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Hepatic and Haemato-biochemical Alterations in Juvenile *Mozambique tilapia* (*Oreochromis mossambicus*) on Pawpaw (*Carica papaya*) Seed Meal

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Abstract

Pawpaw seed meal (PSM) can be used as an antifertility agent in animals, however there is no information on its potential toxicological effect on Mozambique tilapia (*O. mossambicus*). In this study the effect of PSM on the liver, blood haematological and biochemical parameters of *O. mossambicus* juveniles was assessed to ascertain its suitability as a reproductive suppressant. The PSM was administered at an inclusion level of 10 g and 30 g/kg of a commercial tilapia diet (basal diet, BD) for 30 and 60 days, respectively. The potential toxicological effect was assessed by determining the extent of change in the normal haematological (RBC count, haematocrit, haemoglobin, MCV, MCH, MCHC, thrombocyte count, absolute WBC and differential cell counts) and biochemical (cholesterol, total protein, albumin, globulin and glucose) parameters. Haematological and biochemical profiles of different treatment groups did not differ throughout the course of the investigation. Liver weight and hepatosomatic index values of the treated fish were comparable to those of the control. The absence of any effect of the blood parameters measured in this study also indicate that PSM has no compromising influence on the immune system of the fish, indicating that PSM can be considered as a safe alternative to induce masculinization in Mozambique tilapia, and therefore act as a reproductive inhibitor.

Keyword: Hepatosomatic index, *Mozambique tilapia*, *Carica papaya*, haematology, serum biochemistry

1. Introduction

Mozambique tilapia (*Oreochromis mossambicus*) is one of several tilapia species that are commonly cultured in Sub-Saharan Africa. The tilapia species, especially *O. mossambicus*, are characterized by their easy adaptability to various environmental conditions, and an exceptional tolerance to high salinity (Kamal & Mair, 2005). The ease of propagation makes tilapia one of the most preferred cultured food fish worldwide, with an annual production of over 850 metric tonnes (Coward & Bromage, 2000). Tilapia species consume a wide variety of natural food sources, exhibit a rapid growth rate and as a consequence, tilapia can attain sexual maturity in less than four months, and they spawn easily in captivity. Despite all these positive attributes of tilapia, early maturity and easy spawning predispose the species to overcrowding and subsequent stunting of growth in mixed sex culture systems (Piferrer, 2001). To overcome the negative impact of the precocious breeding strategy of tilapia, an alternative approach is to manipulate the gender of tilapia stock to obtain all-male populations. As the production of mono-sex tilapia becomes highly desirable in the aquaculture industry, efforts are being made to substitute the synthetic hormones employed in the sex reversal of genetic females to phenotypic males with less harmful plant phytochemicals. *Carica papaya* (pawpaw) is one of the plants whose seeds have generated scientific research interests and are being considered as a potential agent for sex reversal, and the inhibition of reproductive activities in tilapia (Omeje et al., 2018) in food fish production. Seeds of *C. papaya* contain some bioactive compounds such as benzyl isothiocyanate, benzyl thiourea, hentriacontane, β-sitosterol, caricin and oleanolic glycoside (Krishna et al., 2008) which have the capacity to inhibit steroidogenesis thereby leading to reproductive impairment (Lakshman & Changamma 2013). Phytochemicals, also referred to as phytoestrogens, can modulate the hormonal systems to the extent that they are regarded as endocrine disrupting compounds

(Rearick et al., 2014). Endocrine disrupting compounds apart from their effect on the endocrine and reproductive systems, also affect other physiological functions of fish. Haematological parameters seems to be the most reliable indicator of alteration in the physiology of fishes (Akinrotimi et al., 2012). This is because blood participates directly or indirectly in almost all biochemical processes in the body such as homeostasis as well as disease processes. Different stressors results in significant alteration in haematological and blood chemical indices. In a nutshell, it is a reliable indicator of systemic response of fish to external stimuli (Tavares-Dias & Moraes, 2007).

Indices such as red and white blood cell counts, packed cell volume and haemoglobin concentrations are all subject to variations in stressful conditions such as disease, unfavourable environmental conditions and contaminants (Mali & Chavan, 2014). Also changes in blood parameters have the potential to be used as an indicator of variation in biochemical processes due to xenobiotic treatments. It is believed that changes of blood constituents could adversely affect the performance of the fish. Haematological parameters are also influenced by such factors as age, species, sex and sexual maturity of the fish (Fazio et al., 2012a; Charoo et al., 2013). Conventionally, assay of blood cell counts (RBC and WBC), packed cell volume (PVC) and haemoglobin are undertaken using Neubauer haemocytometer, micro-haematocrit and cyanomethaemoglobin methods respectively but because of advancement in science, automated systems have gained prominence especially in human and other mammalian blood assays. However, the use of automated systems in the assay of fish blood cells is not widespread because unlike those of mammals, fish blood cells are nucleated (Hrubec et al., 2000). Even though the nucleated nature of fish blood makes calibration and assay using automated systems difficult, nonetheless a comparison of values obtained using automated systems and manual counting using haemocytometer shows no significant difference (Fazio et al., 2012a; Charoo et al., 2013). Apart from haematological parameters, analysis of blood chemistry offers information on biochemical changes in the blood and tissues which are vital for accurate diagnosis of infection or effect of treatment (Patriche et al., 2011). Changes in blood chemical parameters are indicators of stress, health and the nutritional status of fish (Fazio et al., 2012). Total protein, albumin and Ca^{2+} are used to test for liver function and calcium metabolism (Zhou et al., 2009). The purpose of this study was to evaluate the effect of the phytoestrogens contained in pawpaw seeds on the haematological and biochemical parameters of *O. mossambicus*. It also investigated the impact of dietary inclusion of PSM on the morphology of the liver of the fish since the organ takes part in haematopoiesis and also detoxification of xenobiotic.

2. **Materials and Methods**

2.1 Experimental Location and Facilities

The experiment was conducted in a one tier plastic water re-circulatory system built in a glass house at the Aquaculture unit of the Welgavellen experimental farm of the University of Stellenbosch, South Africa. The plastic tanks have a dimension of 70 × 40 × 38 cm (L × W × H) and volume of 90 L. It also has aeration, filtration and water heating facilities incorporated into its design. The physicochemical parameters of the culture water were monitored daily. A digital YSI ProODO (Model: EC300, YSI Inc., Yellow Springs, USA) was used to monitor the dissolved oxygen, temperature and conductivity while Crison ICR12502 pH meter (HACH, USA) was used to monitor the pH. The mean water temperature recorded was 26.03±0.61 °C, dissolved oxygen was 6.77±0.78 mg/L while pH and conductivity were 6.42±0.18 and 210±8.02 µS/cm respectively.

2.2 Experimental Animals and Diets

A total of 1 000 Juvenile *O. mossambicus* with an average weight of 24.81±8.54 g, and mean total and standard lengths of 11.06±1.30 cm and 8.84±1.14 cm, respectively, were obtained from Rivendell hatchery, Grahamstown, South Africa. Upon arrival at the Welgvallen Experimental Farm, the fish were acclimatized for three weeks in a holding facility before the commencement of the experiment. During the acclimatization period they were fed *ad-libitum* twice daily with commercial tilapia diet (Aqua-nutro, Nutroscience (Pty) Ltd., Malmesbury, South Africa). Fresh seeds were obtained from large quantities of ripe *C. papaya* obtained from fruit vendors in Stellenbosch, Western Cape, and dried in-doors. The dried seeds were blended to a fine powder using a laboratory grinder (Knifeter, 1095, FOSS), and stored in Ziploc bags for later use. The standard (basal) diet consisted of a commercial tilapia diet (40% crude protein, Aqua-Nutro, Nutroscience (Pty) Ltd, Malmesbury, South Africa). The pawpaw seed meal (PSM) was added to the basal diets according to the inclusion level for that treatment group. A measured quantity of the basal diet and the experimental pawpaw seed powder were mixed thoroughly in Macadams baking system (model: SM-401). To enable pelleting of the feed, lukewarm water (200 mL/kg of feed) was added to the mixture during mixing. The mixture was pelleted in an extruder and oven-dried in a CFW Envirowatch 5 (model: Ø560) oven, and then stored in airtight containers.

2.3 Experimental Design

There were five experimental treatment groups with four replicates (5 × 4 factorial designs). Each of the replicates had a stocking density of 50 fish. The treatment groups include the control that were fed only basal diet for 60 days (P0M2), group fed 10 g PSM/kg of BD for 30 days (P10M1), 10 g PSM/kg of BD for 60 days (P10M2), group fed 30 g PSM/kg of BD for 30 days (P30M1) and those fed 30 g PSM/kg of BD for 60 days (P30M2). The groups fed the diet containing PSM for 30 day were fed the BD from 31 day to end of the experiment. The fish were fed *ad libitum* three times a day (9.00, 13.00 and 17.00 h) with the experimental diets. Uneaten food were syphoned out and the tanks cleaned every day with 50% water change. On the day of stocking, the entire fish in the whole treatments and replicates were weighed (g) individually with top loading balance (Electronic Balance, UWE, HGS-300, capacity: 300 × 0.01 g, Serial # P9440) and their total and standard lengths measured using a measuring board graduated in centimeters. At the end the experimental periods, fish samples from each replicate (ten males and ten females) were randomly selected, weighed, humanely sacrificed and the abdomen incised and the liver dissected out. The weight of the dissected liver was also measured. The hepatosomatic index (HSI) was calculated using the following formula;

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100 \quad (1)$$

2.4 Blood Haematological and Biochemical Analysis

Blood samples were collected from twenty fish (10 males and 10 females) from each replicate on Day 0, 30 and 60, respectively, for the determination of the haematological parameters and blood chemistry assay. The haematological parameters analysed included the red blood cell (RBC) counts, haemoglobin (HGB), packed cell volume (haematocrit, HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), thrombocytes (platelets, PLT), white blood cell (WBC) counts, neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO). The blood chemical parameters analysed include cholesterol, total protein, albumin, globulin, albumin/globulin ratio, and glucose. Blood samples for haematological parameters were collected from the caudal circulation (Affonso et al., 2002) with the aid of 3ml disposable plastic syringes and a 21 gauge disposable hypodermic needle. The collected blood was put into 2ml purple coloured EDTA vacutainer tubes. The haematological parameters were all determined using an automated system, Abbot CELL-DYN system (model: 3700, USA). Blood samples for serum chemistry assay were collected from the caudal circulation with the aid of 3ml disposable plastic syringes and a 21 gauge disposable hypodermic needle. The collected blood was put into a non-heparinized serum separating vacutainer tube containing gel (yellow tubes) and allowed to clot. The serum was collected by centrifugation at 4000 rpm for 15 minutes at room temperature and then sent to the lab for analysis. The tests were done on a BECKMAN instrument (Model: AU5800, USA) at the Pathcare Veterinary pathology laboratory, Bloemfontein, South Africa.

2.5 Statistical Analysis

The results are presented as mean±SE. The data were tested for normality using Kolmogorov Smirnov's test and analysed by one way analysis of variance (ANOVA) with confidence interval of 95%. Variant means were separated using the Bonferroni (Dunn) test. All statistical analysis was performed using XLSTAT version: 2015.2.02.18165.

3. Results

3.1 Haematological Parameters

The respective haematological parameters of *O. mossambicus* fed various levels of PSM at the days 0 and 30 indicate no significant differences ($p > 0.05$) for the fourteen respective haematological indices assayed. At day 60, the mean RBC ranged between 2.15±0.05 × 10^{12}/L (P30M1) and 2.26±0.05 × 10^{12}/L (P10M1) while the mean haemoglobin ranged between 12.07±0.3 (P30M1) and 12.79±0.3 (P0M2). The mean haematocrit ranged between 32.27±0.78 (P30M1) and 34.27±0.78 (P10M1). Also the mean WBC count ranged between 1.72±0.29 × 10^9/L (P10M2) and 2.45±0.29 × 10^9/L (P30M1). There was also no significant difference ($p > 0.05$) in the haematological parameters recorded on the day 60 (Table 1).

Table 1. Haematological parameters (mean±SE) of *O. mossambicus* fed 10 and 30 g of PSM/kg of BD at Day 60

	P30M2	P30M1	P10M2	P10M1	P0M2
Red blood cell (10^{12}/L)	2.19±0.1	2.15±0.1	2.22±0.1	2.26±0.1	2.23±0.1
Haemoblobin (g/dL)	12.12±0.3	12.07±0.3	12.51±0.3	12.77±0.3	12.79±0.3
Haematocrit (%)	32.27±0.8	32.36±0.8	33.44±0.8	34.27±0.8	34.22±0.8
Mean corpuscular volume (fL)	147.4±1.6	150.1±1.6	151.1±1.6	152.0±1.6	154.1±1.6
Mean corp. haemoglobin (pg)	55.34±0.6	55.88±0.6	56.27±0.6	56.56±0.6	57.47±0.6
Mean corpuscular haem. Concn. (g/dL)	37.63±0.5	37.26±0.5	37.34±0.5	37.28±0.5	37.4±0.5
Red cell distribution width (%)	12.43±0.5	12.49±0.5	12.82±0.5	12.53±0.5	12.18±0.5
Platelets (10^9/L)	6.43±1.3	8.66±1.3	5.35±1.3	6.23±1.3	6.82±1.3
White blood cell (10^9/L)	2.13±0.3	2.45±0.3	1.72±0.3	1.79±0.3	1.91±0.3
Neutrophils (% of WBC)	1.72±0.6	3.74±0.6	3.16±0.6	2.42±0.6	2.97±0.6
Lymphocytes (% of WBC)	84.68±1.6	82.42±1.6	79.89±1.6	82.22±1.6	82.39±1.6
Monocytes (% WBC)	9.16±2.3	13.37±2.3	11.55±2.3	10.56±2.3	10.71±2.3
Eosinophils (% of WBC)	2.14±0.6	2.86±0.6	2.38±0.6	2.40±0.6	2.57±0.6
Basophils (% of WBC)	2.3±0.4	2.02±0.4	3.03±0.4	2.33±0.4	1.36±0.4

3.2 Influence of Gender on the Haematological Parameters

The mean RBC count, haemoglobin, haematocrit and MCHC of *O. mossambicus* was significantly higher in males than in females while WBC, MCV was significantly higher ($p < 0.05$) in females than in males. The MCH, RDW, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were not significantly different ($p > 0.05$) between the sexes (Table 2).

Table 2. Influence of gender on the haematological parameters (mean±SE) of *O. mossambicus*

Parameters	Males	Females
Red blood cell (10^{12}/L)	2.39^a±0.0	2.02^b±0.0
Haemoblobin (g/dL)	13.5^a±0.2	11.41^b±0.2
Haematocrit (%)	35.3^a±0.5	31.32^b±0.5
Mean corpuscular volume (fL)	147.6^b±1.0	154.3^a±1.0
Mean corp. haemoglobin (pg)	56.39^a±0.4	56.1^a±0.4
Mean corpuscular haem. Concn. (g/dL)	38.29^a±0.3	36.47^b±0.3
Red cell distribution width (%)	12.52^a±0.3	12.46^a±0.3
Platelets (10^9/L)	6.04^a±0.8	7.36^a±0.8
White blood cell (10^9/L)	1.47^b±0.2	2.53^a±0.2
Neutrophils (% of WBC)	2.9^b±0.4	2.71^a±0.4
Lymphocytes (% of WBC)	83.28^a±1.0	81.36^a±1.0
Monocytes (% WBC)	11.32^a±1.5	10.82^a±1.5
Eosinophils (% of WBC)	2.04^a±0.4	2.91^a±0.4
Basophils (% of WBC)	2.2^a±0.3	2.21^a±0.3

Note. ^{a}, ^{b} Rows with different superscripts differ significantly ($P < 0.05$).

3.3 Blood Serum Chemistry

The blood serum chemistry profile of *O. mossambicus* juveniles fed 10 and 30 g of PSM/kg of BD and the control at days 30 and 60 post exposure are shown in Table 3. The mean cholesterol level in the serum of experimental fish at day 60 post exposure range between (6.05±0.54 mmol/L) (P30M1) and 9.86±0.56 mmol/L (P0M2 group). The differences in the cholesterol levels between different treatment groups were not significant at day 30 and 60 post exposure. The mean total protein range between 47.8±1.5 g/L (P10M1) and 50.6±1.4 g/L (P0M2) and the values were not significantly different ($p > 0.05$) between treatment groups. There was also no significant difference ($p > 0.05$) in the albumin fraction of the serum protein between different treatment groups which range between 13.9±0.7 g/L (P30M1) and 15.2±0.7 g/L (P30M2).

Table 3. Blood chemistry (mean±SE) of *O. mossambicus* fed 10 and 30 g of PSM for 30 and 60 days

Parameter	Duration	P0M2	P10M1	P30M1	P10M2	P30M2
Cholesterol (mm/L)	Day 30	9.86±0.56	6.64±0.54	6.05±0.54	7.29±0.53	6.85±0.54
	Day 60	17.39±1.45	13.99±1.57	14.16±1.45	14.20±1.50	13.36±1.57
Total protein (g/L)	Day 30	47.79±1.80	44.29±1.74	42.96±1.74	41.06±1.68	39.81±1.74
	Day 60	50.60±1.40	47.80±1.50	48.60±1.40	47.80±1.50	49.4±1.40
Albumin (g/L)	Day 30	14.57±0.90	12.69±0.96	11.43±0.92	11.44±0.89	11.43±0.95
	Day 60	14.20±0.70	14.90±0.70	13.90±0.70	14.40±0.70	15.2±0.70
Globulin (g/L)	Day 30	33.07±1.07	32.31±1.08	31.54±1.04	29.67±1.00	28.36±1.07
	Day 60	36.40±1.00	33.00±1.00	34.60±1.00	33.34±1.00	34.2±1.00
Albumin/Globulin ratio	Day 30	0.44±0.03	0.37±0.03	0.35±0.03	0.39±0.02	0.39±0.03
	Day 60	0.39b±0.02	0.45ab±0.02	0.4 ab ±0.02	0.43ab±0.02	0.46a±0.02
Glucose (mmol/L)	Day 60	27.90±3.10	34.5±3.30	24.60±3.10	27.60±3.80	25.4±3.10

Note. [a], [b] Rows with different superscripts differ significantly ($p < 0.05$).

The mean globulin range between 33.0±1.0 g/L (P10M1) and 36.4±1.0 g/L (P0M2) and the differences between treatment groups were statistically non-significant ($p > 0.05$). The derived albumin to globulin ratio shows the highest mean value of 0.46±0.02 (P30M2) while the lowest mean value of 0.39±0.02 (P0M2) and the difference was statistically significant ($p < 0.05$). The mean glucose concentration range between 24.6±3.1 mmol/L (P30M1) and 34.5±3.3 mmol/L (P10M1) and the differences between groups were not significant ($p > 0.05$) (Table 3).

3.4 Influence of Gender on the Blood Chemistry Profile

The blood chemistry assay indicated that the mean levels of cholesterol, total protein, albumin, and also the derived albumin to globulin ratio were significantly higher in female *O. mossambicus* than in males. Also the mean globulin level of females (36.23±0.63) was significantly higher than those of males (32.38±0.60). Conversely, the glucose level in males (37.44±1.94) was significantly higher than those of the females (18.59±2.04) (Table 4).

Table 4. Influence of gender on the blood chemical parameters (mean±SE) of *O. mossambicus*

Parameter	Males	Females
Cholesterol (mm/L)	10.83^{b}±0.92	18.42^{a}±0.96
Total Protein (g/L)	44.08^{b}±0.88	53.56^{a}±0.92
Albumin (g/L)	11.70^{b}±0.41	17.34^{a}±0.41
Globulin (g/L)	32.38^{b}±0.60	36.23^{a}±0.63
Albumin/Globulin ratio	0.37^{b}±0.01	0.48^{a}±0.01
Glucose (mmol/L)	37.44^{a}±1.94	18.59^{b}±2.04

Note. [a], [b] Rows with different superscripts differ significantly ($p < 0.05$).

3.5 Liver Weight and Hepatosomatic Index

Results of the mean liver weight from *O. mossambicus* exposed to various levels of pawpaw seed meal shows the maximum value (1.48±0.13 g) in P0M2 group followed by P30M2 (1.21±0.13 g) while the minimum (0.85±0.13 g) was in P10M1. The fish that received 10 g of PSM per kg BD for 30 days had a significantly lighter mean liver weight as shown in Table 5. Result of the hepatosomatic index (HSI) of the experimental fish from the different treatment groups shows the maximum mean value (2.21±0.17) recorded among the group that received 10 g/kg of the experimental diet for 30 days. However the differences in the HSI among the groups were not statistically significant ($p > 0.05$).

Table 5. Effect of *C. papaya* on the liver weight and hepatosomatic index (Mean±SE)

Treatment	Body weight (g)		Liver weight (g)	Hepatosomatic index
	Male	Female		
P30M2	103.58±3.48	46.08±3.48	1.21[a]±0.13	1.61±0.17
P30M1	102.51±3.48	45.41±3.48	1.17[a]±0.13	1.46±0.17
P10M2	95.25±3.48	45.71±3.48	1.05[a]±0.13	1.53±0.17
P10M1	92.5±3.48	46.78±3.48	0.85[b]±0.13	1.2±0.17
P0M2	94.82±3.48	48.58±3.48	1.48[a]±0.13	2.21±0.17

Note. [a,b] Rows with different superscripts differ significantly ($P < 0.05$).

4. Discussion

4.1 Influence of Pawpaw Seed Meal on Haematological Parameters

Evaluation of the haematological profile of fish blood is important in aquaculture for the determination of the health status, impact of treatment and experimental procedures (Muttappa et al., 2015). The result of RBC counts in this study shows reduction in the level of RBC among the treatment groups that were fed 30 g/kg diet, however the difference is not statistically significant which means that PSM may not affect the erythrocyte count at an inclusion level of 30 g/kg. However this is contrary to the findings of Ayotunde et al. (2010) who reported significant decrease in RBC counts of *Clarias gariepinus* fingerlings exposed to various levels of *C. papaya* seed extract ranging from 100 to 500 mg/20 L of the culture water. Red blood cells (erythrocytes) contain haemoglobin and its main function is the transport of oxygen and carbon dioxide and abnormal changes in its number may indicate anaemia or stress (De Pedro et al., 2005). It is the most abundant cell type and has proved to be a highly variable blood parameter among fish species (Daneshvar et al., 2012). Reported values of RBC in fish include 3.53×10^6/μL (range: 2.21-4.47 × 10^6/μL) for grey mullet (*Mugil cephalus*) (Fazio et al., 2012b). Hrubec et al. (2000) gave a reference interval for RBC counts in tilapia (*Oreochromis* hybrid) of 1.91-2.83 × 10^6/μL.

Gender is one of the factors believed to influence the haematological parameters of fish species. The mean RBC count of male *O. mossambicus* was significantly higher than that of the females in this study. This observation agrees with the finding of Karimi et al. (2013) who reported significant higher RBC count in male yellow fin sea bream (*Acanthopagrus latus*) compared to the females. It has been postulated that significantly higher RBC, haematocrit and haemoglobin in male fish may be due to higher metabolic rate (Acharya & Mohanty, 2014) since RBC determines the dissolved oxygen carrying capacity of the blood.

Clark et al. (1979) stated that haematocrit values for fish usually vary between 20-35%, with variation rarely exceeding 50%. The mean haematocrit values reported in this study falls within the range reported by Clark et al. (1979). In this study the males had significant higher haematocrit values than females. Differences in haematocrit values between the sexes have been reported for sexually mature yellow seabream (Acanthoparus latus) (Karimi et al., 2013). The range of haemoglobin values recorded in this study fall within the range of values reported by (Chuku & Uwakwe, 2012) for tilapia species. Even though the group that were fed higher inclusion levels of PSM had lower mean haemoglobin than the control, the difference was not statistically significant therefore it can be inferred that PSM has no effect on the haemoglobin up to an inclusion level used in this study. Haemoglobin values are one of the haematological parameters mostly used for evaluating fish health. Haemoglobin profile is indicative of oxygen carrying capacity of the blood and anaemia is indicated by low haemoglobin (Zhang et al., 2007). At an inclusion level used in this study, PSM was not potent enough to induce anaemia in *O. mossambicus* and therefore it can be said to be safe for use in tilapia culture. The erythrocyte indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) recorded in this study were within the range reported by Lucas et al. (2003). White blood cell (leucocytes) count in fish blood varies according to season, age and sex (Charoo et al., 2013). Leucopoenia or leucocytosis are pathological conditions associated with abnormal leucocyte count indicating the possibility of alteration in immune function of the individual (De Pedro et al., 2005). A response of the cellular immune system to infection or treatments manifests as an increase in WBC count (Rapatsa & Moyo, 2013). Fazio et al. (2013) presented data on the mean WBC count for several species of fish, ranging from 0.94±0.14 × 10^9/L for *Gobius niger* to 4.74±0.9 × 10^9/L for *Sparus aurata*. There was no significant difference in the mean WBC count of the different treatment groups indicating no influence of PSM on the respective parameters up to an inclusion level of 30 g/ kg. Apart from absolute count, differential WBC counts are equally

very important in disease diagnosis and determination of impact of treatment or experimental procedures on fish. White blood cells are grouped into granulocytes also called polymorphonuclear leucocytes (made up of neutrophils, eosinophils and basophils) and mononuclear leucocytes (granulocytes) that consist of lymphocytes and monocytes. Lymphocytes are the most abundant of the leucocytes in fish blood constituting more than 85% of the WBC while neutrophils is the most common type of granulocytes (Vázquez & Guerrero, 2007). Neutrophils (also referred to as heterophils) are phagocytic and play a key role in acute inflammation as a response to disease stimulus while lymphocytes play a key role in immune response (Anene et al., 2015). Although Ayotunde et al. (2010) reported that *C. papaya* significantly decreased the level of WBC of *O. niloticus*, results from this study did not indicate a similar effect on the WBC count in *O. mossambicus*. In this study, the PSM also had no influence on the differential leucocyte counts (neutrophils, lymphocytes, monocytes, eosinophils and basophils). Lymphocytes were the most abundant of the leucocytes while basophils were the least in terms of percentage composition in the present study. These findings were in agreement with Antache et al. (2014) who reported that lymphocytes constitute 96.4% of the WBC of *O. niloticus* while neutrophils and monocytes were 1.73 and 1.06% respectively. Thrombocytes (platelets) is second to erythrocytes in terms of abundance in fish blood where it plays key role in blood clotting (Vázquez & Guerrero, 2007). In this study, there was no significant difference in the mean number of thrombocytes between treatment groups, and also no distinct trends established in the values that can be attributed to the effect of PSM.

Abnormal decreases in RBC, HGB, PCV and erythrocyte indices (MCV, MCH and MCHC) indicates anaemia (De Pedro et al., 2005) while decreased WBC means reduced disease fighting capacity of the fish (Rapatsa & Moyo, 2013). It has been suggested that xenobiotic treatments that result in the increase of these blood parameters are associated with an improved immune system functioning, compared to treatments that result in their decrease, which are associated with a compromised immune response (Anene et al., 2015). The haematological parameters recorded in this study did not show that the immune system was depressed due to inclusion of pawpaw seed meal in the diet of *O. mossambicus*.

4.2 Influence of Pawpaw Seed Meal on Serum Biochemical Parameters

Serum biochemical parameters are indicators of the health status of animals including fish and it varies among fishes as a result of species, age, diet and sampling methodology (Akbary, 2014). Elevated levels of total protein may indicate structural alteration of the liver with concomitant reduction in deamination whereas hypo-proteinemia (decrease in total protein level) may be as a result of inhibition of protein synthesis in the liver (Al-Asgah et al., 2015). The total protein values reported in this study were within the range (2.8-6.0 g/dL) of total protein reported for shortnose sturgeon (*Acipenser brevirotrum*) by Knowles et al. (2006). Blood protein has been used by scientists to determine the physiological conditions of test animals and has been thought to be one of the indicators of fish immune system (Demir et al., 2014). According to Charoo et al. (2013) there is a tendency of the protein components of blood to increase when fish is stressed due to starvation. The total serum protein consist of mainly the albumin and globulin fractions (Upadhyay et al., 2014). The main function of the albumin fraction of serum protein is the transport of lipids (exogenous fatty acids and endogenous metabolites), and it is reported to increase when fish is exposed to toxicants (Upadhyay et al., 2014). A mean albumin concentration of 1.6±0.46 g/dL has been reported in *O. mossambicus* (Demir et al., 2014). The difference in the mean albumin concentration between treatment groups was not significant which shows that the diet did not predispose the treated fish to osmoregulatory dysfunction. The globulin fraction of the serum protein are associated with innate response against external stimuli (disease, stress and treatment) in fish (Upadhyay et al., 2014). Globulins are made up of alpha, beta and gamma (α, β, and γ) globulins. Gamma globulins consist mainly of immunoglobulins and are synthesized in plasma cells.

The derived albumin to globulin ratio (A/G) of the group fed 30 g of PSM /kg of BD for 60 days (P30M2) was significantly higher than that of the control group fed the basal diet (P0M2) in this study. Kaleeswaran et al. (2012) reported an increased A/G ratio in Indian major carp (*Catla catla*) fed an ethanolic extract of *Cynodon dactylon*, while Sharafeldin et al. (2014) also reported an elevated A/G ratio in *O. niloticus* exposed to acute and chronic profenofos, an organophosphorus insecticide. However Hasheesh et al. (2011) reported no significance difference in the A/G ratio of *O. niloticus* treated with 17α-methyl testosterone while Kumar et al. (2011) reported decreased A/G ratio in *O. mossambicus* exposed to endosulfan. Abnormal increases in A/G ratio may occur as a result of disturbances in liver function or failure to excrete albumin if the animal is stressed due to xenobiotic treatment (Sharafeldin et al., 2014). Elevated A/G ratio may be due to decrease in globulin fraction of the total protein. The implication of decreased level of globulin may be an indication of immune suppression since globulins are precursors of immunoglobulins which play a vital role in immune function of an organism.

Cholesterol concentrations in the study had no distinctive trend and the differences between treatment groups were insignificant. Cholesterol concentrations are controlled by the liver through regulation of the lipoprotein metabolism therefore abnormal increase in the level of cholesterol may be due to disorders of lipoprotein metabolism. Cholesterol is the precursor of steroid hormones therefore sexual development and nutritional statuses are among the factors that determine its level in the blood (Patriche et al., 2011). Cholesterol level was not affected by the experimental diet in this study. Glucose is the source of energy for the cellular metabolism and its levels are used as indicators of stress and nutritional status (Demir et al., 2014) in fish. This study did not establish any distinct trend in the level of glucose between treatment groups, indicating no adverse influence of pawpaw seed meal on the carbohydrate metabolism of the fish. The biochemical profiles are influenced by the gender of the fish among other factors. The serum levels of cholesterol, total protein, albumin, and albumin/globulin ratio were significantly higher in female *O. mossambicus* than in males in all the treatment groups while the globulin level of females was significantly higher. This finding was in agreement with findings of Acharya & Mohanty (2014), who reported higher values of the total protein, albumin, globulin, glucose and cholesterol in female *Clarias batrachus* and *Heteropneustes fossilis* than their male counterparts. However, this findings was contradicted by Charoo et al. (2013) who reported higher total protein, albumin and glucose in male rainbow trout (*Oncorhynchus mykiss*) than in the females in their study. Conversely, the glucose level in males was significantly higher than those of the females in all the treatment groups in the present study which agreed with the findings of Charoo et al. (2013).

4.3 Influence of Pawpaw Seed Meal on Hepatosomatic Index

The ratio of liver weight to body weight termed the hepatosomatic index (HSI) is valuable in the study of effect of treatments on fish species (Ighwela et al., 2012). Evaluation of the HSI makes it possible to identify pathological conditions such as atrophy, hypertrophy or hyperplasia associated with certain disease conditions and exposure to toxicants (Rearick et al., 2014). The liver is the organ whose functions include the metabolism of carbohydrates, proteins and fats, the storage of glycogen, vitamins and iron, and most importantly the detoxification of drugs and toxins. It is this latter function that makes the liver the target for attack by toxicants (Rearick et al., 2014). Results from the present study recorded liver weights of P10M1 being significantly lower than those of the control and other treatment groups. However there was no significant difference in the HSI between treatment groups in this study, in contrast, Ighwela et al. (2014) recorded an increased HSI in *O. niloticus* fed high levels of dietary maltose.

5. Conclusions

Results obtained in this study suggests that the dietary inclusion of PSM has no negative effects on the immune system of *O. mossambicus*, and that there is a possibility of including the seed meal up to 30 g/kg of basal diet of *O. mossambicus* to inhibit their precocious maturation and indiscriminate reproduction without compromising the health status of treated fish.

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