



SOME HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF BLOOD OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FED ON DIETS CONTAINING WATERMELON (*CITRULLUS LANATUS*) SEEDMEAL

*Jimoh, W.A¹., Shittu, M.O¹., Ayelaja, A.A¹., Ajasin, F.O.¹, Okemakin, F.Y³, Abdusalami, S.A². and Adekunle. O.F.⁴

¹Department of Fisheries Technology, Federal College of Animal Health and Production Technology, PMB 5029, Ibadan

²Fisheries and Aquaculture Unit, Department of Biological Sciences, Crescent University, Abeokuta, Ogun State

³Department of Biology, The Polytechnic, Ibadan

⁴Department of Animal Health, Federal College of Animal Health and Production Technology, PMB 5029, Ibadan

Correspondenceauthor:jawabus@gmail.com; +234806 228 7099

ABSTRACT

The haematological and biochemical profile of blood of Nile tilapia (*Oreochromis niloticus*) fed on diets containing watermelon (*Citrullus lanatus*) seedmeal were evaluated using packed cell volume (PCV); haemoglobin content (Hb), white blood cell count (WBC), red blood cell count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), blood glucose, cholesterol, total protein, albumin and globulin as indices. 150 tilapia fingerlings of average weight $6.12 \pm 0.05g$ were acclimatized for a week, weighed and allotted into five dietary treatments; DT1, DT2, DT3, DT4 and DT5 containing 0, 15, 30, 45 and 60% *Citrullus lanatus* replacement levels with soybean meal, respectively. The diets were isonitrogenous and isolipidic. Each treatment was replicated three times with ten fish per replicate. Fish were fed 5% body weight on two equal proportion per day. The results from the study indicated that there was no significant difference ($p > 0.05$) in the haematological and biochemical parameters of the blood of fish fed on the various dietary treatments.

Keywords: Blood biochemistry, *Citrullus lanatus*, haematology, tilapia, watermelon

INTRODUCTION

Monitoring fish health can be done using haematological and biochemical profile of its blood (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). Bahmani *et al.*, (2001) reported that analysis of haematological and biochemical indices in the blood of farmed fish is good for identifying the health status of farmed fish as they provide reliable information on metabolic disorders and deficiencies. Banerjee *et al.*, (2002) reported that blood composition is moderately constant under normal condition with little variation. However, the composition of blood can be changed by dietary treatment, malnutrition and disease condition (Feist *et al.*, 2000). Ferreira *et al.*, (2007) reported that biochemical parameters provide early warning of potentially harmful changes in stressed organisms. Bello-Olusoji *et al.* (2006) further explained that changes in haematology of fish in response to stressing agents are indicators of the stressful stage of fish producing useful information to curb any unfavourable condition that may affect the fish health. Saravanan *et al.* (2011) used haematological, ionoregulatory, biochemical and enzymological parameters of Indian major carp, *Cirrhinus mrigala* to evaluate the toxicity of neem leaf extracts

(*Azadirachta indica* A. Juss). Soybean meal is one of the conventional plant protein source feed ingredients used in fish feed (El-Sayed, 1999). However, the use of soybean meal is limited because of the various uses to which it is put; as food for human being and ingredients for other livestock. The use of alternative plant protein sources which are less expensive would be beneficial in reducing feed cost when used to replace soybean meal (Barros *et al.*, 2002). Watermelon is a drought tolerant crop which belongs to the family Cucurbitaceae. Razavi and Milani (2006) reported that water melon is cultivated in a wide range of tropical, semi tropical and arid region of the world. The seeds of watermelon have nutritional quality comparable to other oilseed proteins including soybean and other conventional legumes (Mustapha and Alamin, 2012). Wani *et al.* (2011) reported that watermelon seedmeal contains adequate amount of nutritional protein that could be used as protein source feed ingredients in the production of animal feed. Investigating possible impact of feeding a lesser known feed ingredients to fish on the health of fish is a reasonable attempt to forestall perhaps possible nutritional disease that could negatively affect aquaculture production and development. According to Southgate (1993), disease is considerable constraint in production, development and expansion in the aquaculture industry.

The use of haematology in evaluating a less expensive and readily available plant protein sources, of little or no significance for direct human consumption to replace soybean meal have been investigated. Prominent among which are sunflower and sesame as protein source feed ingredients for *Clarias gariepinus* (Fagbenro *et al.*, 2013); *Luffa cylindrica* as protein source feed ingredients for *Clarias gariepinus* (Jimoh *et al.*, 2012), *Citrullus lanatus* as protein source feed ingredients for *Clarias gariepinus* (Jimoh *et al.*, 2013). There exist paucity of information on the use of watermelon seedmeal in tilapia diets especially the effect it has on its blood biochemistry and haematology. This study therefore, examines the haematological and biochemical profile of blood of Nile tilapia (*Oreochromis niloticus*) fed on diets containing watermelon (*Citrullus lanatus*) seedmeal.

MATERIALS AND METHODS

Sources and Processing of Ingredients.

Sample of dried water melon seeds were obtained in Bodija market, Ibadan, Oyo state. The water melon seed was rinsed with water and boiled for 15 minutes after which it was sundried for some days and then ground in a hammer mill and the oil therein was removed using the pressure generated from locally made screw press (cassava-presser type). The cakes therefore were analysed for their proximate composition (AOAC, 1990). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled screened to fine particles size and triplicate samples were analyzed for their proximate composition (AOAC, 1990).

Table 1: Proximate composition of the protein feed ingredients

Parameter	Fish meal	Soybean Meal	**CLM
Moisture	9.75	10.70	9.69
Crude Protein	72.4	45.74	19.11
Crude Lipid	10.45	9.68	15.35
Crude Fibre	-	5.10	4.97
Ash	8.32	4.48	5.39
*NFE	-	30.00	45.49

*Nitrogen Free Extract

** *Citrullus lanatus* Meal

Experimental Diets

Based on the nutrient composition of the protein feedstuffs (Table 1), the experimental diets were formulated (Table 2) containing soybean meal which was replaced by cooked water melon seed meal at the rate of 0, 15, 30, 45, and 60. The diets were isolipidic and isonitrogenous containing 40% crude protein and 10% lipid with fish meal (72%), soya

bean meal (45%), fish oil, vitamin premix and starch serving as ingredients. The feedstuffs were ground and water was added to aid binding after which it was introduced into a pelleting and mixing machine to obtain a homogenous mass and then passed through a mincer to produce 2mm size pellet which was immediately sundried at 30 - 32°C. After drying for three days, the diet was kept in a cool place.

Table 2: Gross composition (g/100g) of experimental diets containing *Citrullus lanatus* seedmeal fed to *Oreochromis niloticus*

Ingredients	CTR	DT2	DT3	DT4	DT5
Fishmeal	19.44	19.44	19.44	19.44	19.44
Soybean Meal	33.333	28.33	23.33	18.33	13.33
Watermelon	-	11.77	23.55	35.22	47.09
Corn	10.00	10.00	10.00	10.00	10.00
*Fish Premix	2.50	2.50	2.50	2.50	2.50
Fish Oil	2.50	2.50	2.50	2.50	2.50
Starch	32.33	25.46	18.68	11.91	5.13
Total	100.00	100.00	100.00	100.00	100.00

* Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg, Vitamin K₃, 800mg; Vitamin B₁, 600mg; Vitamin B₂, 2,000mg; Vitamin B₆, 1,600mg, Vitamin B₁₂, 8mg; Niacin, 16,000mg; Caplan, 4,000mg; Folic Acid, 400mg; Biotin, 40mg; Antioxidant 40,000mg; Chlorine chloride, 120,000mg; Manganese, 32,000mg; Iron 16,000mg; Zinc, 24,000mg; Copper 32,000mg; Iodine 320mg; Cobalt, 120mg; Selenium, 800mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland.

Experimental Fish and System

The experiment was conducted at the hatchery unit of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The tilapia fingerlings were obtained from Masopa fish farm, Ibadan, Oyo state and transported live to the project site in an aerated bag. The initial average weight of the fish was 6.12±0.05 and a total of 150

tilapia fingerlings were acclimated for 7 days prior to the feeding trial while being fed on a commercial pelleted diet. Ten juveniles were allotted into each tank with 3 replicates per treatment. Experimental diet was assigned randomly to the tanks and each were fed 5% body weight per day in two equal proportions between 9.00 –10.00am and 5.00 – 6.00 pm for 56days.

Haematological Examination

One test organism was removed, from each tank for blood analysis. 5-10ml blood per fish was collected from cardiac puncture in juvenile using 2ml disposable heparinised syringe treated with ethylene diamine tetra acetic acid (EDTA) as anti-coagulant. The blood was stored at -4°C in deep freezer prior to analysis. The blood analysis follows the method described by Svobodova *et al.*, (1991).

Packed Cell Volume (PCV)

Non-clotted blood was drawn by capillary action into micro haematocrit tubes; one end of the tubes was sealed with synthetic sealant. The sealed tube was centrifuged in a micro haematocrit centrifuge. Centrifugation lasted for 5 minutes at 10500 rpm. The packed cell volume was measured using micro haematocrit reader and expressed as percentage.

Mean Corpuscular Haemoglobin Concentration (MCHC)

This refers to the percentage of haemoglobin in 100ml of red blood cell. This was calculated by dividing the haemoglobin content (in g/100ml) by the PCV/100ml of red blood cell according to the formulae:-

$$\text{(gm/dl)}$$

Mean Corpuscular Volume (MCV)

The value of the mean corpuscular volume was calculated from the haematocrit value (PCV) (%) and the Red Blood Count (RBC) ($10^6/\text{mm}^2$), according to the following formulae

$$\text{(fl)}$$

Mean Corpuscular Haemoglobin (MCH)

Mean corpuscular haemoglobin concentration, expressed the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemoglobin value (Hb) and from the red blood cells according to the following formulae:-

$$\text{(Picogramme) (pg)}$$

Blood Cell Count (Red Blood Cell (RBC) and White Blood Cell (WBC))

Haemocytometer was used in blood cell counts. The apparatus consists of a counting chamber; a cover slit white and red blood and a plastic mouth piece for drawing the fluid into pipette. The blood diluting fluid was prepared as described by Svobodova *et al.*,

(1991). The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope.

$$\text{RBC} = \text{Number of cells counted} \times 3 \times 10 \times 200 \text{ (} 10^6 \text{ mm}^3 \text{)}$$

$$\text{WBC} = \text{number of cells counted} \times 0.25 \times 10 \times 20 \times 20 \text{ (} 10^3 \text{ mm}^3 \text{)}$$

Biochemical Tests

The serum total protein was determined by the Biuret method (Reinhold, 1953) using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method (Doumas and Biggs, 1971). The globulin and albumin- globulin ratio were determined according to the method of Coles (1986). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods, respectively using commercial kit (Quimica Clinica Applicada, S.A). Blood glucose was determined according to the method of Toro and Ackerman (1975).

Statistical Analysis

Data obtained from the experiment conducted was subjected to one way Analysis of Variance (ANOVA). Where the ANOVA reveals significant difference Duncan multiple range tests was used to compare differences among individual treatment means.

RESULTS

Proximate Composition of Experimental Diets fed to *Oreochromis niloticus*

Table 3 reveals the proximate composition of experimental diets fed to *Oreochromis niloticus*. There was no significant difference ($P > 0.05$) in moisture, protein, lipid, fibre, ash and Nitrogen Free Extract (NFE). All the fish responded well to the dietary treatment given to them.

The table of proximate composition of the experimental diets showed that the various diets prepared were isonitrogenous, isocaloric and isolipidic as there was no significant difference ($P > 0.05$) in the crude protein and crude lipid content of the diets. The protein and lipid requirement of *Oreochromis niloticus* was met by the 35 and 10% provided in the experimental diets. Jauncey and Ross, 1982; Luquet 1991).

Table 3: Proximate composition (g/100g) of experimental diets containing *Citrullus lanatus* seedmeal fed to *Oreochromis niloticus*.

Parameters	CTR	DT2	DT3	DT4	DT5
Moisture	9.66±0.51	9.59±0.59	9.56±0.50	9.88±0.33	9.52±0.52
Crude Protein	35.22±0.05	35.14±0.16	35.23±0.33	35.22±0.06	35.17±0.23
Crude Lipid	10.16±0.09	10.15±0.06	10.08±0.03	10.04±0.27	10.19±0.13
Crude Fibre	4.37±0.36	4.17±0.08	4.12±0.03	4.15±0.05	4.13±0.05
Ash	5.15±0.20	4.90±0.28	4.66±0.50	5.12±0.37	5.09±0.16
NFE	35.43±0.53	36.0±0.51	36.34±0.86	35.57±0.57	33.90±0.61
Total	100	100	100	100	100

Figures in each row without superscripts are not significantly different ($P > 0.05$) from each other

The haematological profile of the blood of *O. niloticus* fed diet containing *Citrullus lanatus* seed meal is as shown in Table 4. Fish fed diet DT3 had the highest Packed Cell Volume (PCV) while the fish diet CTR had the lowest Packed Cell Volume (PCV). There was no significant difference ($p < 0.05$) in the PCV of fish exposed to different dietary

treatments. The blood of fish fed diet DT3 had the highest Hb content while the blood of fish fed diet control has the lowest Hb content, no significant variation ($P > 0.05$) was recorded in the Hb content of the fish blood exposed to various dietary treatments. Same trends of result was applicable to RBC, WBC, MCV, MCH, MCHC.

Table 4. Haematological changes in the blood of *Oreochromis niloticus* fed diets containing *Citrullus lanatus* seedmeal

	CTR	DT2	DT3	DT4	DT5
PCV (%)	12.50±0.71 ^a	14.50±0.71 ^a	19.00±7.07 ^a	15.50±2.12 ^a	16.00±0.00 ^a
Hb (gm/dl)	4.10±0.14 ^a	4.08±0.28 ^a	6.30±2.40 ^a	5.15±0.78 ^a	5.30±0.00 ^a
RBC (10 ⁶ /mm ³)	0.80±0.13 ^a	0.98±0.08 ^a	1.40±0.57 ^a	1.20±0.44 ^a	1.40±0.11 ^a
WBC (10 ³ /mm ³)	106.50±7.78 ^a	130.50±14.85 ^a	106.00±7.07 ^a	127.50±21.92 ^a	116.50±21.92 ^a
MCV (fl)	160.27±35.98 ^a	148.20±5.61 ^a	136.67±4.72 ^a	134.94±31.62 ^a	114.66±9.26 ^a
MCH (p□)	52.47±10.65 ^a	49.04±1.36 ^a	45.22±1.10 ^a	44.72±9.86 ^a	37.98±3.07 ^a
MCHC (gm/dl)	32.82±0.72 ^a	33.10±0.33 ^a	33.10±0.33 ^a	33.20±0.47 ^a	33.13±0.00 ^a

Row means with the same superscript are not significantly different ($p>0.05$) from each other

Biochemical changes in the blood of *O. niloticus* fed diets containing *C. lanatus* seedmeal is presented in Table 5. There was no significant difference ($p>0.05$)

in the blood protein, albumin, globulin, total cholesterol and blood glucose.

Table 5. Biochemical changes in the blood of *Oreochromis niloticus* fed diets containing *Citrullus lanatus* seed

Parameters	CTR	DT2	DT3	DT4	DT5
Total Protein (g/dL)	7.20±0.00 ^a	7.75±0.92 ^a	10.80±5.66 ^a	7.90±1.27 ^a	8.30±0.42 ^a
Albumin (g/dL)	5.05±0.07 ^a	5.34±0.76 ^a	7.55±4.03 ^a	5.55±0.92 ^a	5.80±0.28 ^a
Globulin (g/dL)	2.15±0.07 ^a	2.40±0.76 ^a	3.20±1.70 ^a	3.25±0.35 ^a	2.50±0.14 ^a
Total Cholesterol (mg/dL)	18.50±5.23 ^a	18.50±15.70 ^a	22.20±20.93 ^a	16.00±1.70 ^a	15.45±11.38 ^a
Blood Glucose (mg/dL)	37.00±2.83 ^a	40.00±0.00 ^a	53.00±48.08 ^a	48.00±25.46 ^a	50.00±11.31 ^a

Row means with the same superscript are not significantly different ($p>0.05$) from each other

DISCUSSION

The results of the experiments indicated observed increase in haematological parameters of the Nile tilapia fed diets containing *Citrullus lanatus* seedmeal which conforms to the similar report by Jimoh *et al.*, (2013) for *Clarias gariepinus* fed on the same seedmeal. The values recorded for haematological parameters of the Nile tilapia fed diets containing *Citrullus lanatus* seedmeal were all within the range of normal haematology of a healthy fish, (Fagbenro *et al.*, 1993). Fagbenro *et al.* (1993) a range of 3.61-6.54 g/mm³ for haemoglobin; 15-31% for Haematocrit (PCV); 1.31-3.23 (10⁶/mm³) for RBC; 0.80-73.6 (10³/mm⁴) for WBC; 17.28-26.14 (%) for MCHC. According to Lenfant and Johansen (1972), RBC greater than 1x10⁶/mm³ is considered high and is indicative of high oxygen carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and with high activity. The reduction in the MCV with increasing plant protein based diets recorded in this studies is in consonance with the report of Kumar *et al.*, (2010) on plant protein in carp diets and Hemre *et al.*, (2005) on plant protein in Salmon diets. It was explained that the plant protein feed ingredients could induce early release of immature erythrocyte (Kumar *et al.*, 2010). Increase in white blood cell as observed in the fish fed on *Citrullus lanatus* diets is attributed to increase in the production of leucocytes. This is in consonance with the findings of Akinwande *et al.* (2004). The increase in other parameters of the blood of fish fed test diets with respect to that of fish fed control diets agrees with report of Akintayo *et al.*, (2008) who fed the toasted sunflower seed meal to *Clarias gariepinus* and

Yue and Zhou (2008) who fed cotton seed meal to juvenile hybrid Tilapia, Barros *et al.* (2002) for channel catfish and El-Saidy and Gaber (2004) for Nile Tilapia. Das *et al.* (2004) reported that the concentration of blood plasma protein is an indicator to general health condition of fish. Although, Abdali *et al.* (2011) reported that a reduction in plasma protein is an indicator of the effect of toxins in the kidney, spleen and liver the results obtained in this study showed a non significant increase in the total plasma protein of Nile tilapia fed test diets when compared to fish fed control diet. The decrease in protein level with higher levels of plant protein based diets may be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis (Singh and Singh, 2002) and may be due to mobilization of protein to meet energy requirements and to sustain increased physiological activity (Martinez *et al.*, 2004).

There was a general non significant ($P>0.05$) increase in other biochemical parameters in fish fed test diets relative to fish fed control diets. This is desirable because reduction in blood glucose level might be as a result of hypoxic condition induced by feeding diets containing higher concentration of anti-nutrients. The increase in blood glucose level recorded in this study agrees with the findings Kumar *et al.* (2010) who observed higher blood glucose concentration in fish exposed to plant protein based diets. Other researchers with similar observations are Kikuchi *et al.*, 1994; Kikuchi, 1999). Increase in the blood glucose concentration might be resulted from an increase in plasma catecholamine and corticosteroid hormones (Pickering, 1981).

So also reduction in blood cholesterol was observed Kumar *et al.* (2010) and its occurrence may be related to its utilization in the manufacture of cortisol arising from stress created by consumption of diets containing higher concentration of anti-nutrients. Other researchers who reported decrease in blood cholesterol of fish fed on plant protein based diets are Kaushik *et al.*, (1995); Yamamoto *et al.*, (2007).

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De Schrijver (1990) reported that plant products have ability to reduced blood cholesterol.

CONCLUSION

In conclusion, it is possible to replace soybean meal by *Citrus lanatus* in the diet of *Oreochromis niloticus* as it does not have effect on the haematological and biochemical parameters of the blood of fish.

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