23 ± 0.29	7.47 ± 0.39	7.1 ± 0.61	5.9 ± 0.49	7 ± 0.68
White Blood Cell (cells $\times 10^9$ /L)	13.27 ± 1.91	13.13 ± 1.07	14.2 ± 2.5	12.2 ± 1.22	14.27 ± 1
Red Blood Cell (cells × 10^{12}/L)	2.83 ± 0.28	3.07 ± 0.27	3.07 ± 0.54	2.33 ± 0.34	3.17 ± 0.47
Platelet (cells × 10^9/L)	137.67 ± 17.9	132 ± 24.7	121.67 ± 19.2	135 ± 7.64	131 ± 29.3
Neutrophils (%)	34.67 ± 3.38	31.67 ± 5.78	31.67 ± 4.41	30.33 ± 3.28	32.33 ± 3.28
Lymphocyte (%)	56.67 ± 4.18	59 ± 3.06	61 ± 5.86	61 ± 2	56 ± 2.65
Monocyte (%)	5 ± 0	4.33 ± 1.76	5 ± 2.52	4.67 ± 1.33	7.67 ± 1.76
Eosinophils (%)	5.33 ± 0.88	5 ± 1.53	2.33 ± 1.2	4 ± 1	3.67 ± 0.67
Mean Corpuscular Haemoglobin (pg)	25.55 ± 1.44	24.35 ± 1.39	23.23 ± 2.42	25.38 ± 3.18	22.08 ± 2.41
Mean Corpuscular Volume (fL)	76.48 ± 7.23	72.39 ± 5.98	80.52 ± 19.35	76.03 ± 7.7	66.2 ± 7.7
Mean Corpus. Hemoglobin Conc. (g/dL)	33.4 ± 0.99	33.44 ± 1.44	28.82 ± 4.74	33.4 ± 2.55	33.33 ± 2.66

The Lymphocyte (%) was highest in group 3 (61 ± 5.86) and lowest in group 5 (56 ± 2.65).

The values of Monocyte in this present study were highest in group 5 (7.67 ± 1.76) and lowest in the group 4 (4.67 ± 1.33). Eosinophils (%) had the highest value in group1 (5.33 ± 0.88) and lowest in group 3 (2.33 ± 1.2).

There was an increase in RBC values in treatment groups against the control group (2.83 ± 0.28). The highest RBC value was recorded in group 5 (3.17 ± 0.47) and the lowest for group 4 (2.33 ±0.34). Groups 2 and 3 similar values (3.07 ± 0.27, and 3.07 ± 0.54 respectively).

The values of this present studies show slights variations, but no clear trend or significant changes are observed. The highest MCH value was group1 (25.55 ± 1.44) and the lowest was the group 5 (22.08 ± 2.41).

The values of MCH increase in the groups in this order group1< 4, <2< 3< 5. The MCV value was highest in group 3 (80.52 ± 19.35) and lowest in group 5 (66.2 ± 7.7). There was no significant difference (P>0.05) in the different groups of the experimental diet of *P. americana* fed *Oreochromis mossambicus*. The MCHC in the present studies showed no significant difference (p>0.05) amongst the various groups. The highest MCV value was for group 4 (33.4 ± 2.55) while the lowest was group3 (28.82 ± 4.74). The use of haematological techniques has proved valuable for fishery biologist in assessing the health of fish (Fagbenro and Adeparusi, 2003).

Table 2: Liver function parameters for *O. mossambicus* fed graded level of *P. americana*

Parameters	**Group1 (0%)**	**Group2 (5%)**	**Group3 (10%)**	Group4 (15%)	**Group5 (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

Enzymes assays such as AST, ALT, and ALP are parts of standard laboratory test to detect the health status of the animals (Saka *et al.,* 2011). Changes in these enzymes activities could serve as a pointer to the changes in health condition of the animal as a result of exposure to different treatment agent (Shalaby *et al.,* 2006). AST levels in groups 2,4 and 5 were 67.67 ± 12.1, 72.33 ± 10.8, and 42 ± 13.1 respectively. Notably, the AST level in the group 3 was 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 group 5 suggest the therapeutic potential of the treatment against hepatotoxicity.

The ALT levels in all treatment groups (groups 2,3,4,5) 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 health food supplement.

It is concluded that avocado seed meal is a good feed supplement and can be added to feed without causing any hematological nor hepatotoxicity to the animal.

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