

**EFFECTS OF POND FERTILIZATION AND SUPPLEMENTARY FEEDING ON  
GROWTH PERFORMANCE AND ECONOMIC RETURN OF NILE TILAPIA  
(*Oreochromis niloticus*)**

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## EXTENDED ABSTRACT

Nile Tilapia (*Oreochromis niloticus*) is the most cultured fish species in tropical and subtropical countries. Desirable characteristics of farmed Nile tilapia include tolerance to a variety of aquaculture environments and consumption of a wide range of natural food organisms. In developing countries including Tanzania, Nile tilapia is cultured under semi-intensive system. Under this system, supplementary feeding is imperative for optimum growth. However, commercial feeds are very expensive and feed cost accounts for 40 to 70% of the culture operational costs. The best way to reduce production costs is fertilization of ponds to stimulate natural food production that can be eaten by fish. This minimizes the amount of supplementary feeds provided without significantly affecting the growth of the fish, and hence, increases yield and profitability.

A study was carried out to evaluate the effects of pond fertilization alone (T<sub>1</sub>), feeding alone (T<sub>2</sub>) and combination of pond fertilization plus supplementary feeding (T<sub>3</sub>) on water physico-chemical parameters, growth performance and profitability of pond cultured Nile tilapia. The study also assessed the quantity and quality of periphyton found in the ponds subjected to the three treatments. The experiment was conducted in nine earthen ponds, each with an average size of 177 m<sup>2</sup> for 180 days. Sex reversed Nile Tilapia (*O. niloticus*) fingerlings with an average size of 0.9 g were collected from Ruvu fish farm and stocked at a density of 3 fish/m<sup>2</sup> seven days after initial fertilization of ponds. Urea and Diammonium phosphate (DAP) were applied into the ponds under fertilization treatments at a rate of 3 g/m<sup>2</sup> and 2 g/m<sup>2</sup> one week before stocking and then weekly during the experimental period. Mash feed containing 25.1% crude protein (CP) was fed twice daily at 1000 and 1600 hours. During the first two months, the fish were fed at a feeding rate of 10% and 5% of fish body weight (FBW) for T<sub>2</sub> and T<sub>3</sub>, respectively. After two months, the

amount of feed was reduced to 5% and 2.5% for T<sub>2</sub> and T<sub>3</sub>, respectively. The fish were fed at these feeding rates up to the end of the experiment.

Pond water physico-chemical parameters i.e. dissolved oxygen (DO), pH, temperature, total dissolved solids (TDS), conductivity and salinity were measured weekly at dawn while Secchi disk readings were measured weekly after dawn hours. Diurnal measurements were done at three hours intervals for 24 hours at the beginning of the experiment and then at three months intervals up to the end of the experimental period. A total of 500 ml of water samples were collected weekly for alkalinity, total nitrogen (TN), nitrate and phosphorus determination. For periphyton collection, eight nets, each with 20 µm-mesh size and an area of 1250 cm<sup>2</sup> were placed full submerged in water in each pond for periphyton to attach. The nets were taken out from pond water after every two months and put in a bucket containing water and then scrubbed to collect periphyton and zooplankton. The periphyton samples were stored in vials for determination of dry matter, organic matter, crude protein, phosphorus and ether extract. Four ml of the periphyton solution were taken and preserved at 4% concentration of formalin for species identification. A random sample of 30 fish from each pond was taken biweekly and each fish was measured individually for body weight and length. After being measured the fish were returned back to their respective ponds. At the end of the experiment feed conversion ratio (FCR), feed conversion efficiency (FCE), fish body weight gain, growth rate (GR), specific growth rate (SGR), condition factor (K) and proximate chemical composition of the fish body were determined.

The data were analysed using one-way ANOVA to assess the effect of treatment on water physico-chemical parameters, fish body weight gain, growth rate, specific growth rate, proximate chemical composition, survival rate and gross margin. R studio software

version 3.5.0 (2018) was used to analyse the data. Duncan's new multiple range test and Tukey's were used to assess the significance of the differences between pairs of the treatment means at  $p = 0.05$ .

Results indicate that ponds subjected to fertilization alone ( $T_1$ ) had significantly higher ( $p \leq 0.05$ ) dawn dissolved oxygen (DO) ( $4.35 \pm 0.04$  mg/l), pH ( $8.24 \pm 0.01$ ) and Secchi disk reading ( $25.3 \pm 0.1$  cm) than the ponds under feeding alone ( $T_2$ ) and combination of fertilization plus supplementary feeding ( $T_3$ ). Ponds under fertilization alone had the lowest values for water conductivity ( $1322 \pm 3.28$  mg/L), salinity ( $0.660 \pm 0.0$  mg/L) and TDS ( $670 \pm 1.70$ ). Furthermore, the results show that phosphorous ( $0.33 \pm 0.01$  mg/L), TN ( $20.82 \pm 0.24$  mg/L) and nitrate ( $11.85 \pm 0.12$  mg/L) concentrations were higher ( $p \leq 0.05$ ) in the ponds under fertilization alone than in the ponds under other treatments. Water alkalinity was lower ( $181.97 \pm 3.25$  mg/L) in the ponds under the combination of fertilization plus feeding treatment than in other treatments. Higher values of water alkalinity were observed under the ponds subjected to feeding alone ( $194.39 \pm 2.43$  mg/L) and fertilization alone ( $191.82 \pm 2.45$  mg/L) treatments, but the difference of water alkalinity between the two treatments was insignificant ( $p > 0.05$ ). Mean water temperature during the experimental period did not differ significantly among the treatments. The values of DO, pH and temperature within 24 hours showed the peak values at 1500 hours while the lowest values were observed at 0600 hours in all treatments.

Results on growth performance indicate that fish cultured under the treatment of combination of pond fertilization plus supplementary feeding ( $T_3$ ) had significantly higher daily weight gain ( $1.5 \pm 0.1$  g/day), feed conversion efficiency (FCE) ( $0.5 \pm 0.0$ ) and gross margin ( $28\ 499\ 967 \pm 3\ 173\ 413$  TZS/ha/year) than the fish reared under the other

treatments. The survival rate of the fish reared in ponds subjected to different treatments did not differ significantly ( $p > 0.05$ ). In addition, fish reared in ponds under feeding alone showed higher FCR ( $4.1 \pm 0.3$ ) than those grown under combination of fertilization plus supplementary feeding ( $2.0 \pm 0.1$ ). The cost of producing one kg for fish reared in ponds under feeding alone (TZS 8 446  $\pm$  380.6) was significantly higher ( $p < 0.05$ ) than cost of producing one kg for fish reared under fertilization alone (TZS 5 284  $\pm$  327.4) or fertilization plus supplementary feeding treatment (TZS 5 824  $\pm$  166.7). However, the costs of producing one kg of fish did not differ significantly ( $p > 0.05$ ) between fertilization alone treatment and combination of fertilization plus supplementary feeding. Fish condition factor (K) differed significantly among the treatments. The fish cultured under fertilization alone had the highest condition factor ( $2.54 \pm 0.0$ ) while those reared under feeding alone showed the least value ( $2.05 \pm 0.0$ ). The highest periphyton biomass ( $47.35 \pm 7.64$  g DM/m<sup>2</sup>) was obtained in ponds under combination of fertilization plus supplementary feeding treatment. Ether extract (EE) was significantly higher in fish body muscles ( $18.33 \pm 0.19\%$ ) and periphyton ( $1.84 \pm 0.07\%$ ) in samples from ponds subjected to fertilization alone than in the samples from ponds under feeding alone and combination of fertilization plus supplementary feeding. Positive correlation was observed between CP and EE of fish body muscle and those of periphyton. Higher values of CP for both fish ( $69.14 \pm 0.33\%$ ) and periphyton ( $11.40 \pm 0.16\%$ ) were observed in ponds under the combination of fertilization plus supplementary feeding than in other treatments.

The analysis of correlation between fish growth rate and periphyton quantity and quality revealed that as periphyton quantity (biomass, OM) and quality (CP) increased, fish growth rate also increased. Periphyton community composition differed ( $p \leq 0.05$ ) among the treatments. Higher species abundance was observed in the ponds subjected to combination of fertilization and supplementary feeding. The phytoplankton classes

observed were Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae and Zygnematophyceae while zooplankton classes were Eurotatoria, Heterotrichea and Oligohymenophorea. Therefore, from this study it is concluded that the combination of weekly fertilization plus supplementary feeding at 2.5% of the fish body weight is the best feeding strategy. This is because it results into higher periphyton quantity (biomass and organic matter), quality (crude protein) and species composition which ultimately lead to higher fish growth, carcass quality (crude protein) and gross margin compared to the other treatments. The chemical composition of fish body muscles is closely related to periphyton chemical composition.

## DECLARATION

I, **SALIMU SHABANI**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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Date

The above declaration is confirmed by;

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## **DEDICATION**

I dedicate this work to my beloved wife Sharifa, my son Sabr and my daughter Sarvia for immense sacrifices and prayer throughout my study period. Also, this work is strongly dedicated to my father Mr. Shabani Salehe Msangi and the late mother Ms. Azama Athumani Maeda who worked so hard to build the foundation and pillars of my education.

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## LIST OF ABBREVIATIONS AND SYMBOLS

#	Number
%	Percentage
µL	Microlitre
µm	Micrometre
µS	MicroSiemens
°C	Degree Celsius
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
C	Carbon
cm	Centimetre
CO <sub>2</sub>	Carbon dioxide
CP	Crude Protein
DAARS	Department of Animal, Aquaculture and Range Sciences
DAP	Diammonium Phosphate
DED	District Executive Director
df	Degree of freedom
DHA	Decosahexaenoic acid
DM	Dry Matter
DO	Dissolved Oxygen
E	East
EE	Ether Extract
EFAs	Essential Fatty Acids
EPA	Eicosapentaenoic acid
F	F-test
FAO	Food and Agriculture Organization of the United Nations
FBW	Fish body weight
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
g	Gram
GM	Gross margin
GR	Growth Rate
ha	Hectare
HCL	Hydrochloric acid

HLPE	High-Level Panel of Experts on Food Security and Nutrition
K	Condition factor
kg	Kilogram
L	Litre
m <sup>2</sup>	Meter square
mg	Milligram
ml	Millilitre
mm	Millimetre
MSc.	Master of Science
N	Nitrogen
nm	Nanometre
NO <sub>3</sub>	Nitrate
OM	Organic Matter
<i>p</i>	Probability
P	Phosphorous
pH	Hydrogen ion concentration
r	Correlation coefficient
rpm	Revolution per minute
S	South
se	Standard error
SGR	Specific Growth Rate
Sq	Sum of square
SR	Survival Rate
SUA	Sokoine University of Agriculture
TDS	Total Dissolved Solids
TN	Total Nitrogen
TOSCI	Tanzania Official Seed Certification Institute
TZS	Tanzanian shillings
URT	United Republic of Tanzania
USA	United States of America

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background Information

Fish culture is a fast growing and quickest food production sector in the world and is among the viable solutions towards global nutritional deficiency and poverty alleviation (Lowe *et al.*, 2012; HLPE, 2014; Kapinga *et al.*, 2014; . Globally it contributes approximately 10% of the protein intake supplied by fisheries sector (FAO, 2012). In Tanzania, aquaculture is based on the culture of Nile tilapia. The Nile tilapia (*Oreochromis niloticus*) is the most cultured species due to a number of characteristics that make it attractive for pond and tank culture Rice *et al.*, 2006). Nile Tilapia can be farmed in fresh, brackish or salt water either in the ponds, tanks or cage systems. Under captivity it performs better and have higher growth rate, resistance to disease and feeds at low trophic level (Grammer *et al.*, 2012; Lowe *et al.*, 2012; . Also, Nile Tilapia tolerates poor water quality parameters such as turbidity and low dissolved oxygen up to 1 mg/L (Lamtane *et al.*, 2008; Grammer *et al.*, 2012).

Tilapia culture system practiced in tropical and subtropical countries is mostly extensive or semi-intensive. The latter is particularly common in developing countries because it provides a wide range of options for management and higher results into fish yield and profit compared to the extensive systems (Shailender *et al.*, 2013). Management strategies under semi-intensive production system involve the use of fertilizers to encourage natural productivity of phytoplankton and to improve the levels of dissolved oxygen in the fish pond. Fish yields from such systems have been found to be higher than those from natural unfertilized systems (Altun *et al.*, 2006).

Fish culture in Tanzania is mainly done in fresh water under pond culture system. In recent years there has been an increase in fish production from aquaculture (FAO, 2016). Fish production is challenged by high costs of feed, inadequate supply of fingerlings, poor access to fish farming inputs, poor water quality and extension services (URT, 2015; URT, 2016).

### **1.1.1 Water physico-chemical parameters and fish growth**

Water physico-chemical parameters such as dissolved oxygen (DO) and pH depends greatly on the concentration of phytoplankton (Bhatnagar and Devi, 2013). The plankton, especially phytoplankton supports photosynthesis which increases DO level in the water column. Dissolved oxygen is an important variable for the survival, growth and distribution of fish. Low growth rate, starvation and mortality of the fish may occur due to oxygen depletion (Bhatnagar and Devi, 2013; Makori *et al.*, 2017). Dissolved oxygen level of above 3 mg/L is essential for good fish growth (Bhatnagar and Devi, 2013). Low levels of oxygen can be caused by over-fertilization or turbidity of the water which restricts light penetration, hence, limit photosynthesis. Photosynthesis is crucial for primary productivity which increases DO in the pond (Bhatnagar and Devi, 2013). Furthermore, the level of DO may vary depending on the rate of microbial decomposition of organic matter which utilizes oxygen (Makori *et al.*, 2017). This suggests that feeding may lower DO due to decomposition of uneaten feeds. On the other hand, fertilization may increase the availability of natural food, thereby increasing the DO in the fish pond.

Optimum pH for tilapia growth ranges between 6.5 and 8.5 (Bhatnagar and Devi, 2013; Makori *et al.*, 2017). Water pH in most cases is influenced by carbon dioxide (CO<sub>2</sub>) concentration in the water, since they are negatively correlated. Feeding of fish in the pond is very likely to decrease water pH due to decomposition of uneaten feeds which releases

CO<sub>2</sub>. In addition, fish and other organisms release CO<sub>2</sub> as the end product of metabolism. During the day, algae and plants remove CO<sub>2</sub> from water through photosynthesis, this makes the pH to rise. At night when photosynthesis stops, the CO<sub>2</sub> levels increase again and pH is decreased. Since DO and pH are positively correlated, the increase in DO concentration and pH tends to increase biomass of fish (Tucker and D'Abramo, 2008).

High or low temperatures affect the metabolism and physiological activities of the fish because fish are cold blooded animals, this means that their body temperature varies with the environment (Bhatnagar and Devi, 2013). Temperature higher than 30°C increases biochemical activities of aquatic organisms and leads to high demand for oxygen. Concurrently high temperature may decrease DO solubility and increase the level of ammonia. Temperature lower than 20.27°C may reduce biochemical activities, hence, affect growth and health of the fish (Bhatnagar and Devi, 2013; Shoko *et al.*, 2016).

Conductivity is an index of total ionic content of the water and has been shown to affect fish production when stray from the range of 30 to 5000 µS/cm for pond fish culture (Bhatnagar and Devi, 2013). The suitable level of conductivity for higher fish production varies depending on fish species (Makori *et al.*, 2017). The use of feed and/or fertilizer increases conductivity due to the increase of organic matter load in the water from either uneaten feeds and/or decayed plankton which may decompose and add ionic content in the water.

Alkalinity measures the concentration of the bases, mainly carbonate and bicarbonate in the water. Levels less than 20 mg/L limits primary productivity which is important for natural food generation and water quality improvement (Stone *et al.*, 2013). Alkalinity increases with bacterial decomposition of organic materials that generate carbon dioxides



(CO<sub>2</sub>) and dissolves magnesium and calcium carbonate to magnesium and calcium bicarbonate (Priyadarshini *et al.*, 2011). Fertilization of the pond has been reported to increase alkalinity level of the water through increasing phytoplankton bloom which finally increases the organic matter in the pond (Bhatnagar and Devi, 2013). Normally the amount of organic matter in the water, from either leftover feeds or plant residues, determines the alkalinity levels due to microbial activities during decomposition (Thakur *et al.*, 2004). Salinity level affects the metabolic rate of tilapia. The tolerance level depends on species, strain, adaptation time and environmental factors (El-Sayed, 2006), but the level ranges between 0 and 7000 mg/L (Azevedo *et al.*, 2015). The low to optimum salinity levels lead to an isotonic environment which promotes the best growth rate due to the energy economy and none interference of enzymatic activities in the intestine (Azevedo *et al.*, 2015).

### **1.1.2 Pond fertilization and supplementary feeding**

Artificial feed is among the major constraints in aquaculture for both small and commercial farmers as it accounts 40 -70% of the production costs. Therefore, reducing the amount of feeds during the production cycle may decrease the production costs, hence, increase profit. Pond fertilization increases the availability of natural food for fish. Natural food produced through pond fertilization can support fish growth to some extent. The fertilizers applied in ponds stimulate the growth of microscopic plants called phytoplankton (algae) which are a source of food to zooplankton and insects, both of which serve as food for fish. In this way, fertilization of a pond results in higher fish growth rate than that can be obtained in unfertilized pond because of the increased number of algae and zooplankton. Furthermore, pond fertilization reduces the dependence on artificial feed, thereby reducing the cost of feeds. In attempt to reduce the feed costs, fish farmers apply fertilizer to stimulate natural food production (plankton) in the pond during

the grow-out period (Hasan, 2001). Under semi-intensive production system, most farmers fertilize the earthen ponds by using locally available feed stuffs and/or use plant organic manures as fertilisers which are of different types depending on the availabilities (Kaliba *et al.*, 2006). The differences in quantity and quality of locally available feeds and fertilizer may affect both water quality, condition factor and growth performance of the fish.

## **1.2 Problem Statement and Justification**

For proper growth, fish requires suitable water quality, sufficient quantity and good quality food (Abdel-Tawwab *et al.*, 2007; Slawski *et al.*, 2011; Makori *et al.*, 2017). Food can be obtained naturally from the pond (plankton) or provided as supplementary diet. The latter feed is very costly and accounts for approximately 40 -70% of the total production costs (Jabir *et al.*, 2012; Abdel-Warith, 2013). The high feed costs are mainly due to the high price of fishmeal and soybean meal as a result of increased competition for the same resources among livestock, human and fish production. The high price of commercial feed makes it unaffordable to most famers to feed their fish with the required amount and quality feed (Kaliba *et al.*, 2006; Slawski *et al.*, 2011). The best way to reduce the costs of production, at the same time attain a fast growth rate and increase economic return is to use natural and artificial feeds. Studies shows that, the use of both natural and artificial feeds can minimize the amount of supplementary feed without compromising the growth performance of fish (Prabaharan and Murugan, 2012; Abdel-Warith, 2013).

The study done in Cambodia and Kenya revealed that, pond fertilization plus supplementary feeding strategy is an effective practice in semi-intensive system (Manyala *et al.*, 2015). Fish under this strategy perform better in terms of growth, feed conversion efficiency and economic return compared to those from ponds with fertilization or feeding

alone. A similar study conducted in Thailand on Nile tilapia showed that, fish from ponds applied with fertilizer plus supplementary feeding at 50% satiation resulted in higher growth and yield than the fish cultured in either fertilization alone or feeding alone (Diana *et al.*, 1994).

In Tanzania most smallholder farmers culture tilapia using semi-intensive system. However, the information on the effects of the combination of pond fertilization plus supplementary feeding on fish growth, economic return and pond productivity is lacking. In the ponds subjected to the combination of fertilization plus supplementation, in addition to artificial feed, fish will consume natural food (phytoplankton and zooplankton). This may reduce the amount of feed, hence, lower production costs. Therefore, this study was intended to investigate the effects of pond fertilization, feeding full amount (5% FBW) of the required feeds and the combination of pond fertilization plus feeding half (2.5% FBW) of the required feed on growth performance and economic return of *O. niloticus* cultured in ponds. The study intended to identify the best feeding strategy which can be promoted to smallholder farmers.

### **1.3 Objectives**

#### **1.3.1 General objective**

To investigate the effects of pond fertilization and supplementary feeding on growth performance and profitability of Nile tilapia (*O. niloticus*).

#### **1.3.2 Specific objectives**

- i. To determine the water physico-chemical parameters and periphyton species composition and abundance in ponds under inorganic fertilizer application

alone, concentrate feeding alone and the combination of fertilization plus concentrate feeding.

- ii. To determine the effects of fertilizer application alone, concentrate feeding alone and the combination of fertilization plus concentrate feeding on *O. niloticus* growth performance, body chemical composition and yield.
- iii. To determine the gross margin of fish reared in ponds under inorganic fertilizer application alone, concentrate feeding alone and the combination of fertilization plus concentrate feeding.

#### **1.4 Hypothesis**

**1.4.1 H<sub>0</sub>:** There is no significant difference in growth performance and gross margin of Nile tilapia cultured under fertilization alone, feeding alone and combination of fertilization and supplementary feeding.

**1.4.2 H<sub>0</sub>:** There is no significance difference in water physico-chemical parameters from the ponds subjected to fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding.

#### **1.5 Description of the Study Location**

This study was conducted for 180 days in nine earthen ponds located at Tindiga village, Kilosa district in Morogoro region (Fig. 1). Tindiga village is located approximately 13 km from Kilosa town. Kilosa district lies between latitude 5° 55'S and 8° 53'S and longitude 36 °30'E and 37°30'E. The area experiences eight months of rainfall from October to May, with the highest amount of rainfall received between March and April. Mean annual rainfall ranges between 800 and 1400 mm and the average temperature is 25 °C per year (Kajembe *et al.*, 2013).

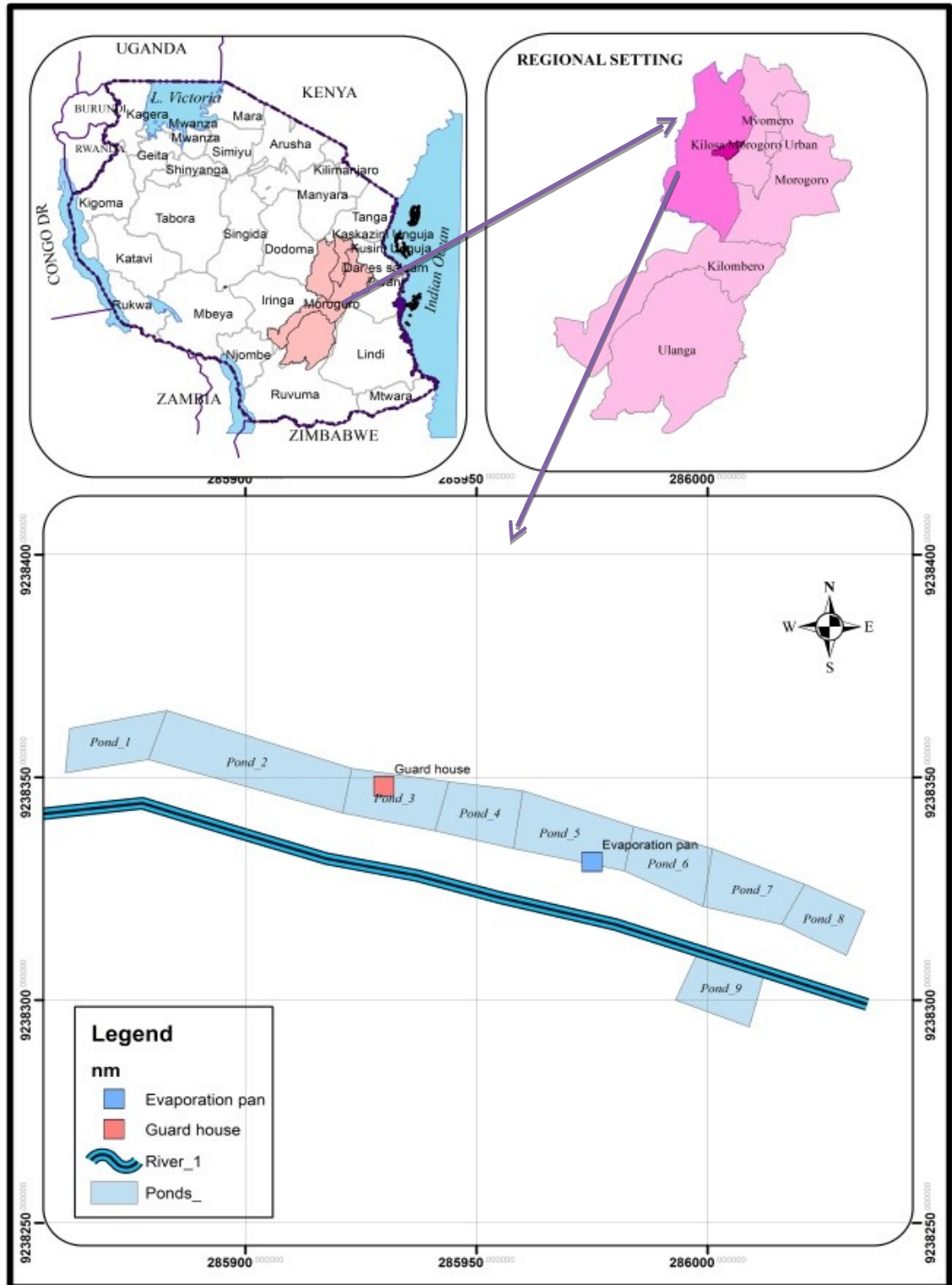


Figure 1: Map of the study area showing pond layout at Kilosa district

## **1.6 Dissertation Organization**

This dissertation adopted a publishable manuscript format and is comprised of four chapters. Chapter one gives general background of the study. Chapter two and three consists of the manuscripts with titles “Effects of inorganic fertilizer application and supplementary feeding on water physico-chemical parameters, growth performance and yield of Nile Tilapia (*Oreochromis niloticus*) cultured in earthen ponds” and “Influence of inorganic fertilizer application and supplementary feeds on periphyton biomass, quantity and species composition”. Chapter four gives a general conclusion and recommendations.

**REFERENCES**

**CHAPTER TWO**

**2.0 MANUSCRIPT I**

**Effects of inorganic fertilizer application and supplementary feeding on water physico-chemical parameters, growth performance and yield of Nile Tilapia (*Oreochromis niloticus*) cultured in earthen ponds**

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**Abstract**

This study was carried out to assess the growth performance, water physico-chemical parameters and profitability of culturing Nile tilapia (*O. niloticus*) in ponds under fertilization alone (T<sub>1</sub>), feeding alone (T<sub>2</sub>) and a combination of fertilization plus supplementary feeding (T<sub>3</sub>). Sex-reversed Nile tilapia fingerlings with an average weight of 0.9 gram were stocked in earthen ponds at a density of 3 fish/m<sup>2</sup>. Urea and Diammonium phosphate (DAP) fertilizers were applied in pond water at weekly intervals at a rate of 3 g/m<sup>2</sup> and 2 g/m<sup>2</sup>, respectively. Mash feed containing 25.1% crude protein (CP) was fed at 10% for T<sub>2</sub> and 5% of fish body weight (FBW) for T<sub>3</sub>, twice daily for the first two months, then the amount of feed was reduced to 5% and 2.5% of body weight for T<sub>2</sub> and T<sub>3</sub>, respectively. Pond water dissolved oxygen (DO), pH, temperature, total dissolved solids (TDS), conductivity, salinity and Secchi disk reading were measured weekly. Diurnal (24 hours) measurements of DO, pH and temperature were done at three hours intervals at the beginning of the experiment and then after every three months. Also, alkalinity, nitrate, total nitrogen (TN) and phosphorous were analyzed. A random sample of 30 fish was taken from each pond biweekly for individual fish weight and length measurements. Feeding rates were readjusted according to fish weight. The experiment took 180 days.

Dissolved oxygen (DO), pH and Secchi disk reading significantly ( $p < 0.05$ ) varied among the treatments with the highest values ( $4.35 \pm 0.04$  mg/L), ( $8.24 \pm 0.01$ ) and ( $25.3 \pm 0.1$  cm) respectively, being observed in T<sub>1</sub>. Ponds under T<sub>1</sub> had the lowest values for water conductivity ( $1322 \pm 3.28$  mg/L), salinity ( $0.660 \pm 0.0$  mg/L) and TDS ( $670 \pm 1.70$ ), but had the highest values for phosphorous ( $0.33 \pm 0.01$  mg/L), TN ( $20.82 \pm 0.24$  mg/L) and nitrate ( $11.85 \pm 0.12$  mg/L). Alkalinity was lower ( $181.97 \pm 3.25$  mg/L) in the ponds under



T<sub>3</sub>. Within a period of 24 hours, DO, pH and temperature attained the peak at 1500 hours and the lowest values were observed at 0600 hours. The fish reared under T<sub>3</sub> showed higher ( $p < 0.05$ ) body weight gain ( $194.1 \pm 4.5$  g), growth rate (GR) ( $1.5 \pm 0.1$  g/day) and gross margin ( $28\,499\,967 \pm 3\,173\,413$  TZS/ha/year) than those under the other treatments. The FCR was higher ( $4.1 \pm 0.3$ ) for the fish subjected to T<sub>2</sub> and lower ( $2.0 \pm 0.1$ ) for the fish reared under T<sub>3</sub>. The highest condition factor (K) ( $2.54 \pm 0.0$ ) was observed for the fish reared in ponds under T<sub>1</sub> whereas fish under T<sub>2</sub> had the lowest value ( $2.05 \pm 0.0$ ). It is concluded that, the combination of fertilization plus supplementary feeding (T<sub>3</sub>) is the best strategy for rearing *Oreochromis niloticus*, since it reduces feed utilization, does not affect water quality parameter beyond the acceptable range and results into higher growth performance and profit.

**Keywords:** *condition factor, fish growth rate, gross margin, water physico-chemical parameters*

## 2.1 Introduction

Growth performance of fish depends on the availability of essential nutrients from either natural food (plankton) and/or supplemented feed. Fertilizers applied in pond water releases nutrients which promote the growth of phytoplankton as primary producers. The growth of phytoplankton in ponds water increases the level of dissolved oxygen, pH and total phosphorus (Qin *et al.*, 1995; Priyadarshini *et al.*, 2011). In addition, phytoplankton traps ammonia excreted by the fish, hence, improves water quality parameters (Priyadarshini *et al.*, 2011).

Phytoplankton is food for zoo-plankton and other herbivore animals which are, in turn, eaten by fish. The presence of natural food in the pond can support fish growth without the need for supplementary feeds. However, as the fish grows and weight increases, the amount of natural food becomes inadequate to sustain the increasing fish weight. This leads to slow growth rate which, in turn, prolongs the production cycle and causes low yield. On the other hand, supplementation of pond cultured fish with an artificial feed can promote fast growth of the fish, hence, higher yield at harvest. Artificial feed is the most expensive input in intensive or semi-intensive aquaculture systems due to the high competition of the same resources among human, livestock and fish production (Opiyo *et al.*, 2014). The best way to reduce feed costs is to combine fertilization plus supplementary feeding. This strategy uses minimal feed, thereby reduces production costs (Prabaharan and Murugan, 2012).

In Nepal, Cambodia, Kenya and Thailand the study done to assess the effects of pond fertilization plus supplementary feeding on growth performance of Nile tilapia revealed that fish cultured under the combination of fertilization plus supplementary feeding performs better in terms of growth performance and yield compared to those grown under

fertilization alone (Diana *et al.*, 1994; Manyala *et al.*, 2015). In Tanzania the combined effects of pond fertilization plus supplementary feeding on water physico-chemical parameters, growth performance, yield and economic return of fish are unknown. This study, therefore, assessed the effects of inorganic fertilizer application alone, feeding alone and the combination of fertilizer application plus feeding on pond water physico-chemical parameters, fish growth performance, yield and profitability of Nile Tilapia cultured in earthen ponds.

## **2.2 Materials and Methods**

### **2.2.1 Description of the study location**

The study was conducted for 180 days at Tindiga village, Kilosa district, Tanzania. Kilosa district lies between latitude 5° 55'S and 8° 53'S and longitude 36 °30'E and 37°30'E. Tindiga village is located 13 km from Kilosa town. The area receives rainfall for an average of eight months. The rainfall falls from October to May, with the highest amount received between March and April. Mean annual rainfall ranges between 800 and 1400 mm and the average temperature is 25 °C per year (Kajembe *et al.*, 2013).

### **2.2.2 Pond and fish management**

Nine earthen ponds with an average size of 177 m<sup>2</sup> were used (Appendix 1, plate 1). The ponds were randomly allocated to three treatments, with three replicates per treatment. The treatments were pond fertilization alone (T<sub>1</sub>), feeding alone at 5% of fish body weight (FBW) (T<sub>2</sub>) and pond fertilization plus feeding at 2.5% FBW (T<sub>3</sub>). Urea and Diammonium phosphate (DAP) inorganic fertilizers were applied weekly at 3 g and 2 g per m<sup>2</sup>, respectively, in the ponds under both T<sub>1</sub> and T<sub>3</sub>. Prior to stocking all ponds were drained, dried, refilled with water, fertilized and left for seven days. The surface water that drains through a canal adjacent to the ponds was used to fill the experimental ponds.

Sex-reversed Nile tilapia fingerlings with an average weight of 0.9 g were collected from Ruvu fish farm and stocked at a stocking density of 3 fish /m<sup>2</sup>. Mash feed was compounded as shown in Table 1. The feed ingredients were ground in a hammer mill machine with an average of 2 mm sieve size. Fish were fed twice per day at 1000 and 1600 hours at the levels of 10% and 5% of FBW for the first two months, then the amount of feed was reduced to 5% and 2.5% for the last four months for T<sub>2</sub> and T<sub>3</sub>, respectively. During fish feeding, the mash feed was broad casted in the pond water surface at specific area for the fish to adapt (Appendix 1, plate 2).

**Table 1: Percentage of ingredients in the formulated feed**

<b>Feed Ingredient</b>	<b>Composition (%)</b>
Wheat bran	50
Fish meal	25
Sunflower seed cake	10
Cotton seed cake	10
Maize meal	4
Mineral premix	1

### 2.2.3 Chemical composition analysis of feeds

Three random samples approximately 250 g each of compounded feed were taken. Feed samples were weighed before being dried at 60 °C and stored in the air tight bottle for analysis. Dry matter and ash contents were determined using standard procedure. Crude protein was determined by Kjeldahl method (AOAC, 2006). Crude fat was determined by Soxhlet extraction method (AOAC, 2006). The proximate analysis of a compounded feed showed the crude protein content (CP) was 25.1% as shown in Table 2.

**Table 2: Proximate composition (%) of compounded mash feed**

<b>Proximate component</b>	<b>Composition (%)</b>
----------------------------	------------------------

Dry matter	91.94
Moisture content	8.06
Organic matter	84.65
Ash	15.35
Crude protein	25.09
Crude fat	9.6

#### **2.2.4 Determination of water physico-chemical parameters**

Pond water physico-chemical parameters i.e. temperature (°C), conductivity ( $\mu\text{S}/\text{cm}$ ), salinity (mg/L), total dissolved solids (TDS), pH and dissolved oxygen (mg/L) were measured. The measurements were taken at the top, middle and bottom of the pond water column weekly at 0600 hours by using multiparameter DO meter (HI 98198 PH/EC/DO Multiparameter HANNA instruments made in Romania). In order to determine 24 hours fluctuations, the same parameters were measured at three hours intervals for 24 hours at the beginning of the experiment, and then after three months and at the end of the experimental period. In addition, 500 ml of water samples were collected weekly from each pond at a depth of 15 cm between 0900 and 1100 hours for alkalinity, total phosphorous and nitrogen determination. The determinations of alkalinity, phosphorous and nitrogen were done by using Titrametric, spectrophotometric and Kjeldahl methods, respectively, as described by Asuero (2013).

#### **2.2.5 Fish sampling and growth performance determination**

To determine growth performance, the fish cultured in the ponds subjected to the three treatments were measured for body weight and length at the beginning of the experiment and then every two weeks throughout the experimental period. A random sample of 30 fish was taken from each pond using a net (1 mm mesh size) and each fish was individually measured. Fish body weight (g) was measured using a portable digital weighing balance while body length (cm) was measured using a measuring board fixed on

a ruler. After measuring body weight and length, the fish were released back into their respective ponds. Death of fish was observed and recorded daily. At the end of the experiment, all fish were harvested by using a seine net with a size of 1.5 m x 15 m and mesh size of 15 mm. The fish harvested were counted to determine the survival rate and yield (Appendix 1- plate 3 and 4). Fish growth rate (GR), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficient (FCE), survival rate (SR) and condition factor (K) were calculated by using the following formulae:

$$\text{GR} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Experimental period (days)}} \dots\dots\dots(1)$$

Where: GR is growth rate (Khalafalla and El-Hais, 2015)

$$\text{SGR} = \frac{[\ln(\text{Final weight (g)}) - \ln(\text{initial weight (g)})]}{\text{Experimental period (days)}} \times 100 \dots\dots\dots(2)$$

Where: ln is the natural logarithm and SGR is specific growth rate (Opiyo *et al.*, 2014; Khalafalla and El-Hais, 2015).

$$\text{FCR} = \frac{\text{Total weight of food consumed (g)}}{\text{Total weight gained by fish (g)}} \dots\dots\dots(3)$$

Where: FCR is feed conversion ratio (Zahid *et al.*, 2013)

$$\text{FCE} = \frac{\text{Weight gained by fish (g)}}{\text{Food intake (g)}} \dots\dots\dots(4)$$

Where: FCE is feed conversion efficiency (Kirimi *et al.*, 2016)

$$\text{SR} = \frac{\text{Total number of fish stocked} - \text{total number of died}}{\text{Total number of fish stocked}} \times 100 \dots\dots\dots(5)$$

Where: SR is survival rate (Khalafalla and El-Hais, 2015)

### **Condition factor (K)**

Condition factor as an indicator of the wellbeing of the fish and was calculated using the formula below:

$$K = \frac{100 \times W}{L^b} \dots\dots\dots(6)$$

Where: K= condition factor; W =weight of the fish at harvest (g); L = length of the fish at harvest (cm); b = The slope of the regression line (also referred to as Allometric coefficient) (.

Note: the slope of the regression line (b) was obtained by using the formula below;

$$W= a L^b \dots\dots\dots(7)$$

Where: W = weight of the fish (g); a = Exponent describing the rate of change of weight with length (The intercept of the regression line on the Y axis); L= Length of the fish (cm); b = The slope of the regression line (also referred as Allometric coefficient).

The log-transformed data gave the regression equation indicated below:

$$\text{Log } W = \text{log}a + b\text{log}L \dots\dots\dots(8)$$

a = Constant; b =The regression coefficient (Ighwela *et al.*, 2011).

**2.2.6 Economic analysis**

Gross margin analysis was used to determine the profitability of the fish cultured under the three treatments. The variable costs recorded per treatment were costs of feed, fertilizer, fingerling and labour. The value of the harvested fish was determined by using the market price of fish/kg. Total harvest, revenue and gross margin were determined as follows:

$$\text{Total weight harvested (kg)} = \frac{\text{Mean final weight (g)} \times \text{total number of fish harvested}}{1000} \dots\dots\dots(9)$$

(Priyadarshini *et al.*, 2011; Kapinga *et al.*, 2014)

$$\text{Revenue} = \text{Total weight harvested (kg)} \times \text{price per kilogram (Tsh)} \dots\dots\dots(10)$$

(Fish price per kg in the study area was TZS 8000 in 2019)

$$\text{GM} = \text{Total revenue from sold fish} - \text{Total variable costs of production} \dots\dots\dots(11)$$

Where: GM = Gross margin (Kapute *et al.*, 2016).

$$\text{Yield (kg/m}^2\text{)} = \frac{\text{Total harvest (kg)}}{\text{Area of the pond (m}^2\text{)}} \dots\dots\dots(12)$$

$$\text{Yield (kg/ha/yr)} = \frac{\text{Yield} \times \text{\# of days in a year (365)} \times 10000 \text{ (m}^2\text{)}}{\text{Experimental period (days)}} \dots\dots\dots(13)$$

Where; yr = year; # =number; (Priyadarshini *et al.*, 2011; Kapinga *et al.*, 2014).

### 2.2.7 Statistical analysis

Data were analysed using R Studio software version 3.5.0 (2018) (Horton and Kleinman, 2015; Tortolero *et al.*, 2016). Before the analysis, the data were checked for normality and transformed whenever necessary to reduce skewedness and increasing error homoscedasticity. One-way ANOVA was used to assess the effects of treatments on growth performance variables, water physico-chemical parameters, production costs, yields and revenue. Initial weight and length were used as a covariate in the analysis for growth performance data. A Tukey's test ( $p = 0.05$ ) was used for Post Hoc analysis to determine the significance difference between pairs of treatment means when the treatment had significant effect. The relationships between fish growth and water physico-



chemical parameters were determined by using Pearson correlation and multiple regression analyses.

The model for testing the effect of treatment on growth performance variables was:

$$Y_{ij} = \mu + T_i + b(X_{ij} - \bar{X}) + e_{ij}$$

Where;

$Y_{ij}$  = is the response (fish weight, length) of  $j^{\text{th}}$  fish received  $i^{\text{th}}$  treatment

$\mu$  = Overall mean

$T_i$  = is the effect of  $i^{\text{th}}$  treatment (fertilization alone, feeding alone, combination of fertilization plus supplementary feeding)

$b$  = is the regression coefficient of Y on X

$X_{ij}$  = is the initial measurement (body weight, length)

$\bar{X}$  = is the mean for initial measurements (mean of body weight, length)

$e_{ij}$  = is the random error term

The model for multiple regression analyses to determine the influence of water physico-chemical parameters on fish growth rate was:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \varepsilon$$

Where;

Y = Response (fish growth rate)

$\beta_0$  = The intercept of the regression line on Y axis

$\beta_1, \beta_2, \dots, \beta_k =$   $k^{\text{th}}$  partial regression coefficient (slope) for different water physico-chemical parameters.

$X_1, X_2, \dots, X_k =$   $k^{\text{th}}$  predictor variable [water physico-chemical parameters (i.e. DO, pH, temperature, conductivity, salinity, TDS, Secchi disk reading)]

$\varepsilon =$  error term

## 2.3 Results

### 2.3.1 Water physico-chemical parameters

Results for pond water physico-chemical parameters are summarised in Table 3. Dissolved oxygen (DO) values at dawn differed significantly ( $p < 0.05$ ) among the treatments. The highest mean DO value ( $4.35 \pm 0.04$  mg/L) was observed in ponds subjected to fertilization alone while the lowest value ( $3.79 \pm 0.03$  mg/L) was observed in ponds under feeding alone. The DO values over the whole experimental period (24 weeks) decreased with time (Fig. 1 (a)) with the highest DO values being observed during the first two weeks and the lowest between 14<sup>th</sup> and 19<sup>th</sup> weeks. pH values differed significantly ( $p < 0.05$ ) among the treatments. The highest pH value was observed in ponds under fertilization alone ( $8.24 \pm 0.01$ ) while the lowest value was found in the ponds under the combination of fertilization plus supplementary feeding ( $8.07 \pm 0.01$ ). During the experimental period of 24 weeks, the pH exhibited an irregular trend. The ponds under fertilization alone had higher pH values while those under the combination of fertilization plus supplementary feeding had lower values (Fig. 1 (b)). Temperature did not differ among the treatments ( $p > 0.05$ ) and the mean temperature ranged from  $25.17 \pm 0.04$  °C to  $25.24 \pm 0.04$  °C. However, throughout the experimental period temperature increased with time (Fig. 1 (c)).

Diurnal variations of DO, pH and temperature are shown in Figure 2 (a), 2 (b) and 2 (c), respectively. The results show that, within 24 hours period dissolved oxygen (DO), pH and temperature had the lowest values at 0600 hours and highest at 1500 hours. The highest values were observed in ponds under fertilization alone and the lowest values in ponds under feeding alone. However, temperature values showed a narrow variation among the treatments within 24 hours period (Fig. 2 (c)).

The results also show that, the mean conductivity, total dissolved solids (TDS) and salinity were significantly lower in ponds under fertilization alone ( $T_1$ ) than those under feeding alone ( $T_2$ ) and the combination of fertilization plus feeding ( $T_3$ ). However, the differences between the ponds subjected to feeding alone and the combination of fertilization plus supplementary feeding were not significant ( $p > 0.05$ ). The mean conductivity, TDS and salinity ranged from  $1322 \pm 3.28$  to  $1360 \pm 3.06$   $\mu\text{S}/\text{cm}$ ,  $670 \pm 1.70$  to  $688 \pm 2.04$   $\text{mg}/\text{L}$  and  $0.660 \pm 0.0$  to  $0.679 \pm 0.0$   $\text{mg}/\text{L}$ , respectively. Furthermore, the ponds under fertilization alone had significantly ( $p < 0.05$ ) higher Secchi disk reading ( $25.3 \pm 0.1$  cm) than those on feeding alone ( $24.4 \pm 0.1$  cm) and the combination of fertilization plus supplementary feeding which had the lowest value ( $23.1 \pm 0.2$  cm).

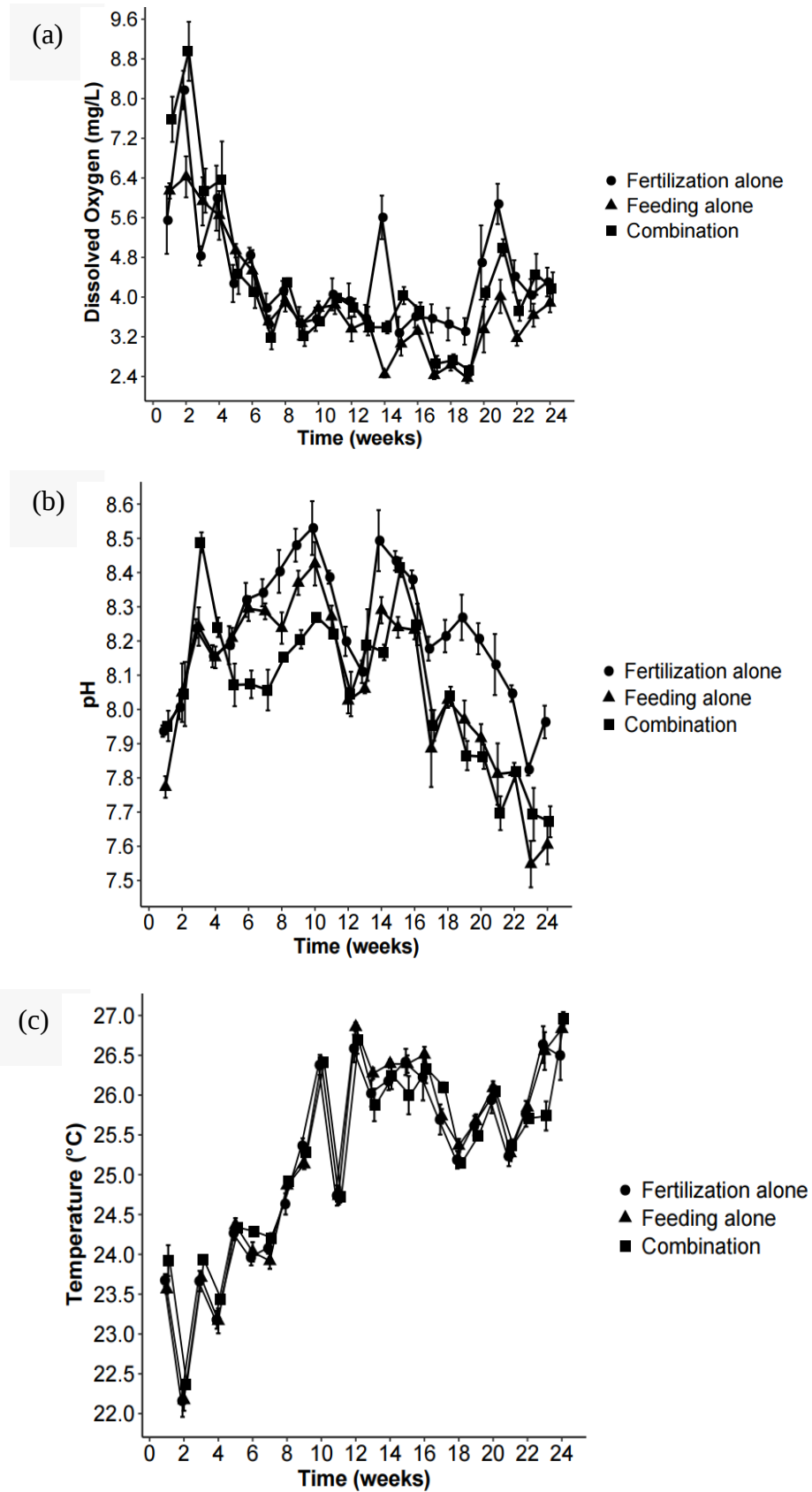
Water alkalinity was significantly ( $p < 0.05$ ) lower in ponds under the combination of fertilization plus feeding than in those subjected to either fertilization alone or feeding alone. The mean alkalinity ranged from  $181.97 \pm 3.25$   $\text{mg}/\text{L}$  in ponds under combination of fertilization plus feeding to  $194.39 \pm 2.43$   $\text{mg}/\text{L}$  in ponds under feeding alone. Total nitrogen ( $20.82 \pm 0.24$   $\text{mg}/\text{L}$ ), nitrate ( $11.85 \pm 0.12$   $\text{mg}/\text{L}$ ) and phosphorous ( $0.33 \pm 0.01$   $\text{mg}/\text{L}$ ) were significantly ( $p < 0.05$ ) higher in ponds under fertilization alone than in those under feeding alone and the combination of fertilization plus feeding.

**Table 3: Dawn water physico-chemical parameters (Mean  $\pm$  se) for ponds under fertilization alone, feeding alone and the combination of fertilization plus feeding**

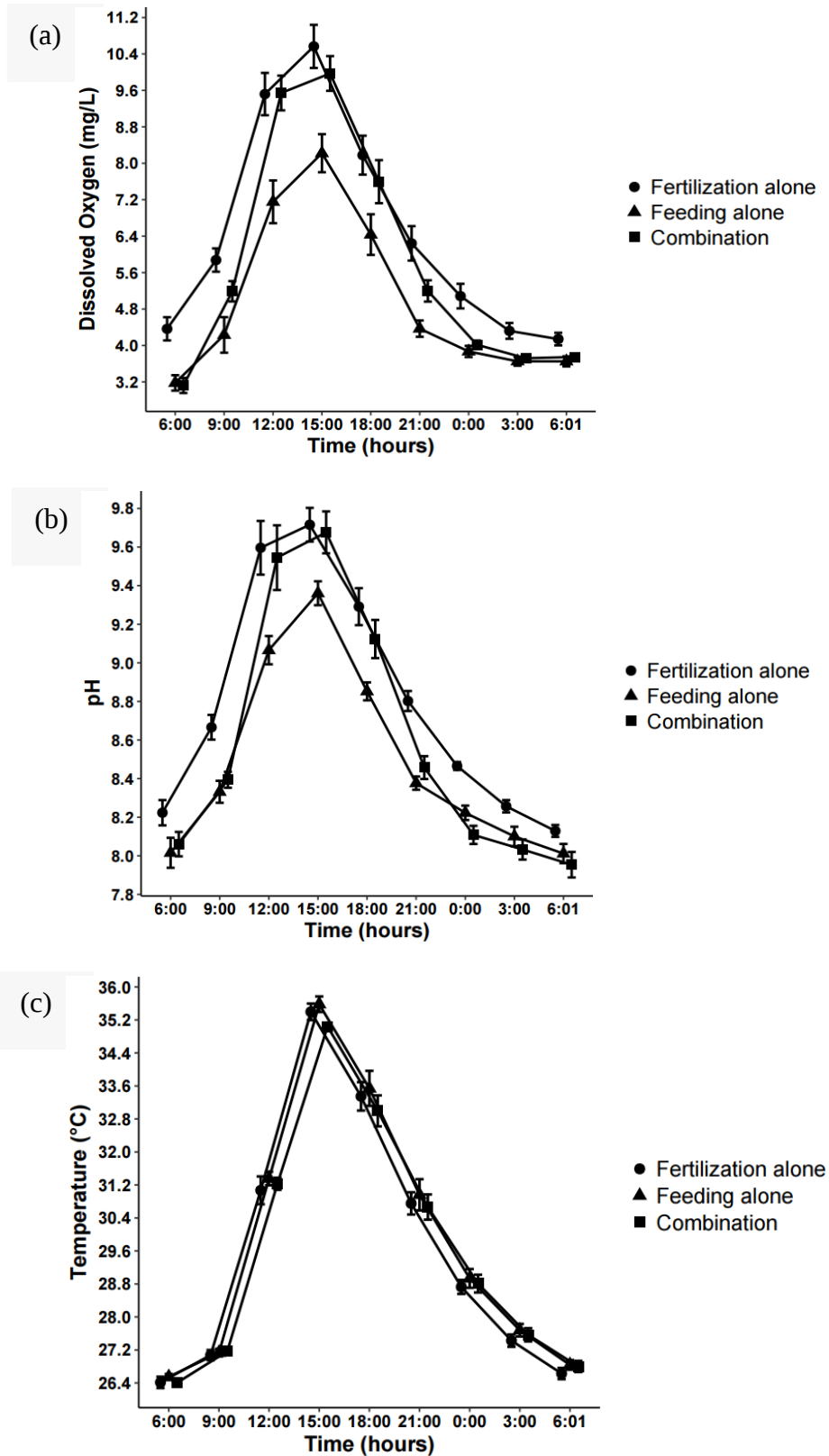
Water physico-chemical parameter	Treatments		
	Fertilization alone (T <sub>1</sub> )	Feeding alone (T <sub>2</sub> )	Combination (T <sub>3</sub> )
DO (mg/L)	4.35 $\pm$ 0.04 <sup>a</sup>	3.79 $\pm$ 0.03 <sup>c</sup>	4.21 $\pm$ 0.05 <sup>d</sup>
pH	8.24 $\pm$ 0.01 <sup>a</sup>	8.10 $\pm$ 0.01 <sup>b</sup>	8.07 $\pm$ 0.01 <sup>c</sup>
Temperature (°C)	25.17 $\pm$ 0.04 <sup>a</sup>	25.24 $\pm$ 0.04 <sup>a</sup>	25.22 $\pm$ 0.04 <sup>a</sup>
Conductivity ( $\mu$ S/cm)	1322 $\pm$ 3.28 <sup>b</sup>	1360 $\pm$ 3.06 <sup>a</sup>	1358 $\pm$ 4.16 <sup>a</sup>
TDS	670 $\pm$ 1.70 <sup>b</sup>	688 $\pm$ 1.56 <sup>a</sup>	688 $\pm$ 2.04 <sup>a</sup>
Salinity (mg/L)	0.660 $\pm$ 0.0 <sup>b</sup>	0.679 $\pm$ 0.0 <sup>a</sup>	0.679 $\pm$ 0.0 <sup>a</sup>
Secchi disk depth (cm)	25.3 $\pm$ 0.1 <sup>a</sup>	24.4 $\pm$ 0.1 <sup>b</sup>	23.1 $\pm$ 0.2 <sup>c</sup>
Alkalinity (mg/L)	191.82 $\pm$ 2.45 <sup>a</sup>	194.39 $\pm$ 2.43 <sup>a</sup>	181.97 $\pm$ 3.25 <sup>b</sup>
TN (mg/L)	20.82 $\pm$ 0.24 <sup>a</sup>	20.28 $\pm$ 0.43 <sup>b</sup>	16.69 $\pm$ 0.33 <sup>c</sup>
Nitrate (mg/L)	11.85 $\pm$ 0.12 <sup>a</sup>	11.19 $\pm$ 0.10 <sup>b</sup>	10.86 $\pm$ 0.10 <sup>b</sup>
Phosphorous (mg/L)	0.33 $\pm$ 0.01 <sup>a</sup>	0.15 $\pm$ 0.01 <sup>b</sup>	0.18 $\pm$ 0.01 <sup>b</sup>

\*<sup>abc</sup> = Means with the same superscript letter in the same row are not significantly different ( $p > 0.05$ ).

Note: se = standard error, DO = Dissolved oxygen, TN = Total nitrogen, TDS = Total dissolved solids.



**Figure 1: Dawn (Mean  $\pm$  se) fluctuations of (a) DO, (b) pH and (c) temperature during the experimental period in earthen ponds under fertilization alone, feeding alone and combination of fertilization plus feeding**



**Figure 2: Diurnal variations (Mean  $\pm$  se) of (a) DO, (b) pH and (c) temperature in earthen ponds under fertilization alone, feeding alone and combination of fertilization plus feeding**

### 2.3.2 Growth performance and survival rate of Nile tilapia

Growth performance, survival rate and FCR of *O. niloticus* cultured in ponds under the treatments of fertilization alone, feeding alone and combination of fertilization plus feeding are presented in Table 4 and Fig. 3. Generally, fish cultured in ponds subjected to a combination of fertilization plus supplementary feeding (T<sub>3</sub>) showed significantly ( $p < 0.05$ ) higher mean growth performances (weight gain =194.1 ± 4.5 g, length gain =18.6 ± 0.2 cm and growth rate =1.5 ± 0.1 g/day) than those reared in ponds under feeding alone and fertilization alone (Table 4). However, the difference in specific growth rate between the fish grown under feeding alone (T<sub>1</sub>) and those under the combination of fertilization plus supplementary feeding (T<sub>3</sub>) was not significant ( $p > 0.05$ ). The condition factor (K) varied significantly among the treatments, with the highest value (2.54 ± 0.0) being observed in fish under fertilization alone and the lowest value (2.05 ± 0.0) in fish under feeding alone.

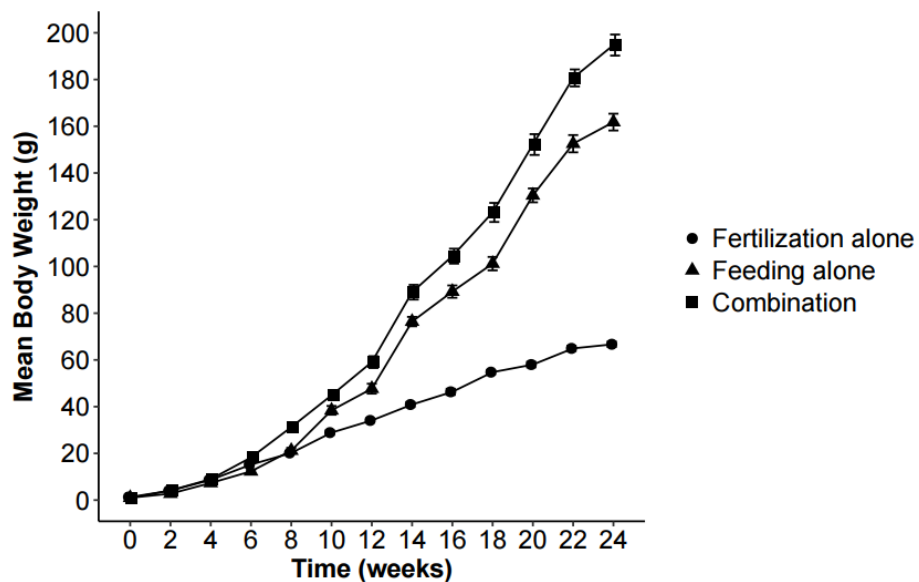
**Table 4: Growth performance (Mean ± se) of *O. niloticus* cultured in earthen ponds under fertilization alone, feeding alone and the combination of fertilization plus feeding**

Growth variable	Treatments		
	Fertilization alone (T <sub>1</sub> )	Feeding alone (T <sub>2</sub> )	Combination (T <sub>3</sub> )
Initial body weight (g)	1.1 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	0.7 ± 0.0 <sup>b</sup>
Initial body length (cm)	3.7 ± 0.1 <sup>a</sup>	3.5 ± 0.1 <sup>b</sup>	3.3 ± 0.1 <sup>b</sup>
Final body weight (g)	66.7 ± 1.1 <sup>c</sup>	161.8 ± 3.6 <sup>b</sup>	194.8 ± 4.5 <sup>a</sup>
Final body length (cm)	15.5 ± 0.1 <sup>c</sup>	20.1 ± 0.2 <sup>b</sup>	21.9 ± 0.2 <sup>a</sup>
Weight gain (g)	65.6 ± 1.1 <sup>c</sup>	160.9 ± 3.6 <sup>b</sup>	194.1 ± 4.5 <sup>a</sup>
Length gain (cm)	11.8 ± 0.1 <sup>c</sup>	16.6 ± 0.2 <sup>b</sup>	18.6 ± 0.2 <sup>a</sup>
Growth rate (g/day)	0.6 ± 0.0 <sup>c</sup>	1.3 ± 0.0 <sup>b</sup>	1.5 ± 0.1 <sup>a</sup>
Condition factor (K)	2.54 ± 0.0 <sup>a</sup>	2.05 ± 0.0 <sup>c</sup>	2.14 ± 0.0 <sup>b</sup>
Specific growth rate (%)	2.4 ± 0.1 <sup>b</sup>	3.1 ± 0.1 <sup>a</sup>	3.2 ± 0.1 <sup>a</sup>
Survival (%)	90.0 ± 0.0 <sup>a</sup>	89.6 ± 0.02 <sup>a</sup>	89.7 ± 0.04 <sup>a</sup>
FCR	-	4.1 ± 0.3 <sup>a</sup>	2.0 ± 0.1 <sup>b</sup>
FCE	-	0.2 ± 0.0 <sup>b</sup>	0.5 ± 0.0 <sup>a</sup>

\*<sup>abc</sup>= Means with the same superscript letter in the same row are not significantly different ( $p > 0.05$ ).

Note: se=standard error, FCR= Feed conversion ratio, FCE =Feed conversion efficient.

Feed conversion ratio (FCR) and feed conversion efficiency (FCE) were assessed only for treatments which involved feeding ( $T_2$  and  $T_3$ ). The results show that, fish reared in ponds under the combination of fertilization plus supplementary feeding had significantly ( $p < 0.05$ ) lower FCR ( $2.0 \pm 0.1$ ) than those cultured in ponds under feeding alone ( $4.1 \pm 0.3$ ). This resulted into significantly higher FCE ( $0.5 \pm 0.0$ ) for fish reared in ponds subjected to the treatment of the combination of fertilization plus supplementary feeding than those under feeding alone ( $0.2 \pm 0.0$ ).



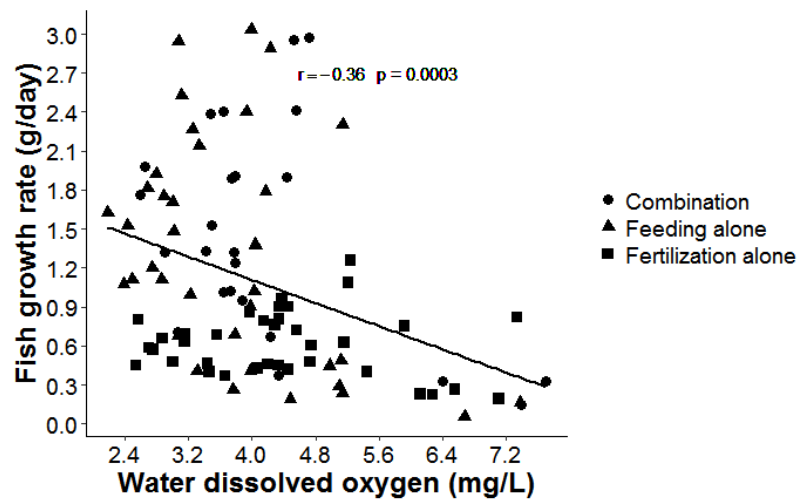
**Figure 3: Growth performance of *O. niloticus* cultured in earthen ponds for six months under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding**

### 2.3.3 Influence of water physico-chemical parameters on growth rate of *O. niloticus*

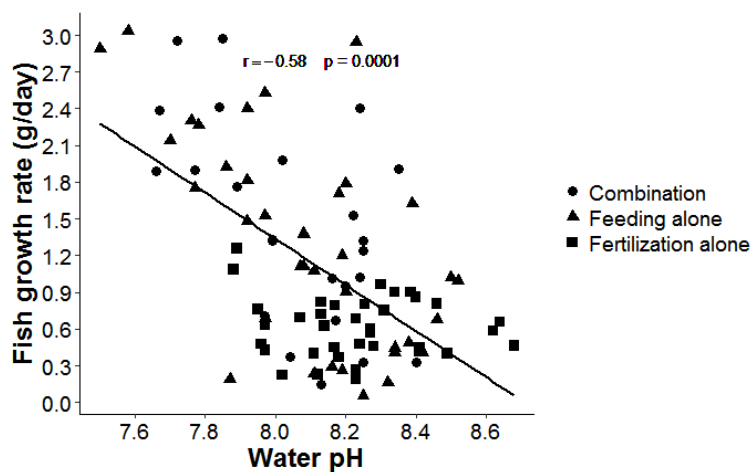
The results for correlation and regression analyses of growth rate and water physico-chemical parameters of fish reared in the ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding treatments are shown in Fig. 4, Fig. 5, Fig. 6 and Table 5. Dissolved oxygen (DO), pH, temperature and Secchi disk reading significantly ( $p < 0.05$ ) influenced the growth rate of *O. niloticus* (Fig. 4, Fig.



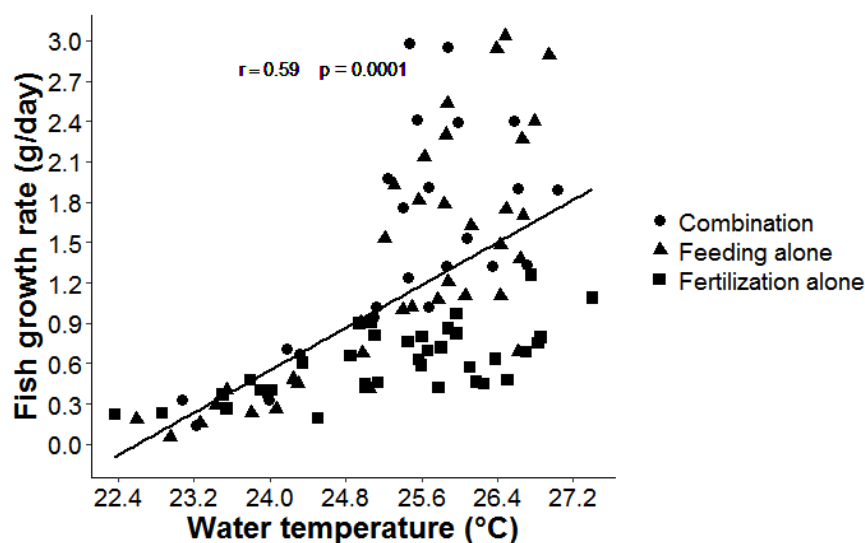
5, Fig. 6 and Table 5.). Nitrate ( $\text{NO}_3$ ), phosphorous, conductivity, TDS and salinity did not significantly ( $p > 0.05$ ) influence the growth rate of *O. niloticus* (Table 5). Fish growth rate increased significantly ( $p < 0.05$ ) as pH, DO and Secchi disk reading in pond water decreased (Fig. 4, Fig. 5) (Table 5). Water temperature positively ( $p < 0.05$ ) influenced fish growth rate (Table 5) (Fig. 6).



**Figure 4: Relationship between growth rate and pond water dissolved oxygen for *O. niloticus* cultured in the ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding treatments**



**Figure 5: Relationship between growth rate and pond water pH for *O. niloticus* cultured in the ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding treatments**



**Figure 6: Relationship between growth rate and pond water temperature for *O. niloticus* cultured in the ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding treatments**

**Table 5: Regression between fish growth rate and pond water physico-chemical parameters for fish cultured in pond**

Water parameter	Partial regression		
	coefficient estimates (b)	se	p-value
pH	-1.32	0.09	<b>0.001</b>
DO (mg/L)	-0.09	0.02	<b>0.001</b>
Conductivity ( $\mu$ S/cm)	0.00	0.00	0.417
TDS	0.01	0.01	0.305
Salinity (mg/L)	0.02	0.00	0.104
Temperature ( $^{\circ}$ C)	0.23	0.02	<b>0.001</b>
Secchi disk (cm)	-0.01	0.00	<b>0.002</b>
Nitrate (NO <sub>3</sub> ) (mg/L)	-0.02	0.01	0.162
Phosphorous (mg/L)	0.20	0.06	0.934

Note: DO =Dissolved oxygen, TDS = Total dissolved solids, se = standard error, b=regression coefficient

### 2.3.4 Fish yield and economic return

Results for variable costs, yield and gross margin for the fish reared in ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding are shown in Table 6. Total variable costs did not differ between ponds under

feeding alone and the combination of fertilization plus feeding but were significantly higher than that for ponds under fertilization alone. Moreover, the cost of producing one kg of fish differed significantly among the treatments. The highest cost (TZS 8 446 ± 380.6) per kg of fish was observed in ponds under feeding alone and the lowest cost was found in the other two treatments. The average cost per kg of fish did not differ significantly between the fish reared under the treatment of combination and those under fertilization alone (Table 6).

The ponds under the combination of fertilization plus supplementary feeding ( $T_3$ ) had the highest fish yield ( $13\,065 \pm 458$  kg/ha/year) and revenue (TZS 104 514 516 ± 3 659 778) while the ponds under fertilization alone treatment had the lowest fish yield ( $4\,602 \pm 376$  kg/ha/year) and revenue (TZ S36 820 392 ± 3 008 558). But the differences in fish yield and revenue between feeding alone and the combination treatments were insignificant. Gross margin differed significantly ( $p < 0.05$ ) (Table 6) among the treatments. The highest gross margin value was obtained from the fish reared under the combination of fertilization plus supplementary feeding treatment (TZS 28 499 967 ± 3 173 413) and the lowest value was observed from the fish reared under the feeding alone treatment (TZS -4 623 126 ± 3 691 410).

**Table 6: Mean variable costs, yield, revenue and gross margin of the *O. niloticus* cultured for six months in earthen ponds under fertilization alone, feeding alone and combination of fertilization plus feeding**

Variable costs (TZS /ha/year)	Treatment		
	Fertilization alone (T1)	Feeding alone (T2)	Combination (T3)
Fingerling Cost	19 131 787 ± 501 028 <sup>a</sup>	18 499 161 ± 840 966 <sup>a</sup>	18 736 991±1 137 351 <sup>a</sup>
Labour cost	1 493 871 ± 43 769 <sup>b</sup>	17 091 062 ± 1 873 473 <sup>a</sup>	21 660 284 ± 129 747 <sup>a</sup>
Fertilizer cost	3 453 833 ± 67 961 <sup>a</sup>	-	3 383 388 ± 51 645 <sup>a</sup>
Feed cost	-	54 796 586 ± 5 683682 <sup>a</sup>	32 233 886 ± 572 883 <sup>a</sup>
<b>Total variable cost</b>	<b>24 079 491 ± 525 407<sup>b</sup></b>	<b>90 386 810 ± 8 013 266<sup>a</sup></b>	<b>76 014 550 ± 486 366<sup>a</sup></b>
Cost/kg of fish (TZS)	5 284 ± 327.4 <sup>b</sup>	8 446 ± 380.6 <sup>a</sup>	5 824 ± 166.7 <sup>b</sup>
<b>Output</b>			
Yield (kg/ha/year)	4 602 ± 376 <sup>b</sup>	10 720 ± 962 <sup>a</sup>	13 065 ± 458 <sup>a</sup>
Total revenue(TZS/ha/year)	36 820 392 ± 3 008 558 <sup>b</sup>	85 763 684 ± 7 696 247 <sup>a</sup>	104 514 516 ±3 659 778 <sup>a</sup>
Gross margin (TZS/ha/year)	12 740 901 ± 2 598 075 <sup>b</sup>	-4 623 126 ± 3 691 410 <sup>c</sup>	28 499 967 ± 3 173 413 <sup>a</sup>

\*Values are expressed as means ± se; <sup>abc</sup>= Means with the same superscript letter in the same row are not significantly different ( $p > 0.05$ ).

## 2.4 Discussion

### 2.4.1 Water physico-chemical parameters

The growth of tilapia in ponds is usually limited by DO as one of the most important water quality parameters for fish survival and growth. In the present study, mean DO values differed significantly among the treatments. The lowest DO values was observed in ponds under the feeding alone treatment and this may be due to the existence of a lot of microbial activities involved in decomposition of leftover feeds (Thakur *et al.*, 2004; Priyadarshini *et al.*, 2011). Aerobic microbes utilize oxygen for the decomposition of the feed residues. The higher amount of the dissolved oxygen in pond water under fertilization alone and the combination of fertilization plus feeding treatments probably was caused by higher concentration of phytoplankton. Large biomass of the phytoplankton results into higher rate of photosynthesis which, inturn, increases the amount of oxygen in the pond water (Thakur *et al.*, 2004).

Variation in DO among the treatments could be caused by the differences in the rate of photosynthesis and respiration in the ponds subjected to different treatments. For the ponds subjected to fertilization alone, there were less microbial activities due to minimum decomposition process. This is because there were no feed remains, hence, low organic matter decomposition and less use of DO in the decomposition of organic matter (Thakur *et al.*, 2004). For the ponds under feeding alone and the combination of fertilization plus supplementary feeding, there were some feeds that were not eaten by the fish. The presence of these feed residues (leftover feeds) resulted into higher microbial activities that was involved in the decomposition process (Thakur *et al.*, 2004; Priyadarshini *et al.*, 2011), hence, low DO, especially at night when fish, algae and microbes both consumes DO and release CO<sub>2</sub>.

Since the ponds under the combination treatment received only 50% of the feeds used for the feeding alone treatment, the amount of uneaten feeds in those ponds was low and consequently the rate of decomposition was lower than in the ponds under the treatment for feeding alone. This resulted into less microbial activities and relatively higher levels of DO in pond water subjected to combination treatment compared to those under feeding alone treatment .

The dawn DO values reported in the present study are lower compared to the values reported by Shoko *et al.* (2016) and Abdel-Warith (2013) which ranged from 6.2 to 13.7 mg/L for the experiment done between 0900 and 1700 hours. It is well established that DO levels fluctuate within 24 hours period and are lowest at dawn and highest at dusks (Francis-Floyd, 2003; Manyala *et al.*, 2015; Makori *et al.*, 2017). These findings support the results of the present study.

The mean value for dawn pH followed the same trend as DO values, with the highest pH values being observed in ponds under fertilization alone. Low pH in the ponds under the treatments of combination and feeding alone could be caused by the accumulation of CO<sub>2</sub>. The presence of uneaten feeds may result into an increase in microbial decomposition activities, hence, high respiration rates (Thakur *et al.*, 2004), especially during night times where both fish, plankton and microbes use oxygen and respire CO<sub>2</sub>. Bhatnagar and Devi (2013) and Makori *et al.* (2017) noted that pH in the water is determined by the concentration of CO<sub>2</sub> which exists in the form of carbonic acid. In addition, the daily pH fluctuation may be caused by the change in the rate of photosynthesis in response to daily photoperiod (Boyd, 1998; Makori *et al.*, 2017). Temperature did not differ significantly among the treatments and was within the range acceptable for tilapia growth. The preferred water temperature for tilapia growth is

approximately between 20.27 to 30 °C (Bhatnagar and Devi, 2013; Manyala *et al.*, 2015; Shoko *et al.*, 2016). Such range agrees with the findings of the present study. In the present study assessment of diurnal temperature fluctuations, revealed that temperature was higher than the optimal range, especially at dusk. This condition might have lowered feed intake and consequently low fish growth rate. It is well documented that as the temperature increases beyond the acceptable range the solubility of DO decreases (Bhatnagar and Devi, 2013).

Ponds under the combination of fertilization plus feeding had the lowest mean Secchi disk reading value than the other treatments. This might be due to high water turbidity and total dissolved solids (TDS) in the ponds under combination treatment. Total dissolved solids (TDS) and plankton are among the factors that increase water turbidity (Bhatnagar and Devi, 2013). Significantly higher TDS values in ponds under feeding treatments, suggest that some of the feeds sank into the water. This is because some particles of the mash feed do not float like floating pelleted feeds, therefore sink to the bottom and decompose and hence, increased TDS of pond water. It has been reported that, high water turbidity, TDS, conductivity, salinity and low Secchi disk reading reflects the amount of suspended clay particles, feed leftover, abundance of plankton and pigments from decomposition of organic matters in the water column (Bhatnagar and Devi, 2013; Sandhya and Benarjee, 2016). Research findings shows that Secchi disk reading decreases as the plankton and TDS increases in the water column (Boyd, 1990; Abdel-Tawwab *et al.*, 2007). Nevertheless, the Secchi disk values observed in this study were in the acceptable range for fish growth of between 15 and 40 cm (Bhatnagar and Devi, 2013; Meiludie, 2013).

Likewise, conductivity and salinity values observed in this study were within the acceptable ranges for fish growth and survival, which is between 30 and 5000  $\mu\text{S}/\text{cm}$  for conductivity and 0 and 7000 mg/L for salinity (Bhatnagar and Devi, 2013; Stone *et al.*,

2013; Azevedo *et al.*, 2015). Lower alkalinity in the ponds under the combination of fertilization plus feeding than in those under the other treatments might be contributed by the low concentration of CO<sub>2</sub> after dawn hours. Water samples were taken after dawn hours when photosynthesis was taking place. Ponds under combination treatment showed low Secchi disk reading which may imply high amount of plankton, thus high photosynthesis which lower CO<sub>2</sub> levels (Mbonde *et al.*, 2017). Interestingly, the alkalinity values obtained in the current experiment for all treatments were in the acceptable range of between 115 and 329 mg/L for fish culture (Manyala *et al.*, 2015). The higher alkalinity mean values in the ponds under fertilization alone and feeding alone treatments indicates the higher capacity of pond water to resist pH fluctuation compared to the ponds under the combination of fertilization plus feeding (Stone *et al.*, 2013).

The higher concentrations of nitrate and total nitrogen in the ponds under fertilization alone than the rest of the treatments probably were caused by application of inorganic fertilizer (urea). The same amount of inorganic fertilizers was applied in ponds under fertilization alone and the combination of fertilization plus feeding treatments. But the ponds subjected to the combination treatment had relatively lower nitrate and total nitrogen concentration perhaps because of higher biomass of phytoplankton and, hence, high consumption of nitrate by phytoplankton. Values of nitrate concentration (NO<sub>3</sub>) in pond water observed in this study were within the acceptable range for fish growth of between 0 and 90 mg/L (Abdel-Tawwab *et al.*, 2007; Bhatnagar and Devi, 2013).

Phosphorous is the major element and performs vital functions including transfers of energy and genetic information in the cells for sustenance, growth and development of algae (McDonald *et al.*, 2010). The higher phosphorous content in ponds under fertilization alone could be caused by input of phosphorous from inorganic fertilizer



(DAP) and the presence of less phytoplankton to consume the phosphorous, thus most of it was left in pond water (Bhatnagar and Devi, 2013). Phosphorous content in pond water observed in the present study was within the standard range of between 0.05 and 1.0 mg/L (Bhatnagar *et al.*, 2004; Stone and Thomforde, 2004).

#### **2.4.2 Growth performance and survival rate of Nile tilapia**

Fish under the combination of pond fertilization plus supplementary feeding treatment had higher growth performance than the fish under the other treatments. This is probably due to consumption of nutrients from both supplementary feed and natural food stimulated by essential nutrients from fertilizers and leftover feeds. This is in agreement with the findings of other researchers (Bahnasawy *et al.*, 2003; Waidbacher *et al.*, 2006; Prabakaran and Murugan, 2012; Abdel-Warith, 2013; Manyala *et al.*, 2015). Also, it has been shown that, in a semi-intensive system, fish can depend on natural food to a limited size, beyond that it requires supplementary diet to attain optimum growth (Abdel-tawwab *et al.*, 2007).

The highest condition factor (K) was obtained for the fish under fertilization alone while those under feeding alone showed the lowest K value. This suggests that, fish under feeding alone grew faster in length relative to weight. Research done by Abdel-Warith (2013) on the effects of fertilization plus feeding, manure plus feeding and feeding alone on tilapia growth also reported higher condition factor for the treatments with low growth rate as in the present study. The differences in condition factor among the treatments may be the result of the differences in feeding strategy that possibly altered the environmental conditions of the pond. However, since condition factor was greater than one in all treatments, the fish in ponds under all treatments were in good health conditions (Nehemia *et al.*, 2012). Survival rate of the fish was not affected by feeding strategies. Fish survival

can be reduced by presence of diseases and/or poor water quality beyond the acceptable range (Lamtane *et al.*, 2008; Bhatnagar and Devi, 2013). Water physico-chemical parameters in the present study were within the acceptable ranges for fish growth. Fish reared under the combination of fertilization plus feeding treatment showed significantly lower feed conversion ratio (FCR) than those cultured under feeding alone. This is because the amount of feed used to feed fish under the combination treatment was 50% of that used under feeding alone treatment. Also the weight gained by fish under the combination treatment was higher than that in feeding alone treatment. The same results have been reported by Opiyo *et al.* (2014) who observed that FCR of the fish decreased when both fertilizer and supplementary feed are used. This suggests that by feeding the combination of natural food and supplementary feed, the efficiency of fish to convert feed into tissue biomass becomes high. The results in the present study revealed that the feed conversion efficiency (FCE) was almost doubled in the fish under the combination treatment compared to those under feeding alone as it has been reported by Manyala *et al.* (2015).

#### **2.4.3 Influence of water physico-chemical parameters on growth rate of *O. niloticus***

In the present study, fish growth rate was negatively correlated with dissolved oxygen. The negative correlation probably might be the result of the effect of experimental time. Previous studies have shown that organic matter in the ponds increase with experimental time (Thakur *et al.*, 2004). The increase of organic matter in the pond is the results of decay of high algae biomass and accumulation of uneaten feeds. The organic matter accumulation in the pond water increases microbial decomposition activities and this demands more dissolved oxygen, thus lower DO levels (Boyd and Hanson 2010). Low DO levels below 3 mg/L in the water may affect fish growth (Sorphen *et al.*, 2010). In the

present study, DO levels supported fish growth as experimental time increases since the DO did not fall below the recommended level.

The highest fish growth rate was observed in ponds under the combination of fertilization plus feeding, which had relatively lower pH compared to the ponds under the other treatments. Previous studies have shown that high fish growth is achieved at the pH of between 6.5 and 8.5 which is close to the average fish blood pH (7.4) (Bhatnagar and Devi, 2013; Makori *et al.*, 2017). Among the three treatments, the ponds under the combination of fertilization plus feeding had pH level close to the fish blood pH. Pond water temperature values in this study were within the acceptable range for fish growth (Bhatnagar and Devi, 2013). The increase in temperature in the present study resulted into an increase in fish growth. Studies have shown that high temperature favours the metabolic and physiological activities of the fish (Bhatnagar and Devi, 2013; Makori *et al.*, 2017). The negative relationship between Secchi disk reading and fish growth rates suggest that, Secchi disk reading decreased as availability of feed in ponds increases. This limits light penetration in ponds water and reduces Secchi disk reading but more feeds become available for fish as reported by Abdel-Tawwab *et al.* (2007).

#### **2.4.4 Fish yield and economic return**

Results for economic analysis show that the cost of producing one kg of fish was higher for the ponds under the treatment of feeding alone than under the combination and fertilization alone treatments. The high unit cost under feeding alone treatment was probably caused by the large quantity of feeds used to feed the fish and this resulted into high feed cost. The feed cost under the feeding alone treatment exceeded that of the combination of fertilization plus feeding by 70%. Feed cost accounted for 61 and 42% of the total variable costs under feeding alone and the combination of fertilization plus

supplementary feeding treatments, respectively. This is in agreement with the findings documented by Jabir *et al.* (2012) and Abdel-Warith (2013) that, feed costs comprise 40 - 70% of total operational costs. Although the unit cost for one kg of fish under fertilization alone was the same as that observed under the combination of fertilization plus feeding treatment, ponds under fertilization alone had lower yield and thus, low gross margin. The fish reared in ponds under feeding alone resulted into a loss due to high feed cost compared with the fish cultured in ponds under the combination of fertilization plus feeding treatment. This is because fish reared in ponds under feeding alone treatment were fed 50% more feed than those under the combination of fertilization plus supplementary feeding.

The gross margin for the fish reared in ponds under the combination of fertilization plus feeding was higher compared to those under feeding alone, suggesting that the combination of pond fertilization plus supplementary diet feeding is the cost-effective strategy for growing tilapia under semi-intensive production system. Similar results have been reported by Thakur *et al.* (2004) and Manyala *et al.* (2015) that the culture of fish in fertilized ponds accompanied with supplementary feeding results in better performance in terms of yield and gross margin. This is mainly the result of reduction of feed costs and efficient utilization of feeds under the combination treatment, hence, high fish growth and yield (Manyala *et al.*, 2015).

## **2.5 Conclusions and recommendations**

This study has shown that, the culture of *Oreochromis niloticus* in earthen ponds subjected to the combination of weekly inorganic fertilizer application plus supplementary feeding at 2.5% of the fish body weight result into faster fish growth and higher food conversion efficient (FCE), than the culture of fish in ponds under fertilization alone or feeding alone

at 5% of the fish body weight. Moreover, the combination of weekly fertilization and feeding at 2.5% of fish body weight reduces feed cost significantly and hence, increases the gross margin compared to feeding alone at 5% of fish body weight. Weekly fertilizer application either under fertilization alone or the combination of fertilization plus feeding does not affect the water quality parameters beyond the acceptable range for optimum fish growth. However, application of fertilizer plus feeding decreases water Secchi disk reading which is negatively correlated with fish growth performance.

It is recommended that further study be done on the effect of fertilization and feeding floating pelleted feeds. The use of floating pelleted feeds will reduce feed leftovers. Moreover, alternative day feeding should be done as this may reduce feed cost and increase profit.

## **2.6 Acknowledgement**

Acknowledgement is made to AQUAFISH INNOVATION LAB for financial support which enabled this research work to be conducted successfully. The authors also acknowledge the assistance of laboratory technicians from the Department of Animal, Aquaculture and Range Sciences (DAARS) and Department of Soil and Geological Sciences at Sokoine University of Agriculture for their supports during sample analyses.

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## **CHAPTER THREE**

### **3.0 MANUSCRIPT II**

#### **Influence of inorganic fertilizer application and supplementary feeds on periphyton biomass, quality and species composition**

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**Abstract**

A study was conducted to assess the effects of fertilization alone (T<sub>1</sub>), supplementary feeding alone (T<sub>2</sub>) and combination of fertilization plus supplementary feeding (T<sub>3</sub>) on periphyton quantity (biomass, dry matter and organic matter) and quality (crude protein, ether extract and phosphorous contents). Sex-reversed *O. niloticus* fingerlings with average weight ranging between 0.7 and 1.1 g were stocked in earthen ponds at 3 fish/m<sup>2</sup> one week after initial pond fertilization. Urea and Diammonium phosphate (DAP) fertilizers were applied in pond water at weekly intervals at a rate of 3 and 2 g/m<sup>2</sup>, respectively. Feeding of fish was done using a diet containing 25.1% crude protein. The fish were feed daily at 1000 and 1600 hours. For the first two months, the fish were fed 10% and 5% of their body weight for T<sub>2</sub> and T<sub>3</sub> treatments, respectively, and then the amount of the feed was reduced to 5% (T<sub>2</sub>) and 2.5% (T<sub>3</sub>) of body weight for the last four months. In each pond eight nets, each with 20 µm-mesh size and an area of 1250 cm<sup>2</sup> were submerged in water to provide substrate for periphyton attachment. The nets were taken out from the water and scrubbed after every two months for periphyton collection. Four ml of the scrubbed periphyton solution were put in bottles and preserved in 4% concentration of formalin for community composition determination. Part of the periphyton solution was centrifuged and preserved for periphyton quality and quantity determination. A random sample of 30 fish was taken biweekly from each pond for individual morphometric measurements. At the end of the study, three fish from each pond were taken for proximate chemical composition analysis. Results show that the ponds under T<sub>3</sub> had higher periphyton biomass ( $47.35 \pm 7.64$  g DM/m<sup>2</sup>), crude protein content ( $11.40 \pm 0.16\%$ ) and organic matter content (OM) ( $25.47 \pm 0.28\%$ ). The periphyton from ponds under T<sub>1</sub> had the highest ether extract content ( $1.84 \pm 0.07\%$ ) and ponds under T<sub>2</sub> had higher phosphorous content ( $0.48 \pm 0.0$  mg/L). The body of fish cultured in ponds under T<sub>3</sub> had higher CP ( $69.14 \pm 0.33\%$ ) and OM ( $96.65 \pm 0.16\%$ ) contents while those reared in ponds

under T<sub>1</sub> had higher ether extract content ( $18.33 \pm 0.19\%$ ) and ash content ( $4.78 \pm 0.1\%$ ). Fish growth rate increased as the periphyton quantity (biomass and OM) and quality (CP and phosphorous) increased, suggesting that in addition to supplementary feed the growth rate of fish was influenced by the quality and quantity of natural food available in the pond. The study also revealed five classes of phytoplankton (Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae and Zygnematophyceae) and three classes of zooplankton (Eurotatoria, Heterotrichea and Oligohymenophorea). In general, both phytoplankton (algae) and zooplankton were more abundant in ponds under T<sub>3</sub> than in ponds under the other treatments. It is concluded that the combination of fertilization plus supplementary feeding (T<sub>3</sub>) produces higher periphyton quantity, quality and species composition and thus promotes higher fish growth rate compared to feeding alone and fertilization alone treatments.

**Keywords:** *algae, growth rate, Nile tilapia, proximate chemical composition, zooplankton*



### 3.1 Introduction

Fish growth performance is a function of the genetic make up and exogenous factors, particularly nutrition and management (Shailender *et al.*, 2013). Fish grown in ponds can be fed supplementary feeds or natural food produced in the pond through fertilization (Abdel-Tawwab *et al.*, 2007; Priyadarshini *et al.*, 2011). Fertilizer application in pond water supplies essential nutrients, especially nitrogen (N) and phosphorous (P) which are the most limiting nutrients for algae (phytoplankton) growth in freshwater ecosystem (Priyadarshini *et al.*, 2011). Moreover, the addition of supplementary feeds in pond water increases nitrogen and phosphorus contents from the decomposition of uneaten feeds which, in turn, results into higher quantity of plankton community in the pond ecosystem (Ssanyu *et al.*, 2011). The concentration of plankton in the water column determines the amount of periphyton (phytoplankton and zooplankton attached into the substrate). Phytoplankton and zooplankton stabilize the aquatic ecosystem and minimize the fluctuations of water quality through photosynthesis, respiration and assimilation of ammonia (Thakur *et al.*, 2004). In addition, periphyton supply some important enzymes that improve the utilization of the supplemented feed, thus increase feed utilization efficiency (Jhingran, 1991).

The quantity and quality of periphyton improve fish growth rate and biochemical composition of the fish (Job *et al.*, 2015). The periphyton quantity and quality can be manipulated through fertilization and management of water quality (Gatenby *et al.*, 2003). The quality of algae (phytoplankton) is influenced by the concentration of nitrogen (N) and phosphorus (P) in pond water, rather than energy (carbon). The presence of carbon (C) in the cell wall of algae lowers digestibility of periphyton (Sternner and Hessen, 1994; Sternner and Elser, 2002). Lipids from natural food are the imperative nutrients for fish as they contain essential fatty acids (EFAs) and energy which are required for fish growth

and healthy tissue maintenance (Tidwell *et al.*, 2007; Kaiser *et al.*, 2012; Roy and Pal, 2014). Algae contain essential fatty acids which includes docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These essential fatty acids must be provided in the diet because they cannot be synthesized by the fish body (Tidwell *et al.*, 2007). Also, zooplankton as a constituent of natural food has been reported to contain high crude protein and lipid contents which are important for fish growth (Kar *et al.*, 2017).

Biomass and community composition of the periphyton differ depending on nutrients availability, water quality and consumption pressure caused by other aquatic organisms (top down effects) (Abdel-Tawwab *et al.*, 2007; Priyadarshini *et al.*, 2011). Normally fish feed selectively depending on the quantity and quality of natural food. Higher plankton quality and biomass in ponds may lead to improved fish growth performance (Abdel-Tawwab *et al.*, 2007). The presence of high quality natural food in a semi-intensive production system is vital for fish growth, because the availability of high quality natural food in pond water minimizes the dependence on supplementary feed, hence, reduces the production costs and increases profit. This is desirable, especially for smallholder farmers who have low capital (Abdel-Warith, 2013).

A study done in Egypt to assess the effect of supplementation of fertilized ponds cultured with tilapia showed that the combination of supplementary feeding plus fertilizer application results into more plankton abundance and species composition than fertilization alone or feeding alone (Abdel-Tawwab *et al.*, 2007; Abdel-Warith, 2013). In Tanzania, the information on the effects of inorganic fertilizer application and supplementary feeds on periphyton quantity, quality and species composition is lacking. This study, therefore, was conducted to assess the effects of inorganic fertilizer application

alone, feeding alone and their combination on periphyton quality, quantity and their influence on fish tissue biochemical composition.

## **3.2 Materials and Methods**

### **3.2.1 Description of the study location**

The experiment was conducted in earthen ponds for 180 days at Tindiga village, Kilosa district, Tanzania. The village is approximately 13 km from Kilosa town. Kilosa district lies between latitude 5° 55'S and 8° 53'S and longitude 36 °30'E and 37°30'E. The area where the ponds are located is subject to seasonal flooding and characterized by poorly drained soils with black cracking clays during the dry period. The district receives rainfall between October and May. The mean annual rainfall ranges from 800 to 1400 mm (Kajembe *et al.*, 2013).

### **3.2.2 Management of ponds and fish**

The experiment was conducted in nine earthen ponds, each with an average area of 177 m<sup>2</sup>. The treatments were fertilization alone (T<sub>1</sub>), supplementary feeding alone (T<sub>2</sub>) and the combination of fertilization plus supplementary feeding (T<sub>3</sub>), each treatment was replicated three times. The inorganic fertilizers used were urea and Diammonium phosphate (DAP) and they were applied at weekly intervals in ponds under treatments T<sub>1</sub> and T<sub>3</sub> at the rate of 3 g and 2 g per m<sup>2</sup>, respectively.

Sex-reversed Nile tilapia fingerlings with weight ranging from 0.7 to 1.1 g were collected from Ruvu fish farm and stocked seven days after initial pond fertilization at a stocking density of 3 fingerlings /m<sup>2</sup>. Feeding in treatments T<sub>2</sub> and T<sub>3</sub> was done twice per day using a diet containing 25.1% crude protein (CP). Fish were fed at the feeding level of 10% and 5% of the fish body weight (FBW) for T<sub>2</sub> and T<sub>3</sub>, respectively, for the first two months.

Then the amount of feed was reduced to 5% and 2.5% for T<sub>2</sub> and T<sub>3</sub>, respectively, for the last four months of the experimental period.

### 3.2.3 Fish sampling and growth rate determination

Fish were sampled biweekly using a net with 1 mm mesh size. A random sample of 30 fish was taken from each pond and each fish was individually measured for body weight (g) and length (cm), using a portable digital weighing balance and board fixed with a ruler respectively. After taking measurements the fish were released back into their respective ponds.

Growth rate (GR) was calculated using the following formula:

$$\text{GR} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Experimental period (days)}}$$

Where: GR is growth rate (Opiyo *et al.*, 2014; Khalafalla and El-Hais, 2015).

### 3.2.4 Determination of chemical composition of fish carcass

At the end of the experiment three fish from each pond were collected, put in the ice box and transported to a laboratory and stored in the deep freezer at -18 °C. Then, the fish were thawed at room temperature, cleaned, eviscerated, filleted and deboned as described by El Shehawy *et al.* (2016) and Zahid *et al.* (2013). The fish were dried at 60 °C for 24 hours. The dried fish samples were homogenised by grinding in the motor to an average sieve size of 2 mm, and then put in bottles for dry matter, ash, crude protein and ether extract determination (AOAC, 2006). Crude protein (CP) was determined by Kjeldahl method (AOAC, 2006; Agwa *et al.*, 2012; Job *et al.*, 2015; Kwikiriza *et al.*, 2017). Ether extract was determined by the Soxhlet extraction method (AOAC, 2006; Kwikiriza *et al.*, 2017). Duplicate sub-samples, each weighing approximately two gram, were taken and burned for 3 hours at 550 °C and digested in 6 Normality of HCL for phosphorous determination

using Spectrophotometer analysis. The absorbance of the samples and standard solution were read in the Spectrophotometer at the wavelength of 420 nm (APHA, 1992).

### **3.2.5 Periphyton quantity and community composition analysis**

Eight nets, each with 20  $\mu\text{m}$ -mesh size and an area of 1250  $\text{cm}^2$  were placed (fully submerged in water) in each pond for periphyton to attach. The nets were taken out from the water and scrubbed to collect algae and zooplankton after every two months. Four ml of the periphyton solution were taken and preserved in 4% concentration of formalin solution for species identification. In the laboratory 10  $\mu\text{L}$  of the periphyton sample, in duplicate, was taken by using a pipette and placed in the neubor chamber slide for enumeration by using microscope at 10x magnification. For each treatment the number of zooplankton and phytoplankton were determined and expressed as a mean number of taxa (Dimowo, 2013).

The rest of the scrubbed periphyton were allowed to settle and the water was decanted to concentrate the periphyton. The samples were put in 500 ml bottles, transported in an ice cool box to a laboratory and stored in deep freezer at  $-18^\circ\text{C}$  for laboratory analysis. In the laboratory periphyton samples were centrifuged at 3000 rpm for 10 minutes, then dried in an oven at  $60^\circ\text{C}$  for 24 hours for biomass and proximate chemical compositions analysis (Golueke and Oswald, 1965; AOAC, 2006).

### **3.2.6 Statistical analysis**

Data were analysed by using R Studio software version 3.5.0 (2018) (Horton and Kleinman, 2015; Tortolero *et al.*, 2016). Before the analysis of variance, the data were checked for normality and transformed whenever necessary to increase error homoscedasticity. One-way ANOVA was used at 5% significance level to assess the effect

treatment on periphyton quantity (biomass, DM, OM), quality (CP, EE, and Phosphorous) and fish body chemical composition. Post Hoc analysis was done by using Duncan's new multiple range test to determine the significance of the differences between treatment means when the treatment had significant effect. In addition, correlation and multiple regression analyses were used to determine the relationships between periphyton proximate chemical composition values and fish growth performance.

The model for multiple regression analyses was as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \varepsilon$$

Where,

$Y$  = Response (fish growth rate)

$\beta_0$  = The intercept of the regression line on Y axis

$\beta_1, \beta_2, \dots, \beta_k$  =  $k^{\text{th}}$  partial regression coefficient (slope)

$X_1, X_2, \dots, X_k$  =  $k^{\text{th}}$  predictor variable [periphyton proximate chemical composition (i.e. Biomass, CP, EE and phosphorous)]

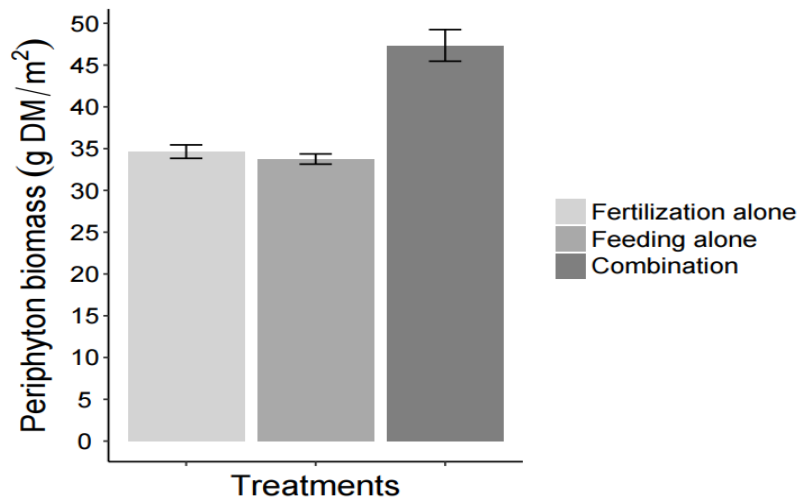
$\varepsilon$  = error term

### 3.3 Results

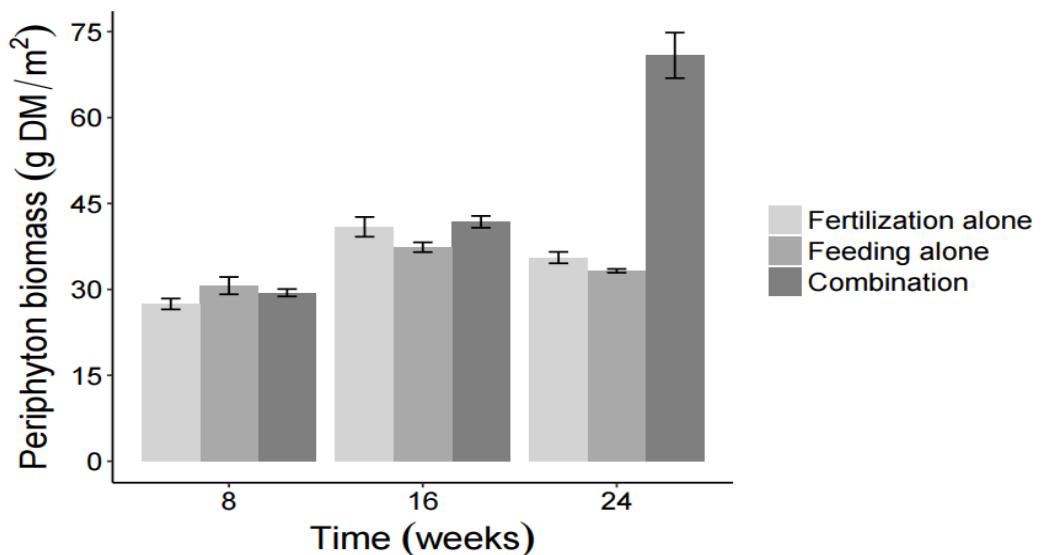
#### 3.3.1 Periphyton Biomass

Results on biomass of periphyton found in the ponds under different treatments are shown in Fig. 1 and Fig. 2. Periphyton biomass differed significantly ( $p < 0.05$ ) among the treatments. The ponds subjected to combination of fertilization plus supplementary feeding ( $T_3$ ) had higher mean periphyton biomass ( $47.35 \pm 7.64$  g DM /m<sup>2</sup>) than those under feeding alone and fertilization alone (Fig. 1). The means of periphyton biomass

between ponds under fertilization alone ( $34.64 \pm 3.21$  g DM /m<sup>2</sup>) and feeding alone ( $33.76 \pm 2.43$  g DM /m<sup>2</sup>) did not differ ( $p > 0.05$ ) (Fig. 1). The mean periphyton biomass from the ponds under the combination of fertilization plus supplementary feeding treatment increased as the experimental time increased while for the other treatments periphyton biomasses decreased toward the end of the experiment (Fig. 2).



**Figure 1: Comparison of periphyton biomass (Mean ± se) in ponds under fertilization alone, feeding alone and combination of fertilization plus supplementary feeding**



**Figure 2: Variation in periphyton biomass (Mean  $\pm$  se) over time in ponds subjected to fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding**

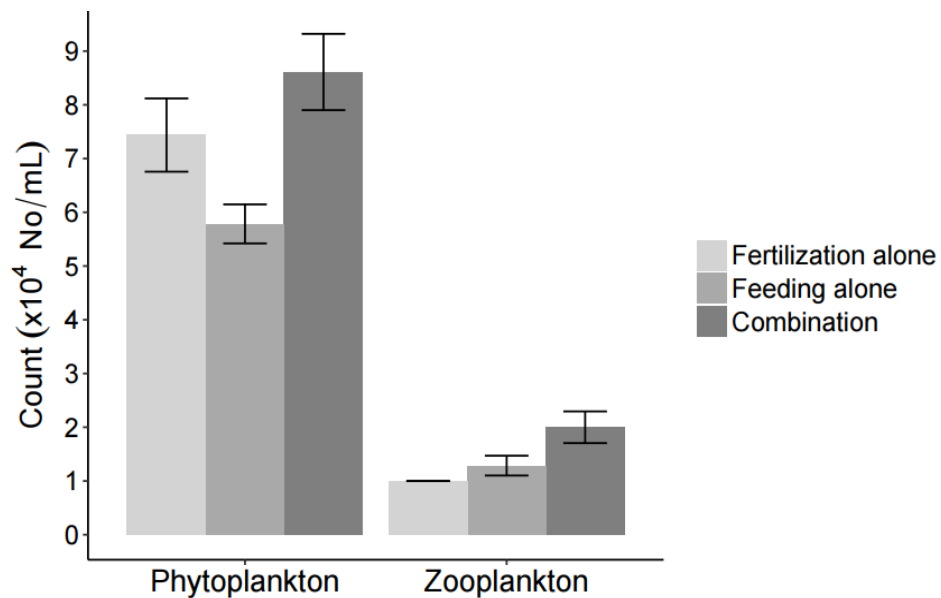
**3.3.2 Periphyton species community composition and abundance**

Results for community composition and abundance of periphyton species from ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding are shown in Fig. 3, Fig. 4 and Fig. 5. The results show that there was significantly higher phytoplankton abundance than zooplankton abundance (Fig. 3). The ponds under the combination of fertilization plus supplementary feeding had higher number of both phytoplankton and zooplankton than the ponds under the other treatments. The ponds under feeding alone treatment showed the lowest phytoplankton abundance while those under fertilization alone treatment had the lowest zooplankton abundance (Fig. 3). The phytoplankton classes found in the experimental ponds, with the number of recorded genera in bracket, were Bacillariophyceae (2), Chlorophyceae (3), Cyanophyceae (9), Euglenophyceae (4) and Zygnematophyceae (1) (Fig. 4). The dominant genera in each class were as follows: *Frustulia* and *Nitzschia spp.* for Bacillariophyceae, *Closterium sp.* for Chlorophyceae, *Anabaene*, *Microcystis* and *Planktothrix spp.* for Cyanophyceae, *Phucus sp.* for Euglenophyceae and *Staurastrum sp.* for Zygnematophyceae. Among the phytoplankton classes in the present study, Cyanophyceae had the highest abundance in all treatments while Zygnematophyceae class was only observed in ponds under the combination of fertilization plus supplementary feeding.

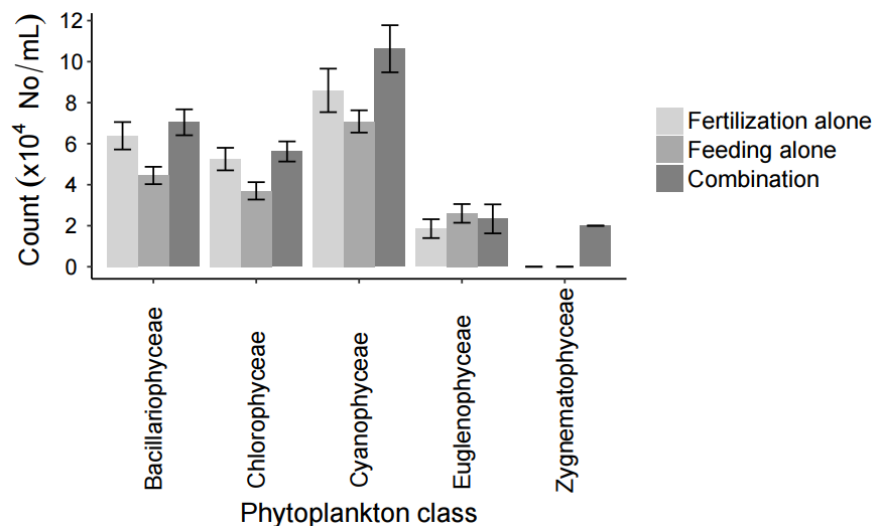
The zooplankton classes observed in the present study, with the number of recorded genera in bracket, were Eurotatoria (2), Heterotrichea (2) and Oligohymenophorea (4) (Fig.5). The dominant genera observed under each class were *Lecane sp.* for Eurotatoria, *Spirostom sp.* for Heterotrichea, *Carchesium* and *Lembadion spp.* for Oligohymenophorea.



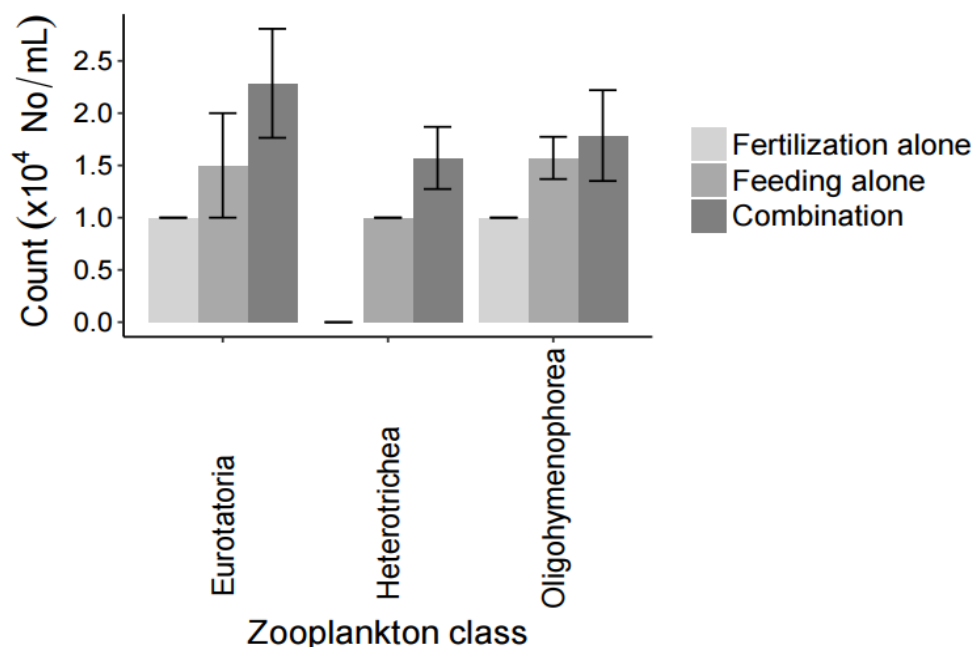
Heterotrichea was observed in ponds under the treatments that included either feeding alone or combination of fertilization plus supplementary feeding. Heterotrichea exhibited significantly higher abundance in ponds under the treatment for combination ( $T_3$ ) than in ponds under feeding alone ( $T_1$ ) (Fig.5). In general, the ponds under the combination of fertilization plus feeding showed the highest abundance of zooplankton, followed by those under feeding alone ( $T_2$ ) and fertilization alone ( $T_1$ ) (Fig. 5).



**Figure 3: Phytoplankton and zooplankton abundance in ponds under fertilization alone, feeding alone and combination of fertilization plus supplementary feeding**



**Figure 4: Phytoplankton abundance in ponds under fertilization alone, feeding alone and combination of fertilization plus supplementary feeding**



**Figure 5: Zooplankton abundance in ponds under fertilization alone, feeding alone and combination of fertilization plus supplementary feeding**

### 3.3.3 Periphyton proximate chemical composition

Table 1 summarises the mean values for proximate chemical composition of periphyton collected from ponds under fertilization alone, feeding alone and combination of fertilization plus feeding. Periphyton from the ponds subjected to the combination of fertilization plus feeding had significantly ( $p < 0.05$ ) higher CP ( $11.40 \pm 0.16\%$ ) and OM ( $25.47 \pm 0.28\%$ ) contents while periphyton from the ponds under fertilization alone had the least CP content ( $8.97 \pm 0.22\%$ ). The difference in OM content of periphyton in the ponds under fertilization alone ( $23.23 \pm 0.33\%$ ) and those from ponds under feeding alone ( $24.78 \pm 0.41\%$ ) was insignificant ( $p > 0.05$ ).

The periphyton ether extract (EE) contents differed significantly ( $p < 0.05$ ) (Table 2) among the treatments. The highest periphyton EE value ( $1.84 \pm 0.07\%$ ) was observed in

periphyton from ponds under fertilization alone ( $T_1$ ) while the lowest value ( $0.97 \pm 0.05\%$ ) was observed in periphyton from ponds under the combination treatment ( $T_3$ ). The highest phosphorous (P) content was observed in the periphyton from ponds under feeding alone and the lowest was found in periphyton from the ponds under fertilization alone ( $T_1$ ) (Table 1).

**Table 1: Proximate chemical composition (Means  $\pm$  se) of periphyton from ponds under fertilization alone, feeding alone and combination of fertilization plus feeding**

Proximate composition	Treatments		
	Fertilization alone ( $T_1$ )	Feeding alone ( $T_2$ )	Combination ( $T_3$ )
DM (%)	$96.27 \pm 0.06^a$	$96.37 \pm 0.08^a$	$95.38 \pm 0.10^b$
CP (%)	$8.97 \pm 0.22^c$	$10.68 \pm 0.25^b$	$11.40 \pm 0.16^a$
OM (%)	$23.23 \pm 0.33^b$	$24.78 \pm 0.41^b$	$25.47 \pm 0.28^a$
EE (%)	$1.84 \pm 0.07^a$	$1.43 \pm 0.08^b$	$0.97 \pm 0.05^c$
P (mg/L)	$0.35 \pm 0.01^c$	$0.48 \pm 0.0^a$	$0.41 \pm 0.0^b$

\* <sup>abc</sup> = Means with the same superscript letter in the same row are not significantly different ( $p > 0.05$ ).

### 3.3.4 Relationship between periphyton quantity, quality and fish growth rate

Table 2 presents the regression of fish growth rate on periphyton biochemical composition. The results show that the growth rate of fish reared in the ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding was influenced by periphyton biomass and biochemical composition.

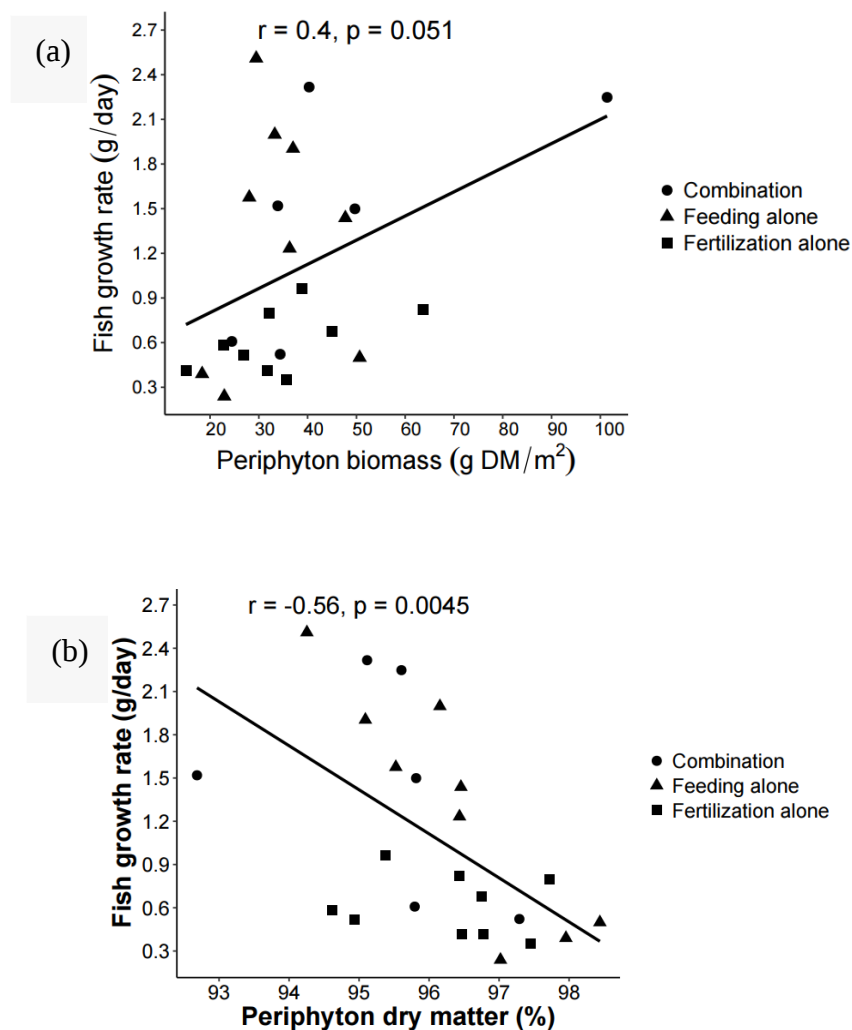
**Table 2: Regression of fish growth rate on periphyton biomass and proximate chemical composition**

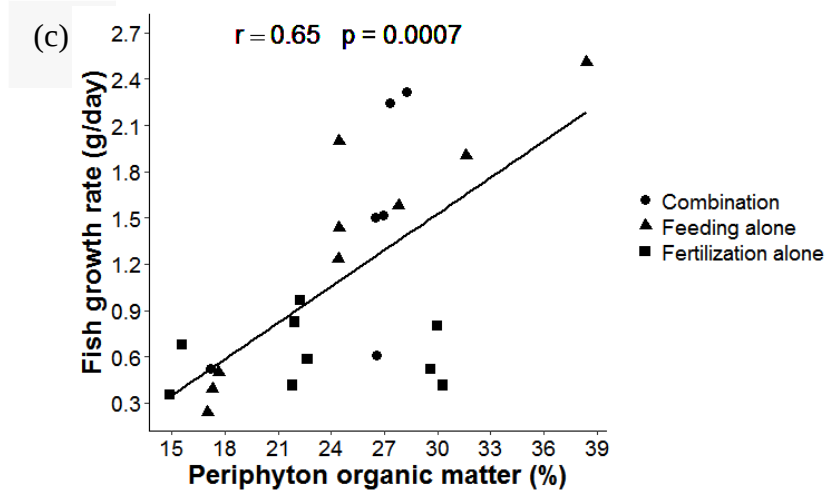
Periphyton variable	Partial coefficient of Regression (b)	se	p -value
Biomass	0.019882	0.003899	<b>0.001</b>
CP	0.110326	0.019560	<b>0.001</b>
EE	-0.025346	0.062457	0.687
Phosphorous	1.518757	0.711005	<b>0.038</b>

Note: CP=Crude protein, EE=Ether extract, se = standard error.

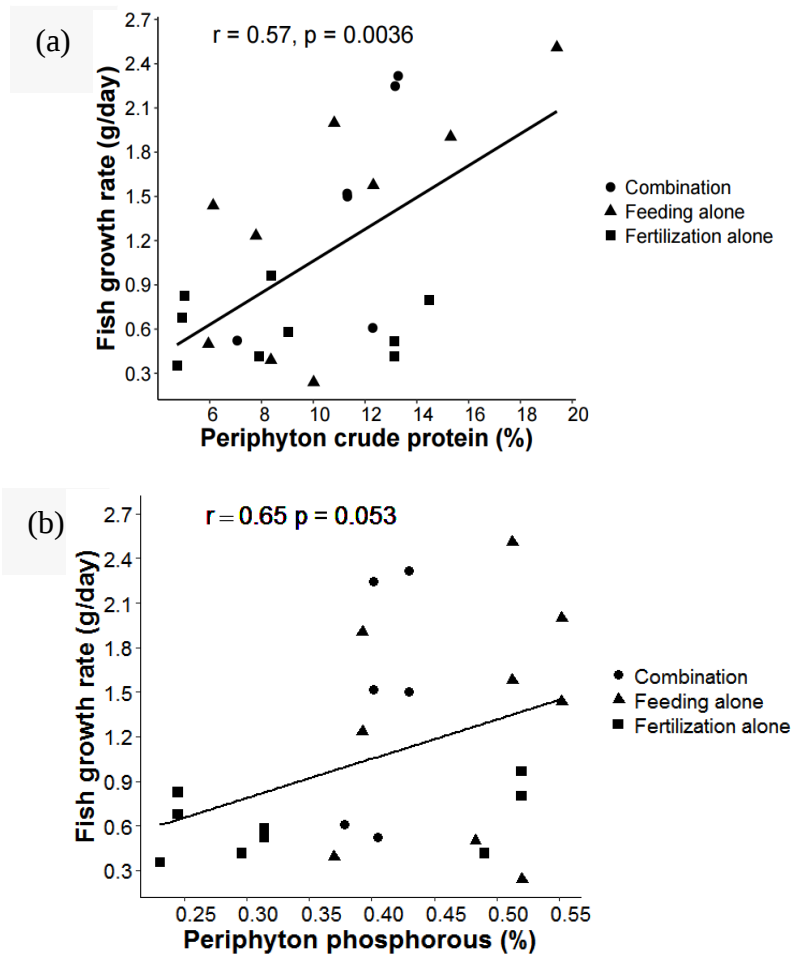
Fish growth rate was significantly ( $p < 0.05$ ) influenced by periphyton biomass, CP and phosphorous contents (Fig. 6 (a), (b) and (c), Fig. 7 (a) and (b) (Table 2).

Fish growth rate was significantly ( $p < 0.05$ ) correlated with periphyton quantity (biomass, dry matter, organic matter) and quality (crude protein and phosphorous). Fish growth rate showed positive correlation with periphyton biomass, organic matter, crude protein and phosphorous, but was negatively correlated with dry matter (Fig. 6 (a), Fig 6 (b), Fig. 6 (c), Fig. 7 (a), Fig. (b)) (Table 2). The influence of the periphyton EE content on the fish growth rate was not significant ( $p = 0.687$ ) (Table 2).





**Figure 6: Relationship between *O. niloticus* growth rate and periphyton quantity (a) Biomass (b) Dry matter and (c) Organic matter**



**Figure 7: Relationship between *O. niloticus* growth rate and periphyton quality (a) Crude protein (b) Phosphorous**

### 3.3.5 Fish body proximate chemical composition

Results for proximate chemical composition of Nile tilapia reared in ponds under fertilization alone, feeding alone and the combination of fertilization plus feeding are shown in Table 3. Fish body proximate chemical composition values differed significantly ( $p < 0.05$ ) among the treatments. Fish grown in ponds under fertilization alone had significantly ( $p < 0.05$ ) lower percentage of DM ( $91.20 \pm 0.59\%$ ), OM ( $95.22 \pm 0.11\%$ ) and CP ( $66.43 \pm 0.45\%$ ) than those from ponds under feeding alone and the combination of fertilization plus supplementary feeding.

**Table 3: Proximate chemical composition (Means  $\pm$  se) of *O. niloticus* cultured in earthen ponds under fertilization alone, feeding alone and the combination of fertilization plus supplementary feeding**

Proximate composition	Treatments		
	Fertilization alone (T <sub>1</sub> )	Feeding alone (T <sub>2</sub> )	Combination(T <sub>3</sub> )
DM (%)	91.20 $\pm$ 0.59 <sup>b</sup>	93.31 $\pm$ 0.75 <sup>a</sup>	94.70 $\pm$ 0.27 <sup>a</sup>
CP (%)	66.43 $\pm$ 0.45 <sup>b</sup>	68.22 $\pm$ 0.35 <sup>a</sup>	69.14 $\pm$ 0.33 <sup>a</sup>
OM (%)	95.22 $\pm$ 0.11 <sup>b</sup>	96.42 $\pm$ 0.23 <sup>a</sup>	96.65 $\pm$ 0.16 <sup>a</sup>
Ash (%)	4.78 $\pm$ 0.11 <sup>a</sup>	3.58 $\pm$ 0.23 <sup>b</sup>	3.35 $\pm$ 0.16 <sup>b</sup>
EE (%)	18.33 $\pm$ 0.19 <sup>a</sup>	16.10 $\pm$ 0.04 <sup>b</sup>	14.47 $\pm$ 0.25 <sup>c</sup>

Means with the same superscript letter in the same row are not significantly different ( $p > 0.05$ ), Note:

DM=Dry matter, CP=Crude protein, EE=Ether Extract, se=standard error.

However, the differences in DM (%), OM (%) and CP (%) contents of fish cultured under feeding alone and combination of fertilization plus supplementary feeding were not significant (Table 3). Furthermore, fish body composition from fertilization alone treatment (T<sub>1</sub>) had significantly ( $p < 0.05$ ) higher ash ( $4.78 \pm 0.11$ ) and ether extract (EE) ( $18.33 \pm 0.19$ ) contents than the fish from the feeding alone (T<sub>2</sub>) and combination of fertilization plus supplementary feeding (T<sub>3</sub>) treatments. The percentage of ash content in fish carcasses did not differ ( $p > 0.05$ ) between the fish cultured under feeding alone ( $3.58 \pm 0.23$ ) and combination of fertilization plus supplementary feeding ( $3.35 \pm 0.16$ ), but EE

(%) content differed significantly between fish under feeding alone and the combination of fertilization plus supplementary feeding (Table 3).

### **3.3.6 Influence of periphyton chemical composition on fish body chemical composition**

Results shown in Table 1 and 3 indicate that, periphyton crude protein and ether extract content influenced fish body crude protein and ether extract. The increase of the periphyton chemical composition (CP and EE) led to the increase of the same chemical composition contents in the fish body.

## **3.4 Discussion**

### **3.4.1 Periphyton biomass**

The ponds under the combination of fertilization plus supplementary feeding had higher periphyton biomass than those under the other treatments. The higher periphyton biomass observed in ponds under the combination of fertilization plus supplementary feeding might be contributed by utilization of nutrients, especially phosphorous and nitrogen from both feeds and fertilizers applied in the ponds, particularly for algae (phytoplankton) bloom (Hietala *et al.*, 2004; Abdel-Tawwab *et al.*, 2007). In most cases phosphorous and nitrogen are limiting nutrients (Sterner and Hessen, 1994; Sterner and Elser, 2002), but very important for promoting algae productivity, high algal biomass implies high primary productivity hence, high fish growth. The application of fertilizer together with the decomposition of uneaten feeds and fish excreta promotes periphyton growth, hence, high biomass production (Gatenby *et al.*, 2003; Abdel-Tawwab *et al.*, 2007; Agwa *et al.*, 2012; Abdel-Warith, 2013; Gilles *et al.*, 2013; Shailender *et al.*, 2013; Tortolero *et al.*, 2016).

### 3.4.2 Periphyton species community composition and abundance

The highest periphyton abundance were recorded in ponds that received both fertilizers and supplementary feed, suggesting that the availability of additional nutrients from the decomposition of leftover feeds might have contributed to the high abundance of plankton (Abdel-Hakim *et al.*, 2013; Abdel-Warith, 2013). Ponds subjected to the treatment of combination of fertilization plus supplementary feeding had higher abundance of Cyanophyceae and Zygnematophyceae than the ponds under the treatment of either feeding alone or fertilization alone. This perhaps was because of the nutrients enrichment from both fertilizer application and decomposed leftover feeds. This is in agreement with the findings reported by Begum *et al.* (2007) and Abdel-Warith (2013) who found that, pond manuring increases the abundance of Cyanophyceae and total phytoplankton abundance than feeding alone.

Furthermore, the high abundance of Cyanophyceae in all treatments in the current study could be due to its tolerance to a wide range of environmental conditions and because of its toxicity. Studies have indicated that fish feed selectively on other algae rather than cyanobacteria (Paerl and Huisman, 2008; However, other studies have shown that Nile tilapia has the capability to select Cyanophyceae as food in addition to Bacillariophyceae, Chlorophyceae and Euglenophyceae (Abdel-Tawwab *et al.*, 2007). In the present study, ponds under feeding alone had less abundance of phytoplankton than the ponds under fertilization. It is well known that, more diversity and higher abundance of phytoplankton is found in the fertilized ponds compared to unfertilized ponds.

Pond fertilization enhances the availability of natural food for fish growth (Zahid *et al.*, 2013; Mosha *et al.*, 2016). The ponds under feeding alone and the combination of fertilization plus supplementary feeding had higher zooplankton abundance than those



under fertilization alone. The high abundance of zooplankton was probably contributed by the presence of uneaten feeds in the pond water (Abdel-Tawwab *et al.*, 2007). Other researchers have shown that the presence of organic fertilizer or matter rather than inorganic increases the availability of zooplankton (Rahman and Rahman, 1999; Mosha *et al.*, 2016). It has been reported that pond water containing the combination of uneaten feeds and inorganic fertilizer has higher zooplankton abundance than those with uneaten feeds or applied with fertilizer alone (Abdel-Warith, 2013).

### **3.4.3 Periphyton proximate chemical composition**

Proximate chemical composition of the periphyton in the present study differed significantly among the treatments. Higher periphyton crude protein (CP %) and organic matter (OM %) contents were observed in the ponds subjected to the combination of inorganic fertilizer application plus supplementary feeding. This probably was influenced by the high abundance of zooplankton in ponds under the combination of fertilization plus feeding. Previous studies have shown that zooplankton are rich in protein while phytoplankton contains more lipid (Kar *et al.*, 2017). The periphyton CP content obtained in this study was within the acceptable range from 9 to 32% CP (Tortolero *et al.*, 2016) while the OM content was lower than the range between 46 and 60% reported by other studies (Leloup *et al.*, 2013). The lower periphyton OM content observed in the present study might be caused by high sand content in the pond water which, in turn, attached together with periphyton on the nets and were collected together with periphyton during sampling. During the experimental period, the pond water had a lot of suspended particles, especially during the rainy seasons and this might have attached to the nets together with periphyton. Low dry matter (DM %) content for periphyton collected from the ponds under the combination of fertilization plus feeding compared to those collected from other treatments may be due to differences in species composition among the treatments. The

lower periphyton DM content may also suggest high moisture content of those periphyton (McDonald *et al.*, 2010). The higher EE content for the periphyton from the ponds under fertilization alone than those from other treatments may be caused by more light penetration due to low turbidity. This is because Secchi disk reading value was high in the ponds under fertilization alone, implying that light penetration was high (Agwa *et al.*, 2012). Light promotes photosynthesis, thereby increases lipid content of the algae as an energy source. Also lipid content can be influenced by pond culture condition (i.e. water quality parameters) (Agwa *et al.*, 2012). Lipid or ether extract content in the present study was slightly lower than the value of 2 - 5% observed in marine algae (Montgomery and Gerking, 1980) and extremely lower than the value of 18% which has been reported in aquaculture periphyton composition (Agwa *et al.*, 2012). This variability might be contributed by differences in species abundance and environmental among the treatments (Narasimman and Murugaiyan, 2012).

Periphyton from the ponds under fertilization alone had lower phosphorous content than those from the other treatments. The lower content of phosphorus, probably, was due to less uptake of phosphorous from the surrounding. The availability of nutrients in the algal cell may be influenced by the quantity of nitrogen (N) and phosphorous (P) in the surrounding environment (Whitton *et al.*, 2016). The source of nutrient in the ponds under fertilization alone was only fertilizer, whereas in ponds under the combination of fertilization plus feeding, nutrients were contributed by both fertilizer and the left over feeds present in the ponds.

#### **3.4.4 Relationships between periphyton quantity, quality and fish growth rate**

Results in the present study show positive and significant relationships between fish growth and periphyton quantity (organic matter and biomass) and quality (crude protein

content). The strong positive relationships between periphyton biomass, CP and OM contents with fish growth rate suggest that biomass, CP and OM are important factors for fish growth. This is because CP is a crucial nutrient responsible for growth and as the percentage of CP increases the growth of the fish increases (McDonald *et al.*, 2010). Studies have shown that fish growth and body composition is reliant on feed composition and availability (Mitra *et al.*, 2007; Job *et al.*, 2015; Simhachalam *et al.*, 2015; Mateen *et al.*, 2016). Moreover, it has been shown that high OM in the periphyton constitutes important nutrients for fish growth (McDonald *et al.*, 2010). The increase in fish growth rate as periphyton phosphorous content increased may be due to the availability of both artificial feeds and/or fertilizer which increased the amount of nutrients (including phosphorus) which is important for algae growth and hence, fish growth. It is well known that fish growth rate is influenced by the amount of phosphorous present in the feed (Job *et al.*, 2015; Simhachalam *et al.*, 2015; Mateen *et al.*, 2016). Presence of phosphorous in the fish tissue enables the formation of energy (ATP) for growth and carrying out different body activities (Paes *et al.*, 2016; Whitton *et al.*, 2016).

#### **3.4.5 Fish body proximate chemical composition**

Crude protein and fat contents are the major nutrients which are used to define the nutritional status of the fish (Oduor-Odete and Kazungu, 2008). Crude protein (CP %) content was higher in fish cultured in ponds under feeding alone and the combination of fertilization plus feeding. The high CP content found in the carcasses of fish cultured in ponds subjected to the treatments which involved feeding of diet is consisted with the findings of previous studies. Previous studies reported that when fish consume more supplementary feeds their body crude protein content also increases because the formulated feed which is offered to the fish has high CP content (Shailender *et al.*, 2013; Zahid *et al.*, 2013). The crude protein content of the feed used in this study is in the range

of 25 - 30% CP that is ideal for tilapia. Also the crude protein contents (CP %) of the fish carcasses for all three treatments fall within the range reported in previous studies elsewhere (Kumar *et al.*, 2011; Kwikiriza *et al.*, 2017). Fish body lipid or ether extract (EE) content significantly differed among the treatments, with the highest value being observed in fish cultured in ponds under fertilization alone. The high lipid content in the fish carcasses may be due to the high consumption of phytoplankton and zooplankton. Studies have shown that high consumption of natural food (plankton) may result into high lipid content in the fish tissue (Shailender *et al.*, 2013; Zahid *et al.*, 2013). The range of the fat content values observed in the present study is similar to the values of fat content which have been obtained in Nile tilapia cultured under pond system (Abdel-Hakim and Ammar, 2009; Olopade *et al.*, 2016).

Fish raised in ponds under fertilization alone had higher ash content than the fish in other treatments. The ash content of the fish body is influenced by the type of the feed used and minerals availability in the water column (Jabir *et al.*, 2012; Kwikiriza *et al.*, 2017). In the current experiment fish reared under fertilization alone solely depended on natural food available in the ponds, which probably consisted of higher proportion of minerals, especially during the rain seasons where most of the ponds had a lot of suspended organic matter. Ash content of the *O. niloticus* flesh obtained in this study falls within the range of 1.76 - 3.83% reported by Jim *et al.* (2017) and Olopade *et al.* (2016) for tilapia species.

#### **3.4.6 Influence of periphyton chemical composition on fish body chemical composition**

Results have shown that, periphyton chemical composition (EE and CP contents) influenced fish body chemical composition (EE and CP contents) since there was a positive correlation between periphyton and fish chemical compositions. Previous studies have shown that fish growth and body biochemical composition may be influenced by

chemical composition of the feed fed to the fish (Shailender *et al.*, 2013; Zahid *et al.*, 2013). In addition, it is reported that the body of fish which consume natural food accumulates more fat while those fed supplementary feed accumulates more crude protein (Shailender *et al.*, 2013; Zahid *et al.*, 2013).

### **3.5 Conclusions and Recommendations**

From the results it can be concluded that, the ponds under combination of weekly fertilization plus supplementary feeding at 2.5% of fish body weight had the highest periphyton quantity (biomass, organic matter), quality (crude protein) and species abundance. The high quantity and quality of periphyton promoted high fish growth rate in ponds under the combination of fertilization plus feeding. The fish body proximate chemical composition was positively correlated with the periphyton proximate chemical composition.

It is recommended that further study be done in different locations and seasons and essential amino acid and fatty acid composition of both periphyton and fish should be analyzed.

### **3.6 Acknowledgement**

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## CHAPTER FOUR

### 4.0 CONCLUSIONS AND RECOMMENDATIONS

From the results of this study, which evaluated the growth performance of sex-reversed *Oreochromis niloticus* cultured in ponds subjected to inorganic fertilizer application alone, feeding alone and the combination of inorganic fertilizer application plus supplementary feeding, the following conclusions can be drawn:

- i. Fish cultured in ponds under the combination of weekly fertilization plus supplementary feeding at 2.5% of body weight have higher final weight, growth rate and gross margin compared to the fish cultured in ponds under either fertilization alone or feeding alone.
- ii. Fish cultured in ponds under the combination of weekly fertilization plus supplementary feeding at 2.5% of body weight have higher feed utilization efficiency than fish cultured in ponds under feeding alone.
- iii. The periphyton from the ponds under the combination of fertilization plus feeding have higher biomass, CP, OM and species composition and abundance compared to the periphyton from the ponds under feeding alone and fertilization alone.
- iv. Combination of weekly fertilization of ponds and supplementary feeding at 2.5% did not affect water physico-chemical parameters beyond the acceptable ranges for Nile tilapia growth.
- v. Diurnal (24 hours) DO, pH and temperature values in ponds water are lowest at 0600 hours and attain peak at 1500 hours.
- vi. Periphyton biomass, Organic matter, crude protein and phosphorous contents influenced fish growth rate and body composition.

Therefore, the combination of weekly pond fertilization plus supplementary feeding at 2.5 % of the fish body weight is the best feeding strategy and can be used by smallholder, farmers since it results in higher growth rate and economic return. Further study on ponds subjected to a combination of fertilization plus supplementary feeding treatment is recommended with the use of floating pelleted feeds at different feeding levels. Further, assessment of periphyton species composition, essential amino acid and fatty acid contents has to be conducted during different seasons (Dry and wet). Also, it is recommended to undertake investigation of fish selectivity on natural food species (phytoplankton and zooplankton) in order to know the preferred species and nutritive values of natural food.



## APPENDICES

**Appendix 1: Plates showing ponds layout, feeding and fish harvesting process at the end of the experiment**



**Plate 1: Pond layout**



**Plate 2: Feeding process**



**Plate 3: Total harvest at the end of the experiment**



**Plate 4: Counting of harvested fish for determination of yield and economic analysis**