



# International Journal of Fisheries and Aquaculture

Volume 8 Number 11 November 2016

ISSN 2006-9839



*Academic  
Journals*

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**ARTICLE**

**Analysis of growth performance and haematological parameters of *Oreochromis niloticus* fed on a varying diet of *Moringa oleifera* Lam. leaf meal as an additive protein source**

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Ian Bbole, Chanda Mumba, Nephter Mupenda and Alexander Shula Kefi

## Full Length Research Paper

# Analysis of growth performance and haematological parameters of *Oreochromis niloticus* fed on a varying diet of *Moringa oleifera* Lam. leaf meal as an additive protein source

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Received 3 May, 2016; Accepted 12 August, 2016

The objective of the current study was to investigate the growth performance and haematological parameters of *Oreochromis niloticus* fed on four different diets at different *Moringa oleifera* leaf meal inclusion levels over a period of 33 weeks. The results indicate that *Moringa* leaf meal causes depressed growth in fish but may increase the immunity of the fish to fight infections and diseases. The four inclusion levels used were: 0% (No *Moringa* - control), 5, 10 and 15%. There was a significant ( $p < 0.05$ ) difference in the final weight of fish in the control diet versus the other three treatments. The Specific Growth Rate (SGR), Mean Weight Gain (MWG) and Condition Factor showed no significant ( $p > 0.05$ ) difference among the fish fed with the four diets. However, the SGR and MWG were highest in the control fish followed by 15% then 10% and lastly 5%. The Gonadal Somatic Index, Hepatosomatic Index and Cardiosomatic Index were not significantly ( $p > 0.05$ ) different among the four treatments. The mean values of haematological parameters showed no significant ( $p > 0.05$ ) difference among the fish fed on the four diets. However, the Mean Cell Haemoglobin in 15% *Moringa* diet was significantly different from the control diet. White Blood Cells were not significantly different among the treatments ( $p > 0.05$ ) though high values were observed in treatments with *Moringa* inclusion. The study has shown that inclusion of *Moringa* to the fish diet results in depressed growth but it improves the immunity of the fish because of an increase in White Blood cells. Further studies to explore the use of *Moringa* in fish diets at rates which do not cause depressed growth but improve immunity are recommended.

**Key words:** Growth performance, haematological parameters, *Moringa oleifera*, *Oreochromis niloticus*, protein source.

## INTRODUCTION

Aquaculture production in Africa has been on a steady increase growing more rapidly in Sub-Saharan countries than the rest of Africa (FAO, 2012). In 2010, Africa contributed 1.2 million tonnes of fish from aquaculture production representing 2.2% of global aquaculture production (FAO, 2012). One major constraint in

aquaculture production has been the cost of fed ingredients. Fish meal has been a major dietary protein source in compounded tilapia feeds (Olsen and Hasan, 2012). Protein requirement in tilapia feeds under intensive culture can represent about 50% of total costs (El-Sayed, 2006). As a result of the high cost and limited

amount of fish meal available, research in fish nutrition has focused on developing alternative cost effective tilapia feeds using plant based sources (El-Sayed, 2006; Olsen and Hasan, 2012).

One plant receiving a lot of attention as a possible replacement of fish meal is *Moringa oleifera*. This is a fast growing plant and it is widely available in the tropics and subtropics and it has several economically important uses for industry and medicine (Richter et al., 2003). The leaves are rich in proteins, vitamins, fatty acids and minerals (Moyo et al., 2011). It has been nicknamed as a 'wonder plant'. Various studies have been conducted to replace fish meal with *Moringa* leaf meal in diets of mainly *Oreochromis niloticus*, *Clarias gariepinus* and other species with varying results (Madalla et al., 2013; Olaniyi et al., 2013; Hlophe and Moyo, 2014; Ncha et al., 2015; Mehdi et al., 2016). However, most studies have shown depressed growth on fish species fed with diets containing *Moringa*.

The haematological parameters of fish are important for evaluating the physiological conditions, disease as well as determining the effect of diet and other environmental factors in cultured fish (De Pedro et al., 2005). Apart from diet composition and environmental factors, reproductive cycles and variations in fish activity can also affect their haematological parameters (Rehulka, 2003). Several studies have reported changes in blood parameters indices of fish as a result of feed (Gabriel et al., 2011; Keri et al., 2012; Ayoola et al., 2013; Ozovehe and Nzeh, 2013; Dieyl and Olumuji, 2014). Therefore, determining the basal parameters of blood is important in order to monitor the health status of fish. For example, packed cell volume (PCV) is a useful indicator of anaemia, hypoproteinemia and leukocytosis of fish (Keri et al., 2012).

The high cost of fish meal which is the major protein source for fish diets has resulted in high feed costs. This has made it difficult for small scale producers to be productive because they cannot afford to acquire the fish feed. Thus alternatives of mainly plant based protein sources which can replace wholly or partly the fish meal is under consideration. Most of the studies conducted on the effect of *Moringa* on fish growth have been done over a shorter period of time compared to the current study. The haematological analysis on fish fed with *Moringa* have mainly been on other species especially *Clarias*. This study focused on *O. niloticus* which is one of the most cultured fish species in the world.

This study evaluated the growth performance and the effect of *M. oleifera* leaf meal on the haematological parameters of *O. niloticus* in order to ascertain the impact this mineral rich plant has on the well-being of fish.

## MATERIALS AND METHODS

### Experimental conditions and design

The experiment was conducted at the National Aquaculture Research and Development Centre (NARDC) (12°49' 0" South, 28°12' 0" East) in Kitwe Zambia, Southern Africa. Twelve hapas measuring 1 m x 1 m x 1 m were placed in a 750m<sup>2</sup> semi-concrete pond (Figure 1). The *Moringa* leaf meal was obtained from local *Moringa* farmers who prepared it by collecting fresh green leaves, washing them to remove dirt, drying them in the shade and grinding into meal form. The treatments were: Control (0%) - no *Moringa*, 5% *Moringa* diet, 10% *Moringa* diet and 15% *Moringa* leaf meal diet. These treatments were replicated thrice and assigned in a Complete Randomized Design. The experiment was conducted for a period of 231 days (33 weeks).

The experimental fish were handled according to the Canadian Council guide to the care and use of experimental animals (CCAC, 1989).

### Stocking and sampling

A total of 15 fish weighing 42.0±2.50 g were stocked according to sex in each hapa. Initial measurements of weight (g), standard and total lengths (mm) were taken at stocking. Sampling to measure weight, standard length and total length were then done fortnightly.

### Feeding

The fish for the first 7 days before the introduction of the experimental diets were feeding on natural food in water. This was done to prepare the fish for the new diets and eliminate variations in weight. The fish were then fed with an isonitrogenous (32% crude protein) feed comprising the following ingredients; fish meal, maize meal, mineral mix, vitamin premixes and soya gold oil. The feeding was done at 3% live body weight at 09:00 hours and 14:00 hours daily. The feed was formulated using Winfeed 2.8 feed package software (Winfeed, 2006). The feed was administered in form of pellets and the feed ration was adjusted fortnightly upon sampling of the fish. The composition of the diet for each treatment is shown in Table 1.

### Water quality

Water quality parameters were taken once per day using a water checker (YSI professional plus) in each hapa. The following parameters were been measured; Temperature, pH, Dissolved Oxygen, Ammonia and Nitrates.

### Measurement of growth performance

The growth parameters were calculated as follows:

$$i) \text{ Specific Growth Rate: } ((\ln W_t - \ln W_i) / t) * 100$$

Where  $\ln W_t$  is the natural logarithm of the final body weight,  $\ln W_i$  is the natural logarithm of initial body weight of fish and  $t$  is the final

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**Figure 1.** Experimental units.

**Table 1.** Diet formulation (DM %) of the four experimental diets.

Ingredients	Control	5% diet	10% diet	15% diet
Maize meal	59.95	56.08	52.3	48.53
<i>Moringa</i> leaf meal	0	5	10	15
Fish meal	38.65	37.42	36.2	34.97
Soya gold oil	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5
Vitamin mix	0.5	0.5	0.5	0.5
Total	100	100	100	100

time of the experiment in days (De Silva and Anderson, 1995).

ii) Mean weight gain:  $((W_f - W_i)/W_i) * 100$

Where  $W_f$  is the mean final weight and  $W_i$  is the mean initial weight of fish (De Silva and Anderson, 1995).

iii) Condition factor (K):  $((W/L^3) * 10^5)$

Where  $W$  is the final mean body weight (g) and  $L$  is the mean standard length (mm) (Ricker, 1975).

iv) Survival rate (SR):

$((\text{Initial number of fish stocked} - \text{mortality}) / \text{Initial number of fish}) * 100$

#### Determination of haematological parameters

A total of 12 fishes were randomly selected from each treatment

and blood samples collected following the procedures by Wedemeyer and Yasutake (1977) at termination of the experiment. Two 2ml of blood was collected using a syringe and needle by piercing the vein located on the caudal peduncle. Each blood sample was placed separately in each sterile vacuum tube, containing Ethylene Diamine Tetraacetic acid (EDTA) as an anticoagulant and sent to the laboratory at Ndola Central Hospital for haematological analysis.

The following haematological parameters were analysed; Red Blood Cell count (RBCs), Haemoglobin Concentration (Hb), Haematocrit (Hct), White Blood Cell count (WBCs), Mean Cell Haemoglobin concentration (MCHC), Mean cell Volume (MCV), and Mean Cell Haemoglobin (MCH).

After the blood samples for haematological analysis were collected the fish were preserved in 10% formalin for 10 days. Then the gonads, liver and heart were collected from each fish for:

i) Gonadosomatic index (GSI) =  $((\text{weight of the gonads (g)} / \text{weight of fish (g)}) * 100$  (Bolger and Connolly, 1989).

ii) Hapatosomatic index (HSI) =  $((\text{weight of liver (g)} / \text{weight of fish$



**Table 2.** The growth parameters of *O. niloticus* fed on four different diets (Means  $\pm$  Standard error).

Parameter	Control	5% diet	10% diet	15% diet
Initial mean weight (g)	44.067 $\pm$ 0.948 <sup>a</sup>	44.178 $\pm$ 0.973 <sup>a</sup>	42.911 $\pm$ 0.911 <sup>a</sup>	41.422 $\pm$ 0.895 <sup>a</sup>
Final mean weight (g)	127.865 $\pm$ 5.158 <sup>b</sup>	98.469 $\pm$ 4.836 <sup>a</sup>	100.595 $\pm$ 6.260 <sup>a</sup>	108.514 $\pm$ 3.020 <sup>a</sup>
Mean weight gain (g)	81.743 $\pm$ 13.340 <sup>a</sup>	54.425 $\pm$ 4.810 <sup>a</sup>	58.557 $\pm$ 11.734 <sup>a</sup>	68.697 $\pm$ 12.180 <sup>a</sup>
Specific Growth Rate (%)	0.451 $\pm$ 0.057 <sup>a</sup>	0.347 $\pm$ 0.027 <sup>a</sup>	0.367 $\pm$ 0.050 <sup>a</sup>	0.419 $\pm$ 0.043 <sup>a</sup>
Condition factor (K)	3.260 $\pm$ 0.065 <sup>a</sup>	3.342 $\pm$ 0.063 <sup>a</sup>	3.382 $\pm$ 0.033 <sup>a</sup>	3.396 $\pm$ 0.033 <sup>a</sup>
GSI	1.366 $\pm$ 0.6618 <sup>a</sup>	1.274 $\pm$ 0.441 <sup>a</sup>	0.569 $\pm$ 0.139 <sup>a</sup>	0.588 $\pm$ 0.390 <sup>a</sup>
HSI	0.702 $\pm$ 0.820 <sup>a</sup>	0.841 $\pm$ 0.950 <sup>a</sup>	0.641 $\pm$ 0.062 <sup>a</sup>	0.756 $\pm$ 0.070 <sup>a</sup>
CSI	0.131 $\pm$ 0.015 <sup>a</sup>	0.114 $\pm$ 0.004 <sup>a</sup>	0.115 $\pm$ 0.010 <sup>a</sup>	0.123 $\pm$ 0.007 <sup>a</sup>
*Survival Rate (%)	82.22	73.33	93.33	77.78

Values within the column having different superscripts are significantly different ( $P < 0.05$ ). \*No statistical analysis was done as determination was on collective fish samples.

(g))\*100 (Htun-han, 1978).

iii) Cardiosomatic indices (CSI) ((weight of heart (g)/ weight of fish (g))\*100.

#### Statistical analysis

The data was analyzed using a Statistical Package for Social Scientists (SPSS) version 15.0. (SPSS Inc., Chicago, IL, USA). Tests among the treatments were done using the ANOVA test and Duncan's Multiple Range tests (Duncan, 1955) was employed were differences occurred at a significance level of 0.05.

## RESULTS

The growth trend of *O. niloticus* revealed a higher final weight in the fish fed with control diet (no *Moringa*) which was significantly ( $p < 0.05$ ) different to the other three diets. The Specific Growth Rate (SGR) and condition factor were not significantly ( $p > 0.05$ ) different among the four diets although the SGR was highest in the fish fed on control diet followed by 15% *Moringa* then 10% *Moringa* and 5% *Moringa* diet was least. The mean weight gain was similarly highest in the control diet followed by 15% then 10% and 5% was least (Table 2 and Figure 2). The GSI, HSI and CSI were all not significantly ( $p > 0.05$ ) different among the four treatments. Furthermore, the final weight of male fish was significantly ( $p < 0.05$ ) higher than that of the female fish among the four treatments but there were no differences between the sexes in each treatment.

The haematological parameters in fish fed on the four different *Moringa* diet levels showed no significant ( $p > 0.05$ ) difference except for MCH were 15% *Moringa* diet was significantly different from both the control and 5% diet (Table 3). The highest blood indices were observed in 10% *Moringa* diet for all the parameters measured except Haematocrit. At 15% *Moringa* diet blood parameter indices reduced in all the parameters taken.

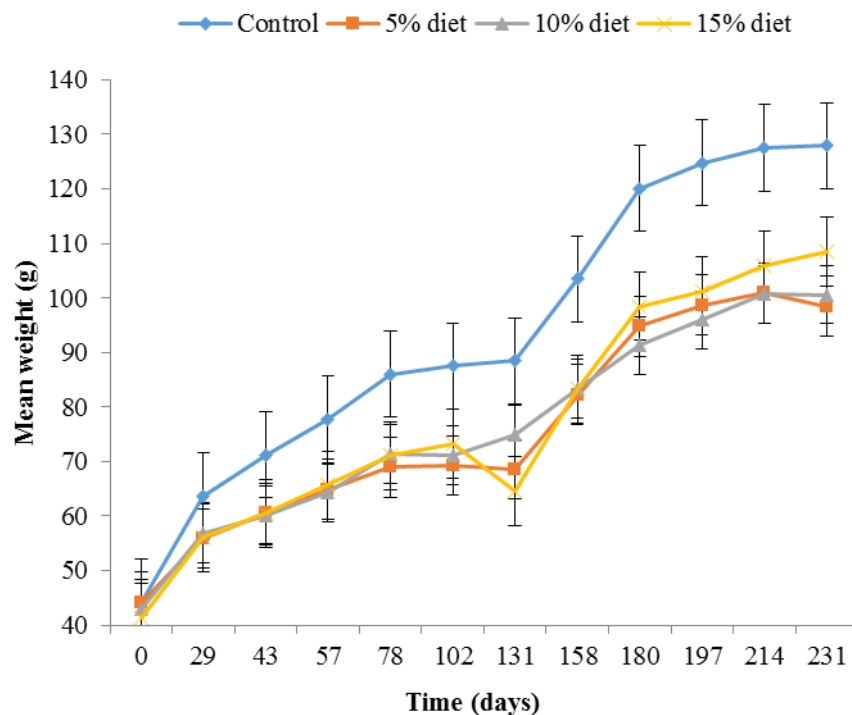
The second order polynomial regression described the best relationship between the blood parameters and the level of *Moringa* inclusion in the diet. The regression coefficient ( $R^2$ ) obtained for RBC and Hb were 0.97 and 0.82 respectively. Those for MCV, MCHC, MCH were 0.83, 0.83 and 0.76 while for WBC and BASO were 0.98 and 0.85 respectively (Figure 3).

## DISCUSSION

The higher growth of fish observed in the control group compared to experimental fish feeding on *Moringa* diet is similar to the findings by Richter et al. (2003). This could be attributed to some anti-nutritional factors such as phenolics, saponin and phytic acids in the *Moringa* leaves as reported by Richter et al. (2003). Considering the longer growing period the fish was subjected to in this study compared to other studies (Bello et al., 2013; Dienye and Olumuji, 2014), it may be concluded that the inclusion of *Moringa* leaf meal in the diet of *O. niloticus* does not result in corresponding somatic growth.

The GSI results obtained among the male fish implies that the reproductive characteristics of the male fish were not affected by the addition of *Moringa* in the diet though the values were lower at inclusion rate of 10 and 15%. The HSI was also similar among the treatments which indicate that *Moringa* leaf meal had no hazardous effect on the fish. The HSI is a useful biomarker to detect hazardous effects of environmental stressors (Sadekarpawar and Parikh, 2013).

The haematological parameters obtained were within or close to the values reported by other authors on *Oreochromis niloticus* and other fish species. The Red Blood Cells (RBC) counts obtained in this study were in the range of 0.92 - 0.98  $\times 10^6/\mu\text{L}$ . This was within the acceptable limits of 0.70 - 28.00  $\times 10^6/\mu\text{L}$  reported for *O. niloticus* kept in captivity (Bittencourt et al., 2003). There was a decrease in the RBC count with increase of *Moringa* above 10% inclusion rate.



**Figure 2.** Growth trend of *Oreochromis niloticus* feeding on four different diets during 231 days experimental period.

**Table 3.** Haematological parameters of *Oreochromis niloticus* fed on different diets containing *Moringa* leaf meal (mean  $\pm$  standard error).

Blood parameters	0% <i>Moringa</i>	5% <i>Moringa</i>	10% <i>Moringa</i>	15% <i>Moringa</i>
Red Blood Cells - RBC ( $10^6/\mu\text{L}$ )	0.959 $\pm$ 0.144 <sup>a</sup>	0.978 $\pm$ 0.121 <sup>a</sup>	0.979 $\pm$ 0.138 <sup>a</sup>	0.917 $\pm$ 0.157 <sup>a</sup>
Haemoglobin - Hb (g/dL)	4.155 $\pm$ 0.636 <sup>a</sup>	4.392 $\pm$ 0.548 <sup>a</sup>	4.750 $\pm$ 0.615 <sup>a</sup>	4.418 $\pm$ 0.754 <sup>a</sup>
Haematocrit - HCT/ PCV (%)	14.445 $\pm$ 2.532 <sup>a</sup>	15.275 $\pm$ 2.182 <sup>a</sup>	15.200 $\pm$ 2.222 <sup>a</sup>	13.418 $\pm$ 2.275 <sup>a</sup>
Mean Cell Volume - MCV (fL)	146.246 $\pm$ 5.121 <sup>a</sup>	151.075 $\pm$ 5.529 <sup>a</sup>	156.190 $\pm$ 6.441 <sup>a</sup>	147.500 $\pm$ 4.044 <sup>a</sup>
Mean Cell Haemoglobin - MCH (pg)	43.500 $\pm$ 0.697 <sup>a</sup>	45.700 $\pm$ 1.607 <sup>a</sup>	52.010 $\pm$ 3.625 <sup>ab</sup>	48.450 $\pm$ 2.763 <sup>b</sup>
Mean Cell Haemoglobin Concentration - MCHC (g/dL)	29.870 $\pm$ 1.028 <sup>a</sup>	30.808 $\pm$ 1.761 <sup>a</sup>	33.710 $\pm$ 2.198 <sup>a</sup>	32.910 $\pm$ 1.453 <sup>a</sup>
White Blood Cells - WBC ( $10^3/\mu\text{L}$ )	360.008 $\pm$ 81.579 <sup>a</sup>	487.943 $\pm$ 63.448 <sup>a</sup>	534.950 $\pm$ 74.698 <sup>a</sup>	416.352 $\pm$ 76.455 <sup>a</sup>
Basophils- BASO ( $10^3/\mu\text{L}$ )	0.0250 $\pm$ 0.006 <sup>a</sup>	0.052 $\pm$ 0.022 <sup>a</sup>	0.080 $\pm$ 0.025 <sup>a</sup>	0.039 $\pm$ 0.009 <sup>a</sup>

Values within the column having different superscripts are significantly different ( $P < 0.05$ ).

This reduction is similar to the reports on inclusion of *Moringa* in the diet of *C. gariepinus* with inclusion levels of above 10% (Ozovehe, 2013; Dienne and Olumuji, 2014). The Haemoglobin (Hb) and Haematocrit (PCV) were in the range 4.16 – 4.75 g/dL and 13.42 – 15.28% respectively. These values are within the reported ranges for *O. niloticus* of 3.99 - 4.93 g/dL and 15 - 45% for Hb and PCV respectively (Bittencourt et al., 2003; Gabriel et al., 2011; Kefi et al., 2013). The Hb and PCV results also compare favourably with results obtained for *Clarias* species (Ayoola et al., 2013). The Hb and PCV results also decrease at the inclusion rate of above 10% *Moringa* similar to RBC counts. The decrease in RBCs, Hb and

PCV with increased *Moringa* inclusion could be as a result of higher anti-metabolites which are toxic in the diet of the fish, especially tannin (Ozovehe, 2013; Dienne and Olumuji, 2014).

The Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) in this study ranged from 146.25 – 156.19 fL, 43.50 – 52.01 pg and 29.87 – 33.71 g/dL respectively. This was within the range of 12.36 – 526.57  $\mu\text{m}^3$ , 5.07 – 120.86 pg and 19.84 – 87.73 g/dL for MCV, MCH and MCHC reported for *O. niloticus* in semi-intensive culture (Bittencourt et al., 2003). The MCV is also within the range of 115-183fL for hybrid tilapia (Hrubec et al., 2000)

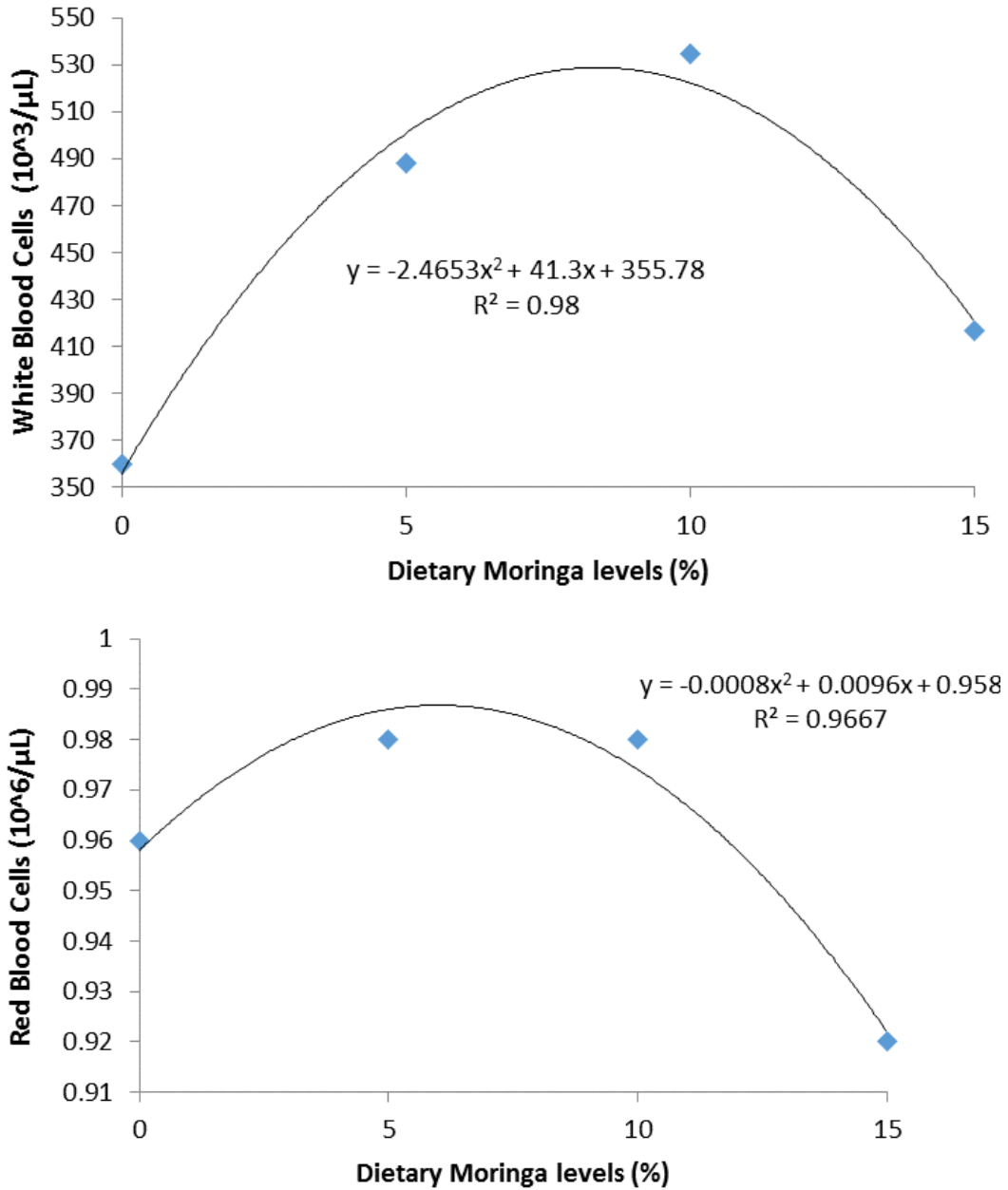


Figure 3. Relationships between white blood cells and Red blood cells to dietary *Moringa* levels.

and 121.67 – 299.33fL for *Oreochromis andersonii* (Kefi et al., 2013). This implies that addition of *Moringa* leaf meal does not negatively affect the MCV, MCH and MCHC.

The White Blood Cell (WBC) results obtained ranged from 360 – 534 x10<sup>3</sup>/μL. The WBC count obtained in this study was higher than reported by most authors on other species (Ozovehe and Nzeh, 2013; Ncha et al., 2015). The diet with *Moringa* inclusion had higher values for WBC compared with the control though not statistically different. This is similar to the positive correlation between increase in *Moringa* inclusion and increase in

WBC obtained on studies of *Clarias* species ( Hlophe and Moyo, 2014; Ncha et al., 2015). The high values of WBC may be an indicator that the fish feeding on *Moringa* diet have high immunity which will be effective in fighting infections (Hrubec et al., 2000).

**Conclusion**

Summarizing, it can be concluded that the addition of *Moringa* to the fish diet could be a viable option. Although it decreases growth it improves the immunity of fish. This

is extremely beneficial because of an increase in White Blood Cells which will eventually improve the capacity of the fish to fight infections and diseases. Further studies are recommended to investigate the level at which the *Moringa* leaf meal does not suppress growth but increase immunity.

### Conflict of Interests


The authors have not declared any conflict of interest.

### ACKNOWLEDGEMENTS

The authors wish to sincerely thank the Government of the Republic of Zambia through the National Aquaculture Research and Development Centre (NARDC) for providing the necessary funds for this study. They also offer gratitude to the Technical staff at NARDC for their tremendous task especially in the collection of data.

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