

**EFFECTS OF FEED ADDITIVES (PREMIX) ON GROWTH  
PERFORMANCE OF *Oreochromis niloticus* (L, 1758) IN  
CONCRETE POND, SEBETA ETHIOPIA**

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**Abstract**

*Lack of suitable formulated diets for *O. niloticus* production in Ethiopia has contributed to the low development of aquaculture production. To address this problem, two iso-nitrogenous (29 % protein) diets were formulated and their effect on the growth performance of *O. niloticus* in unfertilized concrete ponds was evaluated. One of the formulated diets contained vitamin and mineral premix while the other did not. There were significant differences in growth rates and feed conversion ratios between the fish species on the two diets. Fish fed on the feed with premix grew faster ( $p < 0.05$ ) than the fish on the feed with no premix. The reason for the better growth performance of fish fed on the feed with premix can be explained by better metabolic activities, better health and a balanced diet as compared to the fish fed on feed with no premix. Fish fed on formulated feed with premix gave the best economic return.*

**Key words/phrases:** *Oreochromis niloticus*, minerals, premix, vitamins

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## Introduction

Nile tilapia (*Oreochromis niloticus*), which occurs throughout the country, is one of the most dominant and popular indigenous fish species in sub-Saharan water bodies and best candidate species for aquaculture, due to good adaptive abilities, fast growth rate, growth in adverse conditions, diverse feeding habit and preference by the consumers (Litiet *al.*, 2006; KassahunAsaminewet *al.*, 2012; ZenebeTadesseet *al.*, 2012). It is also cultured in fresh and saline waters and in any climatic zones (Lim and Webster, 2006). This fish has been reared for subsistence or commercial purposes and most of the cultural practices are semi-intensive due to its flexible feeding habit and the ability to use more diversified feed resources (El-Sayed, 2006).

Feeding of fish is the main activity in aquaculture production systems and accounts for 40-50% of the total production costs in semi-intensive culture systems (Craig and Helfrich, 2002; FAO, 2006). To develop aquaculture, search for cheaper and locally available feedstuff is necessary. Industrial and agricultural by-products have been used as sources of suitable and cheap feed (FAO, 2006; Munguti *et al.*, 2006, KassahunAsaminewet *al.*, 2012). The production of fish increases by improving feeding quality and health of the fish through supplementing formulated protein rich feed stuffs with vitamins and minerals premix (Lim and Webster, 2006).

Vitamins and minerals are needed in small amounts for normal growth, better health and metabolic activities (Carmen and Geoff, 2007). In Ethiopia, only limited studies have been conducted on the effect of fish feeds based on agro-industrial byproducts on the growth performance of fish

(Kassahun Asaminew *et al.*, 2012; Zenebe Tadesse *et al.*, 2012). However, all these attempts failed to include vitamins and minerals in the formulated fish feed. The present work tried to evaluate the effect of locally formulated fish feed based on agro-industrial byproducts plus vitamins and minerals on the growth performance of *O. niloticus*.

## **Materials and methods**

The study was conducted at National Fisheries and other Aquatic Life Research Center, Sebeta, Ethiopia (8°55.076'N; 38°38.161'E), located 24 km south-west of Addis Ababa, at an altitude of 2240 m above sea level. Two iso-nitrogenous (29 %) diets were formulated from brewery waste, noug cake and wheat bran. The formulated feeds were fed in pelleted form using 2 mm mesh size sieve for the first four weeks and 3 mm mesh size sieve for the later six weeks. Amount of inclusions and proximate compositions of the ingredients of the two tested feed types are shown on table 1.

Table 1. Ingredient and proximate composition (dry weight basis) of locally available feeds.

<b>Ingredient composition</b>	<b>Test diets</b>	
	<b>With premix</b>	<b>Without premix</b>
Brewery waste	35	40
Noug cake	54	51
Wheat bran	9	9
Premix	2	-
Total	100	100
Proximate composition (g per 100 g)		
Dietary protein levels	29	29
Lipid	4.8	5
Crude fiber	22	22

One of the formulated diets was supplemented with commercial (Deutsche Vilomix GmbH) vitamin and mineral premix at 2 % inclusion level (Chapman, 1992). The premix had a composition of different vitamins and minerals (Table 2).

Partitioned concrete ponds (25m<sup>2</sup>) were dried and limed for two weeks and filled with water before the experiment was carried out. After two weeks of conditioning of the ponds, *O. niloticus* fingerlings with mean weight range of 16.7-17 g were stocked with a stocking density of 2 fish m<sup>2</sup>. The duration of the experiment was from November 2013 to mid-March 2014. The fish were fed at 5 % of their body weight daily (half of the feed at 10:00 am and the remaining half at 4:00 pm).

**Table 2.** Vitamins and mineral composition of the premix (per kg of premix).

<b>Vitamins</b>	<b>Content</b>	<b>Minerals</b>	<b>Content</b>
A	4000 000 IU	Iron	25 000 mg
E	40 000 mg	Copper	1200 mg
D3	400 000 IU	Manganese	4000 mg
B1	3000 mg	Zinc	6000 mg
B2	4000 mg	Iodine	400 mg
B6	3000 mg	Selenium	20 mg
B12	8000 µg	Cobalt	200 mg
K3	1200 mg		
Nicotinic acid	30 000 mg		
ca-pantothenate	10 000 mg		
p-amino benzoic folic acid	20 000 mg 1000 mg		
Biotin	300 000 µg		
C	50 000 mg		

A sample of 50 % of the fish stocked was measured every two weeks using seine net to collect data on length-weight increment. Feed was adjusted based on the bi-weekly weight gain. At the end of the experiment, all fish were counted and length-weight data were taken.

Water temperature, dissolved oxygen (Do), pH and conductivity of the experimental ponds were measured three times a day (morning, noon and in the afternoon) using multi-line probe. Water transparency was measured using a black and white secchi-disk. To get information on the natural food conditions of the experimental ponds, chlorophyll A was analyzed using photo spectrometer. Nutrients like phosphorus and ammonia were also analyzed in the laboratory following standard procedures. Total phosphorus were determined by first digesting the unfiltered sample using potassium-peroxodisulphate. A sample of 50 ml was kept into the Schott-bottles and

then 1 ml of hot K-peroxodisulphate solution (the digesting reagent) was added immediately after its preparation.

The digesting reagent was prepared by dissolving 12 grams of K-peroxodisulphate in 100 ml of distilled water, eventually heating to dissolve without boiling the solution. A blank with only the distilled water together with 1 ml of the digesting reagent was also prepared. These bottles together with the samples were weighed before and after autoclaving at 120 °C for 50 minutes to transfer bound phosphorus into orthophosphate (SRP). After autoclaving, the bottles were re-weighed and the weight loss after autoclaving corrected by adding distilled water. The phosphorus available was determined by following the test for the soluble reactive phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ).

**Reagent:**

Potassium persulphate  $\text{K}_2\text{S}_2\text{O}_8$ , 12 g of  $\text{K}_2\text{S}_2\text{O}_8$  was dissolved in 100 ml of hot distilled water.

$\text{NH}_3\text{-N}$  was determined by using the Indo-Phenol blue method. A stock solution of  $1\text{ g L}^{-1}\text{NH}_3\text{-N}$  was prepared by dissolving 3.819 g  $\text{NH}_4\text{CL}$  in 1000 ml of distilled water. Intermediate solution of  $10\text{ mg L}^{-1}$  was prepared by diluting 10 ml of stock solution to 1000 ml of distilled water. Working solution of  $250\text{ }\mu\text{g L}^{-1}$  was prepared by diluting 25 ml of intermediate solution into 1000 ml of distilled water. A volume of 25 ml of sample was used.

**Reagents:**

A) Sodium salicylate solution:

130 g Sodium-Salicylate and 130 g of Trisodiumcitrat-Dihydrat was dissolved in 800 ml of distilled water, then 0.97 g of sodium nitropruside was added and the volume made to 1L.

B) Hypochlorid solution:

32 g of NaOH was dissolved in 1000 ml of distilled water just before use. Then 0.2 g Sodium dichloroisocyanurat was dissolved in 100 ml of the base (reagent B).

2.5 ml of reagent A were added to 25 ml of filtered sample and standard series. After shaking, 2.5 ml of reagent B were immediately added. Eventually the samples were stored in dark for 1.5h at 25°C for color development, and then the absorbance was measured at 655nm.

Plankton were also sampled using plankton nets (30µm for phytoplankton and 50 µm for zooplanktons) and identified by using binocular microscope.

**Statistical Analysis**

Two-way ANOVA was used to test for the presence of significant differences and Duncan's multiple range test was used to identify which means were significantly different from each other. Differences were considered significant at  $p < 0.05$ . All data were analyzed using the SPSS version 18.

## Results and Discussion

### Physico-chemical Parameters

Means and standard deviations of physico-chemical parameters measured during the study period are presented on Table 3.

The minimum and maximum temperatures of the experimental units during the beginning of the experiment in November/December were 16.8 °C in the morning and 20.4 °C in late afternoon. Low water temperature is expected during those months as the regular cool season occurs from October to December in most parts of Ethiopia. However, there was an increasing trend in water temperature after December where the minimum and maximum were 20.4 °C and 24 °C. Temperature of a water body has significant impact on chemical and biological features of the aquatic system.

**Table 3.** Physico-chemical values (mean  $\pm$  SD) of the experimental ponds.

Parameters	With premix	Without premix
Temperature ( °C)	20.4 $\pm$ 0.09 <sup>a</sup>	20.0 $\pm$ 0.13 <sup>a</sup>
Oxygen (mg l <sup>-1</sup> )	12.7 $\pm$ 0.4 <sup>a</sup>	9.4 $\pm$ 0.37 <sup>a</sup>
pH	7 $\pm$ 0.10 <sup>a</sup>	6.8 $\pm$ 0.14 <sup>a</sup>
Conductivity( $\mu$ s cm <sup>-1</sup> )	185.7 $\pm$ 1.98 <sup>a</sup>	182.9 $\pm$ 1.63 <sup>a</sup>
Ammonia- nitrogen (mg l <sup>-1</sup> )	0.12 $\pm$ 0.05 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>a</sup>
Total phosphorus (mg l <sup>-1</sup> )	0.12 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.02 <sup>a</sup>
Secchi depth (cm)	46.9 $\pm$ 3.0 <sup>a</sup>	46.9 $\pm$ 3.0 <sup>a</sup>
Chlorophyll-a ( $\mu$ g L <sup>-1</sup> )	23.4 $\pm$ 3.9	18.2 $\pm$ 2



According to Kassaye Balkew (2012), at lower water temperature or below the critical level, fish could stop feeding and would even die. The metabolic activity and physiological functions of aquatic animals (e.g. feed utilization, feed conversion, growth rates) can be affected by the water temperature (Halver and Hardy, 2002; Azaza *et al.*, 2008; Kassaye Balkew, 2012; Zenebe Tadesse *et al.*, 2012). This was evident from our result where fish growth rate increases as water temperature increases (Fig. 1).

Temperature has also indirect effect on the survival and growth of fish. According to Wetzel (2001), the solubility of dissolved oxygen (DO) which is essential to all forms of aquatic life depends on temperature, pressure and altitude. Dissolved oxygen (DO) concentration in the pond water at three different times of the day ranged between 6 and 14.5 mg l<sup>-1</sup>. DO concentration was higher during the relatively low temperature regime in November-December and started to decline as the temperature increased. The DO concentration was relatively higher when compared to other similar studies (Litiet *et al.*, 2005; Kassaye Balkew, 2012). This could be due to algal growth which had created low grazing pressure on fish utilizing commercial feeds.

The nutrients analyzed in this study were total phosphorus (TP) and ammonia-N. The reason behind measuring these two parameters was because the TP is essential in the algal biomass production, which in turn avails natural food to the fish. On the other hand, knowing the concentration of ammonia in water is important as it is toxic to aquatic life. For example, fish cannot survive when ammonia in unionized form is high in pond water (El-Shafai *et al.*, 2004; Brook Lemma, 2008). However, the concentration of TP and ammonia-N in the present study were 130 and 140

$\mu\text{g L}^{-1}$ , respectively. The concentration of ammonia was within the range that cannot lead to toxicity. This could be due to the fact that the supplemental feed was given in a suspended net plate where the fish can utilize and no feed leftovers could settle down into the bottom to form ammonia. The other probable reason could be the suspended net plate was on the surface of the well oxygenated part of the water column and hence ammonia formation was not fast.

Data on economic performance is shown in Table 4. Both diets indicated positive returns. But diets with premix demonstrated significantly ( $P < 0.05$ ) higher net return.

Table 4. Cost-benefit analysis for the dietary treatments

Items	Test diets		
	unit	With premix	Without premix
Gross revenue	Birr	60000 <sup>a</sup>	50340 <sup>b</sup>
Variable cost	Birr	7250 <sup>a</sup>	7025 <sup>a</sup>
Fixed cost	Birr	5000 <sup>a</sup>	5000 <sup>a</sup>
Total cost	Birr	12250 <sup>a</sup>	12025 <sup>a</sup>
Net return	Birr	47750 <sup>a</sup>	38315 <sup>b</sup>

### Biological variables

#### Phytoplankton species composition

A total of 17 phytoplankton species, of which six were green algae, four diatoms, four blue greens, two euglena and one dinophyta, were identified

during the study period (Table 5). The most frequently observed algal species were *Pediastrum*, *Haematococcus* species and *Euglena* species.

**Table 5.** Phytoplankton species identified during the study period (1-4 indicates frequency of occurrence of species: 1=rare, 2 = sporadic, 3 = common, 4 = abundant).

Group	Species	Relative abundance
Chlorophyta	<i>Pediastrum</i>	4
	<i>Haematococcus</i>	3
	<i>Zygnema</i>	3
	<i>Staurastrum</i>	1
	<i>Coelastrum</i>	1
	<i>Scenedesmus</i>	3
Bacillariophyta	<i>Cymbella</i>	1
	<i>Navicula</i>	1
	<i>Nitzschia</i>	1
	<i>Synedra</i>	1
Cyanophyta	<i>Anabaena</i>	3
	<i>Anabaenopsis</i>	2
	<i>Microcystis</i>	1
	<i>Oocystis</i>	1
Dinophyta	<i>Peridinium</i>	1
Euglenophyta	<i>Euglena</i>	4
	<i>Phacus</i>	2

In aquaculture feed trial experiments, one has to consider the natural food conditions in the experimental ponds. *O. niloticus* is capable of using a wide range of food materials such as plankton (phytoplankton and zooplankton) and also grows well on artificial feed. The phytoplankton community in the present study consisted of high number of Chlorophyta and Bacillariophyta species which can be utilized by the fish. The presence of diverse

phytoplankton species in each experimental pond could be due to the less pressure from the fish because of the supplemental feed. The transparency of the pond water secchi depth greater than half of the pond water depth could favor most phytoplankton species to grow. The other reason for such diverse phytoplankton species could be the absence of large filter feeding cladocerans such as *Daphnia* species (Table 6) (Fernando, 1994; Sarma *et al.*, 2005).

Table 6. List of Zooplankton species identified during the study period (1-4 indicates frequency of occurrence of species: 1 =rare, 2 = sporadic, 3 = common, 4 = abundant).

<b>Group</b>	<b>Species</b>	<b>Relative abundance</b>
Copepods	Cyclopoid copepod	3
	Nauplii	4
Cladoceran	<i>Moinamicrura</i>	2
	<i>Diaphanosomaexcisum</i>	3
Rotifera	<i>Asplanchna</i>	3
	<i>Brachionuscalyciflorus</i>	4
	<i>Brachionusplicatilis</i>	4
	<i>Brachionuscaudatus</i>	2
	<i>Brachionusplicatilis</i>	1
	<i>Trichocerca</i>	3
	<i>Hexarthra</i>	1
	<i>Keratellatropica</i>	2
	<i>Keratellaquadrata</i>	2
	<i>Polyarthra</i>	1
<i>Lecane</i>	1	

### **Zooplankton species composition**

Zooplankton species identified from the experimental ponds belong to three groups: Rotifera, Cladocera and Copepod (Table 6). A total of 14 zooplankton species were identified where rotifers being the species rich contributing to greater than 70% of the total zooplankton taxa. On the other hand copepods were represented only by one species and cladocerans by two species.

Rotifers account for three-fourth of the total zooplankton species identified during the study period. Absence of large *Daphnia* species could favor the diversity of rotifers. On the other hand, such zooplankton species composition also reflects typical tropical aspects where rotifers being the diverse taxa (Green, 1993; Fernando, 1994; Adamneh Dagne *et al.*, 2008).

### **Response of *O. niloticus* to feed additives (premix)**

The mean total weight of fish that consumed feed with premix showed better growth performance throughout the experimental period as compared to the fish that were fed on feed without premix (Table 7) (Fig. 1).

**Table 7.** Growth performances of *O. niloticus* fed on premix and without premix dietary treatments.

Parameters	Treatments	
	Fed with premix	Fed without premix
Stocking weight (gm)	17.30 <sup>a</sup>	16.70 <sup>a</sup>
Harvesting weight (gm)	59.20 <sup>a</sup>	46.00 <sup>b</sup>
Gross yield (kg ha <sup>-1</sup> )	4999.81 <sup>a</sup>	4194.86 <sup>b</sup>
Weight gain (gm fish <sup>-1</sup> day <sup>-1</sup> )	0.33 <sup>a</sup>	0.23 <sup>b</sup>
Feed conversion ratio (FCR)	2.60 <sup>a</sup>	4.40 <sup>b</sup>
Weight gain (gm fish <sup>-1</sup> )	41.90 <sup>a</sup>	29.30 <sup>b</sup>

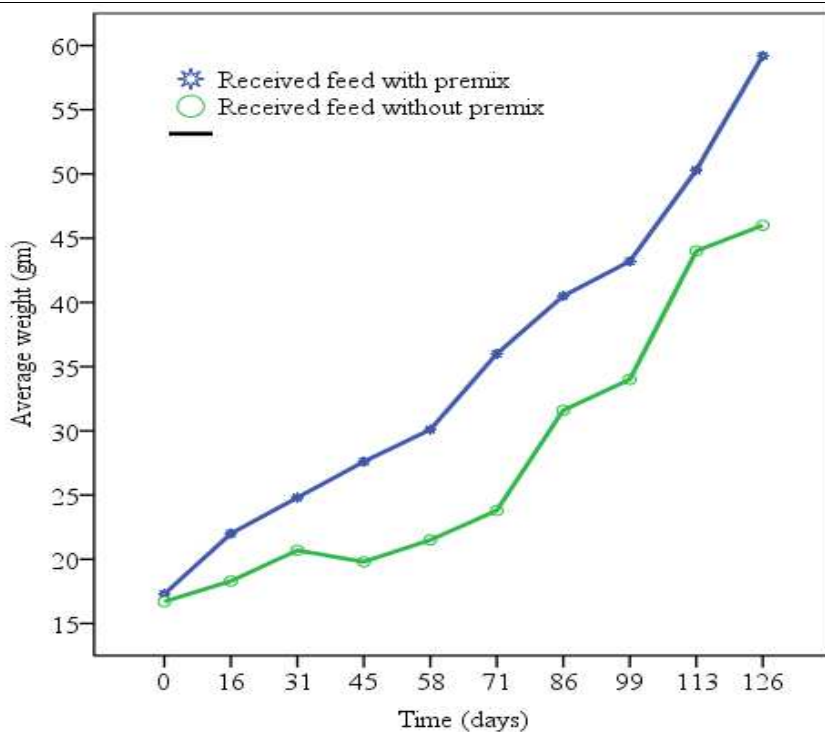


Figure 1. Growth curves of *O. niloticus* for the experimental ponds.

The observed growth differences can be explained due to vitamins and mineral additives (premix) as they are known to contribute for higher metabolic activity, better appetite, protein digestion, lysine oxidation, better health and growth (Pillay and Kutty, 2005; Carmen and Geoff, 2007). The deficiency of such premixes in feed could result in impaired carbohydrate metabolism, poor appetite, muscle atrophy, low hemoglobin and poor growth (Halver and Hardy, 2002; Carmen and Geoff, 2007); thus, the fish fed on formulated feed without premix had poor growth performance. During the culture study period we had also observed that fish consuming feed with premix finish their feed faster than the fish which were supplied feed without premix. In contrast to other studies which were conducted in fertilized ponds (Foteder, 2004; Litiet *al.*, 2005; El-Sayed, 2006; Ispiret *al.*, 2011) we had observed a positive effect of the premix on growth performance. This difference could be due to type of pond used as this study was conducted in unfertilized ponds and thus the culturing pond contained low biomass of natural food, so the fish depended mainly on the supplementary feed.

The growth rate of fish in this study had shown differences in time (Fig. 1). At the beginning of the experiment until the 99 culturing days, the two experimental groups grew slowly. This slow growth rate was due to low water temperature (16.8 - 20.4 °C) of the ponds. Zenebe Tadesse *et al.* (2003) had indicated that water temperature below 20°C can lead to reduced feeding of the fish. The fish relatively grew faster after the 99 days until the end of culturing period. This could be due to an increase in the water temperature (20.4 - 24 °C) which can increase the metabolic activity of the fish. During culturing period social hierarchy and size difference was observed which might be due to use of mixed-sex type in the experiments.

Most of the females were smaller in size and the males were relatively bigger which is also evident from other studies where the growth rate of mixed sex tilapia is lower as compared to male tilapia (AdamnehDagneet *al.*, 2013). This is due to the fact that female *O. niloticus* spent much energy for egg production as compared to males and hence reflected on their size.

Although net returns were positive in both diets, but there was a significant differences in economic returns. Fish fed on formulated feed with premix was the more profitable compared with fish fed on formulated feed without premix.

Generally, supplementation of fish feeds with vitamins and minerals improve the growth rate of *O. niloticus*. But the growth rate of the *O. niloticus* was slower as compared to the other studies conducted in fertilized ponds (Litiet *al.*, 2005 and 2006; Waidbacheret *al.*, 2006; Mohamed, 2009; Padaet *al.*, 2012). This could be because, the low water temperature which coincided with the cold season in Ethiopia and the frequency of fish (every two weeks) sampling which could stress and affect the growth rate of the fish (Halver and Hardy, 2002; Azaza *et al.*, 2008; KassayeBalkew, 2012; ZenebeTadesseet *al.*, 2012).

## **Conclusions**

The present study had shown that supplementation of formulated agro-industrial by-products with vitamins and minerals resulted in better growth performance of *O. niloticus* in unfertilized ponds and also better economic return. Further studies should focus on testing the premix under different



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conditions, like in fertilized ponds, use of single-sex fish and under different agro-ecological conditions.

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