

HAEMATOLOGICAL PARAMETERS AND ORGAN INDICES OF THE NILE TILAPIA (*Oreochromis niloticus*) FED WITH ROSELLE (*Hibiscus Sabdariffa*) SEED MEAL AT VARIOUS INCLUSION LEVELS

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ABSTRACT:

Oreochromis niloticus, weighing an average of 0.60g, were reared in a plastic aquarium measuring 60cm x 50cm x 30cm with a water holding capacity of 120 litres for a period of 28 weeks. To examine the impact of different inclusion levels (0%, 25%, 50%, 75%, and 100%) of (Roselle) *Hibiscus sabdariffa* seed meal on the haematological properties of *O. niloticus*, five experimental diets were used in this study. At the end of the feeding trial, fish from each treatment group were randomly selected for haematological analysis. The blood of each fish was collected using a syringe and needle and stored in ethylenediamine- tetra acetic acid (EDTA) bottles. This was used to determine various parameters: packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) level, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV). The obtained results indicated that the fish fed with a 50% inclusion level of Roselle had the highest packed cell volume of 40.00%. The control group with 0% inclusion level 38.50%, followed by levels of 25%, 75% and 100 % (37.50%; 34.00%; 32.00%) respectively. There were significant differences ($P < 0.05$) observed in all the PCV values. The RBC values exhibited a similar pattern with readings of 4.70 (50%), 4.35 (0%), 4.19 (25%), 3.37 (75%), and 3.10 (100%). The Hb values also followed a consistent trend, measuring at 7.70, 7.15, 8.20, 7.43, and 7.20 for inclusion levels of 0%, 25%, 50%, 75%, and 100% respectively. However, it is worth noting that lymphocytes and white blood cell counts were lower at the inclusion level of 50% (81.00) when compared to the levels of 83.50 (0%), 84.45 (25%), and 89.70 (100%). Based on these findings, it can be concluded that processed *Hibiscus sabdariffa* seed meal can effectively replace fish meal in practical diet for *O. niloticus* at 50% inclusion level without any detrimental effects on the fish's haematological parameters.

Keywords: Packed cell volume, haematological properties, *Oreochromis niloticus*, *Hibiscus sabdariffa*, Inclusion levels

INTRODUCTION

The Nile Tilapia (*Oreochromis niloticus*) is considered one of the most important fish species for aquaculture in the 21st century. It is cultivated in numerous countries worldwide, primarily due to its high-quality meat, rapid growth, and hardiness (Castro *et al.*, 2011). Tilapias exhibit good resistance to diseases, but intensive culture and rapid growth can potentially affect their immune status and increase susceptibility to diseases.

In order to assess the physiological and pathological aspects of fish as well as the effects of nutrition and environmental factors on cultured fish, haematological measures are crucial (De-Pedro *et al.*, 2005). In addition to feed composition and environmental influences, haematological parameters can also be influenced by fish activity fluctuations and reproductive cycles (Rehulka, 2003). According to several research, feeding causes changes in fish's blood parameter indices (Gabriel *et al.*, 2011; Ayoola *et al.*, 2013; Dieyl and Olumuyi, 2014). To evaluate the health state of farmed fish, it is crucial to establish the fundamental blood parameters. According to Ayoola *et al.* (2013), packed cell volume (PCV) in fish is a key marker for anaemia and hypoproteinaemia,

According to Bahmari *et al.* (2001), examining haematological and biochemical indices in the blood of farmed fish can help determine the fish's health status and reveal any metabolic problems or nutritional inadequacies. However, nutritional interventions, malnutrition, and illness states can all affect blood composition (Feist *et al.*, 2000). Biochemical characteristics provide early indications of potentially harmful changes in stressed organisms, (Ferreira *et al.* 2007). Bello-olusoji *et al.* (2006) stress-related alterations in fish haematology are signs of a stressful stage and can be used to help reduce adverse conditions that could affect fish health. Haematological, biochemical, and enzymological indices in Indian main carp *Cirrhinus mrigala* were employed by Saravanan *et al.* (2011) to assess the toxicity of new leaf extracts. This study examines *Hibiscus sabdariffa* seed meal as an alternative to conventional fishmeal. Although, the seed is known for its high protein content, in Nigeria however, large quantities of this crop's seeds are wasted annually on farms, with only a minimal amount being collected and stored for planting purposes. The study therefore evaluates the impact of various inclusion levels of *Hibiscus sabdariffa* seed meal on the haematological

parameters and organ indices of Nile Tilapia (*Oreochromis niloticus*) to determine the plant's effects on fish wellbeing.

MATERIALS AND METHODS

Study Area:

The study was carried out at the Ahmadu Bello University Zaria's Aquaculture Unit, Skill Acquisition Center, and National Agricultural Extension and Research Liaison Services. The region is situated in latitude 11° 09' 06" N and longitude 7° 38' 55" E in Nigeria's Northern Guinea Savannah Zone, at an altitude of 706 m above sea level.

Procurement and Processing of Feedstuff:

Hibiscus sabdariffa seeds were purchased from Sabon-Gari Market; Groundnut cake, maize soybean cake, and bone meal were sourced from a reputable feed while palm oil, salt, maize bran, and wheat offal were purchased from Samaru Market in Zaria. Fish meal, vitamin, and mineral premix were procured from PIMO agro-based Ventures in Zaria. Table 1 shows the proximate composition of the major ingredients in the experimental diets. A total of 2.5 kg of Roselle (*Hibiscus sabdariffa*) seeds were boiled for 30 minutes at 100°C, washed, and kept in an airtight container to ferment for three days. The fermented seeds were sun-dried, ground, and put into the experimental diet at various inclusion levels, as shown in Table 2.

Table 1: Proximate composition of feedstuff used in the experimental diet (%)

INGREDIENTS	CP	CF	CL	Ash	NFE
Fishmeal	72.23	0.10	8.50	10.00	3.96
Roselle seed meal	39.95	6.44	20.65	6.20	29.20
Wheat offal	16.34	12.34	1.69	6.58	22.50
Maize	10.80	3.50	3.60	8.40	64.10
GNC	43.50	6.15	5.36	6.10	31.00

CP=Crude Protein, CF=Crude Fibre, CL=Crude Lipid, NFE=Nitrogen Free Extract, GNC=Groundnut cake

Experimental Diets:

Five iso-nitrogenous diets were formulated using fermented *Hibiscus sabdariffa* seeds. Each experimental diet contained a 35% crude protein level, calculated using Pearson's square method as described by Bolorunduro (2002). The control diet consisted of fishmeal without Roselle seed meal. The *H. sabdariffa* seed meal was used to replace fishmeal at inclusion levels of 0 %, 25 %, 50 %, 75 %, and 100 %. The ingredient composition of the experimental diets is shown in Table 2.

TABLE 2. Gross Compositions of the Experimental Diets.

INGREDIENTS (g/100g)	0%	25%	50%	75%	100%
Fishmeal	52.0	39.0	26.0	13.0	0.00
<i>H.sabdariffa</i>	0.00	13.0	26.0	39.0	52.0
Wheat offal	20.0	20.0	20.0	20.0	20.0
Maize	20.0	20.0	20.0	20.0	20.0
Vit./min.premix	1.0	1.0	1.0	1.0	1.0
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Oil	0.8	0.8	0.8	0.8	0.8
DCP	0.2	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5	0.5
Groundnut cake	5.0	5.0	5.0	5.0	5.0
AIA	0.1	0.1	0.1	0.1	0.1

DCP=Dicalcium phosphates, AIA=Ash-in-ash

Haematological Parameters:

At the end of the week 24 of feeding, fish from each treatment were randomly selected for haematological analysis. Live fish was put on a tray. A damp cloth was used to cover the fish head. Blood was drawn from the caudal vein through the lateral line of fish into

ethylenediamine tetra-acetic acid EDTA bottles by the use of needle and syringe for determination of the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), following Hrubec and Smith (2000).

Packed cell volume (PCV)

The heparinized capillary tubes were 3/4 filled with whole blood and one end sealed with plasticine. The tubes were centrifuged for 5 min in a micro haematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Kelly, 1979)

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

The RBC and total WBC counts were carried out by use of the Neubauer improved counting chamber as described by (Kelly, 1979). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99 ml of 3% aqueous solution of sodium citrate; and 1 ml of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber (Kelly, 1979)

Total white blood cell counts

For white blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid to which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain (Schalm *et al.*, 1975). A total of 100 white blood cells were enumerated and differentiated.

Haemoglobin (Hb) estimation

The cyano-methaemoglobin method as described by Kelly, (1979) was used in the determination of haemoglobin concentration. Well-mixed blood of 0.02 mL was added to 4 mL of modified Dabkin's solution (potassium ferricyanide, 200 mg; potassium cyanide, 50 mg; potassium dihydrogen phosphate 140 mg). The volume was made up to 1 L with distilled water at pH of 7.0. The mixture was allowed to stand for 3min and the Hb concentration was read photometrically by comparing with a cyano-methaemoglobin standard with a yellow-green filter at 625 nm.

Wintrobe Erythrocyte Indices

Calculations of the absolute values or the erythrocyte indices, namely mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were carried out according to the following equations: -

Mean Corpuscular Volume (MCV):

The formula $MCV = \text{Haematocrit} / \text{RBC} \times 100$ was used to calculate the mean corpuscular volume value using the haematocrit value (PCV) (%) and the red blood cell count (RBC) ($10^6/\text{mm}^2$).

$$MCV = \frac{\text{Hct ml}/\mu}{\text{RBC ml}/\mu} \times 10$$

Mean Corpuscular Haemoglobin (MCH):

The concentration of haemoglobin in a unit volume of erythrocytes is expressed by the mean corpuscular haemoglobin concentration. The formula $MCH = \text{Hb}/\text{RBC} \times 100$ was used to calculate the haemoglobin value (Hb) and the red blood cell count.

$$MCH = \frac{\text{Hb}}{\text{RBC}} \times 10$$

Mean Corpuscular Haemoglobin Concentration (MCHC):

This is the quantity of haemoglobin in 100 millilitres of red blood cells. It involved dividing the haemoglobin content (in g/100 ml) by the PCV/100 ml of red blood cells. The formula $MCHC = \text{Haemoglobin}/\text{Haematocrit} \times 100$ was used following Hrubec and Smith (2000).

$$MCHC = \frac{\text{Hb}}{\text{Hct}} \times 100$$

Organs Indices

A sample of the fish from each tank were killed, and the abdominal cavity were opened to remove liver, kidneys, gonads, and spleen, each of the organs were weighted individually. Hepato-somatic index (HSI), kidney somatic index (KSI), gonad somatic index (GSI), and spleen somatic index (SSI) were calculated as follows:

$$HSI = 100 \left(\frac{\text{Liver weight}}{\text{body weight}} \right)$$

$$KSI = 100 \left(\frac{\text{Kidney weight}}{\text{body weight}} \right)$$

$$GSI = 100 \left(\frac{\text{Gonads weight}}{\text{body weight}} \right)$$

$$SSI = 100 \left(\frac{\text{Spleen weight}}{\text{body weight}} \right)$$

Statistical Analysis:

One-way Analysis of Variance (ANOVA) was used to analyse the data. Duncan multiple range tests were used to assess differences between the means of each individual treatment.

RESULTS AND DISCUSSION

Table 3 displays the haematological parameters of *O. niloticus* fed with different inclusion levels of fermented *H. sabdariffa* seed meals. The packed cell volume (PCV) of tilapia fed a 50% inclusion level of *H. sabdariffa* was higher (40.00) compared to 0% inclusion level (38.50), followed by 25% (37.50), 75% (34.00), and 100% (32.00). There was a significant difference in PCV values. Other blood parameters followed a similar pattern: RBC (4.70) for 50% (4.35) for 25%, 4.19 (0%), 3.37 (75%), and 3.10 (100%). The

Hb. values were 7.70, 7.15, 8.20, 7.43, and 7.20 for 0%, 25%, 50%, 75%, and 100%, respectively. However, lymphocyte values and white blood cells (WBC) were higher at 75% and 100% inclusion levels. Lymphocyte

values were 83.50, 84.45, 81.00, 89.00, and 89.70 for 0%, 25%, 50%, 75%, and 100% inclusion levels, respectively.

Table 3: Blood Parameters of *O. niloticus* fed Different Inclusion Levels of Roselle Seed Meals for 28 weeks.

Parameters	0%	25%	50%	75%	100%
RBC	4.19 ±0.05 ^c	4.35±0.05 ^b	4.70±0.05 ^a	3.37±0.05 ^d	3.10±0.05 ^a
WBC	3.85 ±0.26 ^c	3.41 ±0.26 ^d	3.12 ±0.26 ^e	4.20 ±0.26 ^b	4.36 ±0.26 ^a
PCV	38.50 ±0.32 ^b	37.50 ^c ±0.32 ^c	40.00 ^a ±0.32 ^a	34.00 ^d ±0.32 ^d	32.00 ^e ±0.32 ^e
Hb	7.70 ^b ±0.00 ^b	7.15 ^c ±0.00 ^e	8.20 ^a ±0.00 ^a	7.43 ^c ±0.00 ^c	7.20 ^d ±0.00 ^d
MCH	280.38 ^a ±0.33 ^a	240.30 ^b ±0.33 ^b	280.40 ^a ±0.33 ^a	230.15 ^c ±0.33 ^c	210.35 ^d ±0.33 ^d
MCHC	34.00 ^b ±0.14 ^b	32.45 ^c ±0.14 ^c	34.60 ^a ±0.14 ^a	31.42 ^d ±0.14 ^d	30.75 ^e ±0.14 ^e
MCV	90.10 ^b ±0.00 ^b	90.00 ^c ±0.00 ^c	90.50 ^a ±0.00 ^a	89.90 ^d ±0.00 ^d	89.30 ^e ±0.00 ^e
Lymphocytes	83.50 ^c ±0.27 ^c	84.45 ^b ±0.27 ^b	81.00 ^d ±0.27 ^d	89.00 ^a ±0.27 ^a	89.70 ^a ±0.27 ^a

Means on the same row with the same superscripts are not significantly different (p<0.05)

In this experiment, the effect of various inclusion levels of *H. sabdariffa* (Roselle) seed meal on the haematological characteristics of *O. niloticus* was measured. A higher level of dietary *H. sabdariffa* seed meal than 50% (75% and 100% inclusion levels) significantly reduced the concentration of haemoglobin (Hb), packed cell volume (PCV), and red blood cells (RBC). The increased consumption of *H. sabdariffa* seed meal in the diets may be responsible for this decrease due to higher amounts of anti-nutritional components. Protein inhibitors in raw Roselle meal, like trypsin inhibitor, can interfere with the function of digestive enzymes and result in digestion losses (Faris and Singh, 1990). The small decline in haematological markers seen in this study was probably caused by these factors.

Fish productivity, food, and health can all be negatively impacted by anti-nutritional factors (Makkar, 2003; Jiraungkoorskul *et al.*, 2003; Kuntz and Kuntz, 2003). According to Lieiner (2014), trypsin and other protease inhibitors may act as possible anti-nutrients and hinder growth. Nutritionally inadequate meals have been linked to lower levels of haemoglobin concentration, haematocrit, and red blood cell volume, according to Tacon (1992). Khajepour *et al.* (2011) is of the view that, nutritional toxicity in fish is linked to anaemia, which is brought on by a reduction in red blood cell

volume and can cause fish to suffocate and die. The low levels of Hb, PCV, and RBC in *Clarias gariepinus* were attributed by Osuigwe *et al.* (2003) to raw Jack bean seed meal, which has inherent anti-nutritional components. This study was validated by their findings. These findings align with earlier reports by Lin and Luo, 2011; Mistral and Mohanty, 2008; El-more, 2007; and Adeyemo, 2005, Who also witnessed a low level of haemoglobin concentration, haematocrit, and red blood cell volume due to the presence of anti-nutritional factors like protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamin and allergens. Fish fed diets 1, 2, and 3, which included 0%, 25%, and 50% *H. sabdariffa* seed meal, respectively, exhibited higher Hb. concentration, PCV, and RBC counts. This indicates that fish fed 100% (control) fishmeal, 75%, and 50% of fishmeal had a better haematological profile in *O. niloticus* compared to those fed only 25% and 0% fishmeal. This finding supports the work of Jahan *et al.* (2007) who partially replaced fish meal protein with soybean meal protein in the diet of mrigal (*Cirrinus cirrhosis* Ham). In their study, diet 3 which included 50% fishmeal and 50% soybean (Glycine max) seed meal performed better and exhibited the highest haematological profile.

Table 4: Organ Indices of *O. niloticus* fed Inclusion Levels of Roselle Seed Meals.

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Parameters	TREATMENTS				
	0%	25%	50%	75%	100%
Hepatosomatic Index	0.33±0.02 ^a	0.32±0.02 ^a	0.30±0.02 ^a	0.34±0.02 ^a	0.34±0.12 ^a
Kidney somatic Index	0.50±0.02 ^a	0.49±0.02 ^a	0.48±0.02 ^a	0.49±0.02 ^a	0.49±0.02 ^a
Spleen Somatic Index	0.30±0.01 ^a	0.31±0.01 ^a	0.30±0.01 ^a	0.32±0.01 ^a	0.31±0.01 ^a
Gonad Somatic Index	1.62±0.05 ^a	1.61±0.05 ^a	1.60±0.05 ^a	1.61±0.05 ^a	1.62±0.05 ^a

Means on the same row with the same superscripts are not significantly different ($p < 0.05$).

Table 4 presents the results for organ indices of *O. niloticus* fed raw and processed Roselle seed meals. All the organs were within the normal size range for *O. niloticus*, with the liver ranging from 0.30 - 0.34, kidneys between 0.48 and 0.50, spleen between 0.30 and 0.32, and gonads ranging from 1.60 to 1.62 for *O. niloticus* fed Roselle seed meals for twenty-eight (28) weeks.

There were no significant differences in the other internal organ indices of fish fed diets containing 0%, 25%, 50%, and 75% replacement of fishmeal by *H. sabdariffa* seed meal. These findings align with Abdel-warith (2008), who revealed that there were no significant changes in viscera-somatic, gonad-somatic, and spleen somatic indices of tilapia fed 0%, 25%, 50%, and 75% inclusion levels of Jojoba seed meals as complete or partial replacements for fishmeal. The gonad somatic index (GSI) results indicate that the reproductive characteristics of the fish were not affected by the addition of *Hibiscus sabdariffa* seed meal in the diet, although the values were lower at 50% and 0% inclusion levels. This supports the work of Ebole *et al.* (2016) in their study on the growth performance and haematological parameters of *Oreochromis niloticus* fed with varying diets of *Moringa oleifera* (Lam) leaf meal as an additive protein source. The researchers reported that the fish were not affected by the addition of Moringa in the diet, although GSI values were lower at inclusion levels of 10% and 15% (Ebole *et al.*, 2016). The hepatosomatic index (HIS) was also similar among the various treatments and did not exceed the normal size recommended for tilapia. This indicates that processed *Hibiscus sabdariffa* seed meal did not have any harmful effects on the fish. The HIS is a useful biomarker for detecting harmful effects of environmental stressors (Sadekarpawar and Parikh, 2013).

CONCLUSION

Based on these findings, it can be concluded that processed *Hibiscus sabdariffa* seed meal does not have any detrimental effects on the fish's haematological parameters. It is recommended to use the oil seed meal at 50% level of inclusion.

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