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# Periodical Evaluation of the Haematology of African Catfish (*Clarias gariepinus*) Fed Dietary Powdered Avocado Pear (*Persea americana*) Leaves

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## 1. ABSTRACT

The study was conducted to evaluate the haamatology of *C. gariepinus* fed dietary powdered *Persea americana* at the end of four and eight weeks of feeding, and five different diets were evaluated. The control diet (Do) had 0% level of powdered *Persea americana* inclusion, D1 (3%), D2 (6%), D3 (9%) and D4 (12%). At the end of the fourth week, there were no significance difference (P>0.05) in the Packed cell volume (PCV); Haemoglobin (Hb); Red Blood Cells (RBC); Eosinophils (E); Monocytes (M); Mean Corpuscular Haemoglobin (MCH); Mean Corpuscular Haemoglobin Concentration (MCHC); and Mean Corpuscular Volume (MCV). The values of the White Blood Cells (WBC) and the Neutrophils (N) were significantly higher in the control compared to treated diets while values for the Lymphocytes (Lymph) and the Thrombocytes (TCT) fluctuated across the various diets, with D4 having the highest value in both. At the end of the eight week, the M, MCH, MCHC and MCV were similar across the diets, but there were elevations in MCH, MCHC and MCV, while M had a decline across the diets. The PCV, Hb and RBC had higher values (P<0.05) in the control diet compared to the treated diets, though they were not dose dependent. The WBC, N, Lymp and TCT had fluctuating values across the diets, with the values in some of the treated diets been higher (P<0.05) compared to the control. The results shows that *P. americana* diets have no adverse effect on the haamatology of *C. gariepinus*, but the prolong use caused a slight decline in oxygen production capacity of the *C. gariepinus* blood.

***Keywords***: *P. americana, haematology, C. gariepinus.*

## 2. INTRODUCTION

Aquaculture is one of the fastest growing sector in food production worldwide, and was observed to increase from 29.9million tones in 2007 to 41.9 million tonnes in 2012 (FAO, 2014). This is a clear attestation to the fact that the demand for fish increases with increase in population. The world population is expected to be 9 (nine) billion by the year 2050 (UNPD/DESA, 2015), and this means increase for the demand of fish and its product from our fresh and marine waters. The productivity from our natural waters is diminishing each passing day because of massive fishing, and pollution as a result of contamination from industrial and agricultural waste, climate change etc. (Maske and Satyanarayan, 2010). Aquacultural product like fish contains lipids, vitamins, minerals and some essential fatty acids such as Omega – 3, and if consumed at the required quantity eliminates or control some fatal diseases like cancers, cardiovascular diseases, eye defects etc (Ukwe *et al.*, 2018a Kirpal, 2003) and the quest to meet up these demand have given birth to series of employment opportunity in the aquaculture sector (Ukwe *et al.*, 2018a).

Disease presence as a result of bacteria is a bottle neck to a successful aquaculture practice (Rahman *et al.*, 2009) it is responsible for series of mortalities and severe economic losses. In the practice of aquaculture, one of the means of determining the health status of the cultured fish is the assessment of the haematological parameter (Iheanacho *et al.*, 2017; Oniovosa *et al.*, 2017). Synthetic chemotherapeutic drugs have been used in aquaculture to maintain the health of fish and boost its immune system, despite the fact that they encourage antibiotic – resistant pathogens, accumulate in fish flesh, pollute the environment and causes immune suppression (Seyfried, *et al.*, 2010; Armstrong *et al.*, 2005; Reverter *et al.*, 2014) and most farmers in the rural areas do not have knowledge concerning their dosage, duration of use, mode of application etc (Ukwe and Gabriel, 2019). Most countries have prohibited the use of synthetic chemotherapeutic drugs as vaccines as well as placing ban on importation of aquacultural products treated with chemicals (Syahidah *et al.*, 2015). There is serious need to replace the use of chemicals in aquaculture with phytochemicals. Phytochemicals are eco-friendly (biodegradable), always available, far less expensive, free from adulteration, dose not deposite on fish flesh and treat more than one problem on administration (Ukwe and Gabriel, 2019; Olusola *et al.*, 2013). Some of the plants that have been used in aquaculture as phytochemicals includes: *phyllanthus* emblica (Sivagurunathan *et al.*, 2012), *zingiber officinale* (Sivaguruthan *et al.*, 2011), *Aloe vera* (Heidarich *et al.*, 2013), a*zardiracha indica* (Adamu *et al.*, 2017), *carica papaya* (Olusola & Nwokike, 2018) among others.

Avocado pear (*Persea americana*) is found all over the world and it is known for its medicinal and other important values (Purseglove, 1977). It belongs to the family: lauraceae, and genus: persea (Uzukwu *et al.*, 2016). Phytochemicals such as saponnins, tannins, oleric acid, terpenoids, flavonoids among others are found in the extracts of the avocado pear leaves and bark (Ogundare and Oladejo 2014), and these phytochemicals have been reported to boost the fish immune system (Bello, 2014).

The purpose of this work was to assess the efficacy of avocado powdered leaves as immune enhancer in *C. gariepinus*.

## 3. Materials and Methods

**3.1 Study Location:** The research work was done in African Regional Aquacultural Center (ARAC), Allu in Ikwerre Local Government Area of Rivers State, Nigeria.

**3.2 *Clarias gariepinus***: Fish (*Clarias gariepinus)* of weight 90 - 95g and length 20 – 26cm was purchased from Idi-Onyana farms, along Abua –Ahoada Road in Rivers State. They were acclimatized for a period of four weeks, using the methods of Gabriel et al (2011) and were fed 5% body weight per day.

**3.3 Herbal Preparation**: The avocado pear leaves were harvested in Aluu in Rivers State. It was carefully washed, dried, grounded to powered form using electric blender, sieved and stored in an air tight entertainer for use (Alabi *et al.*, 2012; Luky and Jonathan, 2017).

**3.4 Diets Preparation**: About 38.35 ±0.19cp diet (Do) was prepared using maize meal, wheat bran, soyabean meal, fish meal, garri, common salt, palm oil, fish premix, lysine, methonine and vitamin C and was used as control. Four other diets (D1 – D4) were prepared from this, with the addition of 3%, 6%, 9% and 12% powdered avocado pear leaves respectively (Nasir et al, 2018).

**3.5 Experimental Procedure**: Five hundred and twenty five fish 25.88±0.14cm length and 117.80±0.11g weight were used in this research work in triplicates across fifteen tanks of 200L capacity at thirty five fish per tank. Feeding commenced twenty four hours after stocking at 5% body weight per day. Blood samples were collected from the fish (via kidney puncture) at the end of week 4 and 8, and taken to the laboratory for haematological analysis.

**3.6 Haematological Analysis**: The haematological analysis was done using haematological analyzer, model MY – BOOZB, manufactured by MAYA MEDICAL EQUIPMENT LIMITED COMPANY LIMITED in Guangdong, China. The Packed Cell Volume (PCV), White Blood Cells (WBC), Heamoglobin (Hb), Red Blood Cells (RBC) and Thrombocyles (TCT) were determined. The blood indices were calculated using the methods of Adeniran *et al.*, 2017, while the differential counts were determined using the following steps:

(i) The individual blood samples were well mixed, and drops were made on a clean microscope slides and allow to dry.

(ii) The slides were fixed in methanol and then stained with Heisman stain.

(iii) A differential count of 100 to 200 cells was done accordingly and their percentage of occurrence were determined.

## 3.7 Results

The haematological parameters in the fish fed the various experimented diets at the end of week 4 and week 8 are shown in tables 1 and 2. At the end of week 4, there were no significant difference (P<0.05) in the values of the PCV, Hb, RBC, E, M, MCH, MCHC and MCV in the fish fed the treated diets (D1 – D4) compared to the control (Do) meanwhile there were fluctuating results in the WBC, N, LYMP, and TCT across the various diets (Do – D4). The WBC was significantly higher in fish fed control diet (Do) and lower (P<0.05) in fish fed D3, LYMP were significantly higher in fish fed Do, D1 and D3, while the TCT were higher (P<0.05) in fish fed Do, D3 and D4.

At the end of week 8, the PCV, Hb and RBC were significantly higher in the fish fed the control diet (Do) compared to the values in the fish fed the treated diets (D1 – D4) which were fluctuating but in the same range. The M, MCH, MCHC and MCV were similar across the diets (Do – D4). The WBC was significantly higher in fish fed D4, though the values were fluctuating across the diets. The LYMP, N and TCT also had fluctuating values across the various experimented diets. The TCT was higher (P<0.05) in fish fed D2 and D3, the LYMP was significantly higher in fish fed D3 while N were significantly higher in fish fed Do, D1 and D4.

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**Table 1: Haematological Parameters in *C. gariepinus* fed Avocado Pear leaf Supplemented diets for 4 week (Mean ±SD)**

| **Parameters** | **Diets** | | | | |
|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** |
| Packed Cell Volume (%) | 37.00 ±1.00[a] | 36.00±2.00 [a] | 34.00±1.00 [a] | 33.00±1.00 [a] | 33.00±2.00 [a] |
| Haemoglobin (g/dl) | 12.40±0.10[a] | 12.07±0.31 [a] | 11.50±0.20 [a] | 10.67±0.15 [a] | 11.00±1.00 [a] |
| Red Blood Cell (cells x $10^{12}$) | 5.30±0.20[a] | 5.30±0.10 [a] | 4.63±0.12 [a] | 4.32±0.18 [a] | 4.63±0.15 [a] |
| White Blood Cell (cells x $10^{9}$/l) | 15.50±0.10[a] | 14.70±0.20 [b] | 13.10±0.10 [b] | 8.67±0.15 [c] | 12.80±0.20 [b] |
| Neutrophils (%) | 29.00±2.00[b] | 27.33±2.52 [b] | 33.00±1.00 [a] | 35.00±2.00 [a] | 25.00±2.00 [b] |
| Lymphocytes (%) | 60.00±2.00[a] | 62.00±1.00 [a] | 57.00±2.00 [b] | 54.67±0.58 [b] | 65.00±1.00 [a] |
| Eosinophils (%) | 4.00±1.00[a] | 3.00±1.00 [a] | 3.00±0.00 [a] | 3.00±1.00 [a] | 3.33±0.58 [a] |
| Monocytes (%) | 7.00±0.00[a] | 8.00±1.00 [a] | 8.33±0.58 [a] | 8.00±1.00 [a] | 8.00±1.00 [a] |
| Thrombocytes (%) | 237.09±1.53[a] | 185.45±2.52 [b] | 192.48±2.00 [b] | 207.99±2.00 [a] | 242.41±2.00 [a] |
| Mean Corpuscular Haemoglobin (pg) | 69.83±0.75[a] | 67.92±3.31 [a] | 73.41±2.83 [a] | 76.56±4.68 [a] | 71.33±6.01 [a] |
| Mean Corpusc. Haemoglobin Conc. (g/ | 23.42±0.81[a] | 22.77±0.44 [a] | 24.83±0.76 [a] | 24.74±1.35 [a] | 23.79±2.69 [a] |
| Mean Corpuscular Volume (FL) | 33.52±0.81[a] | 33.56±1.06 [a] | 33.83±0.41 [a] | 32.34±0.95 [a] | 33.29±1.02 [a] |

**Means within the same roll with different superscript are significantly different ($p<0.05$)**

**Table 2: Haematological Parameters in *C. gariepinus* fed Avocado Pear leaf Supplemented diets for 8 weeks (Mean±SD)**

| **Parameters** | **Diets** | | | | |
|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** |
| Packed Cell Volume (%) | 44.00±1.00$^a$ | 34.00±1.00$^b$ | 36.00±1.53$^b$ | 35.00±1.00$^b$ | 31.00±1.53$^b$ |
| Haemoglobin (g/dl) | 14.40±0.10$^a$ | 11.67±0.58$^b$ | 12.00±1.00$^b$ | 11.80±0.10$^b$ | 10.27±0.25$^b$ |
| Red Blood Cell (cells x $10^{12}$) | 6.13±0.15$^a$ | 4.60±0.10$^b$ | 4.80±0.10$^b$ | 4.50±0.10$^b$ | 4.20±0.10$^b$ |
| White Blood Cell (cells x $10^9$/l) | 6.60±0.20$^b$ | 6.60±0.10$^b$ | 4.50±0.20$^c$ | 7.10±0.10$^b$ | 9.50±0.20$^a$ |
| Neutrophils (%) | 42.00±1.00$^a$ | 45.00±1.00$^a$ | 38.33±0.58$^b$ | 29.33±0.58$^c$ | 41.83±0.58$^a$ |
| Lymphocytes (%) | 51.33±1.53$^b$ | 52.33±2.53$^b$ | 54.00±1.00$^b$ | 62.00±1.00$^a$ | 52.00±1.00$^b$ |
| Eosinophils (%) | 3.00±0.00$^a$ | 2.33±1.58$^b$ | 2.67±0.58$^b$ | 3.67±0.58$^a$ | 3.33±0.58$^a$ |
| Monocytes (%) | 5.71±0.58$^a$ | 5.63±0.52$^a$ | 5.81±0.51$^a$ | 5.89±0.36$^a$ | 5.33±0.58$^a$ |
| Thrombocytes (%) | 265±1.00$^b$ | 272±2.00$^b$ | 295±1.53$^a$ | 303±1.53$^a$ | 186±2.08$^c$ |
| Mean Corpuscular Haemoglobin (pg) | 71.78±2.81$^a$ | 74.04±4.74$^a$ | 75.67±1.65$^a$ | 77.77±0.50$^a$ | 74.65±4.48$^a$ |
| Mean Corpusc. Haemoglobin Conc. (g/ | 23.49±0.66$^a$ | 25.39±1.71$^a$ | 25.04±2.61$^a$ | 26.23±0.73$^a$ | 24.46±1.04$^a$ |
| Mean Corpuscular Volume (FL) | 32.74±0.52$^a$ | 34.30±0.98$^a$ | 33.14±4.10$^a$ | 33.74±1.14$^a$ | 32.79±0.84$^a$ |

**Means within the same roll with different superscript are significantly different ($p<0.05$)**

### 3.8 Discussion

The well being of fish can be ascertained through the analysis of its haematological parameters (Oniovosa *et al.*, 2017; Olapade and Lombi, 2015). The result of this work indicates that there were no significant differences in the values of PCV, Hb and RBC of the fish fed the treated diets compared to the control at the end of the fourth week. This is an indication that the experimented diets did not negatively alter the PCV, Hb and RBC after four weeks of feeding. Similar result was reported in *Prochilochus lineatus* by Winkaler *et al.,* 2007, but this results disagrees with the Harikrishnan *et al* (2003) in *C. carpio* following herbal treatment. But at the end of the eighth week, the values of the PCV, Hb and RBC were noticeably higher ($P<0.05$) in the fish fed the control diet (D1) compared to the fish fed the experimented diets (D1 – D4) though the values were not dose dependant, fish fed D4 hard the least values. This could be as a result of the prolong use of the dietary *P. americana* powder leaves and its saponin content may have altered the blood parameters (Ogbe and Affiu, 2011), it could also be that the used concentrations with respect to the herb were high and tend to affect the fish haematopoyetic processes (Cope, 2005; Palanisamy *et al.*, 2011), as a result of immunosuppression that may arise from overdose of phytochemicals (Mastan, 2015). This work is in agreement with the result of Zaid *et al* (2020) who fed *C. gariepinus* with dietary Gbewutu, and the work of Gabriel *et al* (2019) when *C. gariepinus* was fed with high concentration of dietary *Aloe vera.* The WBC was lower in *C. gariepinus* fed the treated diets compared to the control at the end of the fourth week, this could be as a result of experimented fish adjusting itself to the presence of the phytochomicals contain in the feed that may have affected the production of the WBC in the kidney, or the release of mature WBC to the blood by the spleen (Kumar *et al.,* 2011; Wedemeyer and Wood, 1974). This work is similar to the works of Savaravanan et al (2011) in

C. mrigala fed neem diets, and Oniovosa *et al.,* (2017) in *C. gariepinus* fed dietary neem. At the end of week 8, the value of WBC dropped in *C. gariepinus* fed all diets, with fish fed D3 and D4 having higher values compared to the control. This could be that the prolong use of the dietary *P. americana* powdered leaves was becoming toxic due to excess phytochemicals (Palanisamy *et al.,* 2011). It could also be as a result of different levels of impurities that may have find their way into the diet during the period of diet preparation (Asif, 2012).The result of this work is similar to the works of Bahrami *et al.,* (2015) in *Cyprinus carpio* fed dietary *stachys lavandulifolia* and Negm *et al* (2015) in *Oreochromis niloticus* fed dietary *zingiber officinale.* The LYMP had fluctuating values at the end of the week 4 and week 8 across all the diets, but some of the values in the treated diets were higher than the control. The presence of high LYMP shows the immunostimulatory effects of *P. americana*, which could be as a result of the presence of physchemicals (Reverter et al, 2014; Ogundare and Oladejo, 2014). The N also had fluctuating values at the end of week 4 and 8, but the values were slightly higher in some of the dietary *P. americana* diet fed *C. gariepinus*, this could also be as a result of the presence of phytochmicals. The results of the LYMP and N are similar to the result of Olapade and Lombi (2015) in *C. gariepinus* fed dietary *Anisophylla Laurna*. Though the values of TCT were fluctuating among the fish fed the various diets at the end of week 4, it was significantly higher in the fish fed the treated diets compared to the control at the end of week 8. This could be as a result of the medicinal values of the phytochemicals present in *P. americana* (Soogs and Barikow, 2014), since the TCT have been reported to be responsible for healing of wounds (Jagadeeswaram *et al.,* 2005) and repair of damage internal organs (Davi and Patrono, 2007). Blood indices (MCH, MCV and MCHC) are indicators of low oxygen in circulation (Ahilan *et al.,* 2004), there were no significant difference ($P<0.05$) in the values of MCH, MCV and MCHC of the fish fed dietary *P. americana* compared to that of the control throughout the period of this work. This is an indication that the *P. americana* at the used concentration is not inimical to oxygen circulation in the *C. gariepinus* (Shalaby *et al,* 2006). This result is in agreement with the report of Dalta *et al.,* (2018) on the effects of different protein source on the haematological parameters of stripped catfish. There were no significant difference in the values of E and M throughout the period of this work, though the value of M was slightly higher in the fish fed the treated diets compared to the control, this could be as the result of the immunopotency of the phytochemicals in *P. americana* (Soongs and Barikow, 2014; Reverta, *et al,* 2014). The fluctuating values in some of the haematological parameters in this work compared to others could be as a result of external factors such as: stock density (Skjervolt *et al,* 2011), sex difference (George and Akinrotimi, 2017) and quality of water used during the experiment (Fazio *et al,* 2013).

### 3.9 Conclusion

The results of this work shows that powdered *P. americana* at the used concentrations did not hinder oxygen production and circulation in *C. gariepinus* during the period of the evaluations. Though, there was a little decline in the production of oxygen at the end of the eighth week which was attributed to prolong use of the treated diets. Some of the immune parameter of the *C. gariepinus* were slightly affected by the treated diets, but they were better at the end of eighth week. *P. americana* leaves are available all through the year, and it is almost free in our farms/bushes. This is the first time it is been used for its important in fish culture and the result is impressive.

However, in order to maximize its potency in fish culture, more work should be done with lesser quantity of the powdered leaves. Extracts of the leaves with different solvents such as ethanol, methanol, aqueous etc (since solvent affects type and quantity of phytochemical extracted) should be used at varying quantities on *C. gariepinus* and other fishes. *Persea americana* powdered leaves is a recommendable herb to be used as a dietary supplement in the culture *C. gariepinus*.

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