

**Research Article**



SOI: <http://s-o-i.org/1.15/ijarbs-2-11-20>

**Use of eco enzymes in Tilapia diets: effects of growth performance and carcass composition**

**Servais Djromahouton TOKPOHOZIN<sup>1</sup>, Jean FALL\*, Abdoulaye LOUM, Mariama SAGNE, Malick DIOUF**

Institut Universitaire de Pêche et d'Aquaculture (IUPA) Université Cheikh Anta Diop UCAD II  
batiment pédagogique/Rez de chaussée BP 5005 DAKAR

\*Corresponding author: [kagoshima77@yahoo.com](mailto:kagoshima77@yahoo.com)

**Abstract**

Aquaculture has developed through improved farming techniques, food quality and availability at lower cost. Through the development of new approaches to improving food, eco-enzymes have been incorporated into the Nile Tilapia feed (*Oreochromis niloticus*) to evaluate their effects. The objectives are the valuation of plant residues, the production of good quality feed and low cost, and the analysis of the effects of eco-enzymes on Nile tilapia growth and survival, and improved digestibility. The completion of this study was to produce eco-enzymes; then to make five (5) diets A, B, C, D, and E iso-protein (30%) containing respectively 0, 2.5, 5, 7.5 and 10% eco-enzymes. The experiment lasted two (2) months and was conducted in an isolated system consisting of 5 treatments with 2 repetitions on tilapia fingerlings of  $4.54 \pm 0.3$  g fed twice daily. Every two weeks fish was weighed to monitor trends. Samples of fish dorsal muscle before and after experiment were made for carcass composition analysis. The results showed that the diet C exhibited better weight gain 151.23% compared to the control 127.75%; better TCS 1.67% / d compared to control 1.50% / d and increased TCA is 3.68 against 4.12 in control. Moreover, the results showed that the diet D had better survival 90% against 45% in control. In short, eco-enzymes have played an important role in improving diet, tilapia growth and survival. Increasing the amino acid profile can do improving the nutritional quality of the food perspective. Also, eco-enzymes could be used to strengthen the immune system of Nile tilapia.

**Keywords:** Eco Enzymes, Tilapia, Diets, Growth Performance

**Introduction**

The world production of fish feed was estimated between 18.7 and 30.7 million tons in 2006. In 2008, 708 million tons of industrial feed for animal diet were produced all over the world, including 29.2 million tons of fish feed (4.1% of the total production of animal feed) (FAO, 2012).

African production, intended for domestic market purposes also increased, with 430,000 tons of farmed tilapia produced in 2008, twice more than in 2000. Egypt is the second largest producer in the world and the first in the African continent (SYPAGUA, 2014).

In Senegal, Nile tilapia production evolved between 1983 and 2009. Its production from aquaculture in Senegal started in 1983 and never reached more than 100 tons per year except in 1999 during which period the production was estimated at 105 tons. Between 2000 and 2014, production of Nile tilapia reached 1090 tons due to private investments, the development of ponds and institutional support from the Government of Senegal through the National Aquaculture Agency (NAA). Because of huge potentials available in relation with the favorable climate, tilapia could have major impacts on aquaculture development in the country (APIX, 2013).

Despite the increase in production of fish feed, the lack of efficient, cheap and available feed on the market remains a serious problem in the farming of aquatic animals. Moreover, there are risks of contamination of these feeds if they are not well preserved.

The need for good quality feed ingredients with improved nutritional value, economic viability, and growing awareness of the environment has led to a rise in the use of exogenous enzymes in the diet of fish and shrimp in recent years. Today, most enzymes are used to improve digestibility of phosphorus and carbohydrates from plant protein sources (Chowdhury, 2014).

In addition, some solutions composed only of enzymes are produced and applied in fish farming in particular in the treatment of water. This is the case of the eco-enzymes or garbage enzymes produced from fresh wastes of plants.

Eco-enzymes are used in many areas for their beneficial effects including the environment, agriculture, livestock, households and Aquaculture. During the production of eco-enzymes, catalase process generates ozone (O<sub>3</sub>), which promotes the CO<sub>2</sub> reduction in the atmosphere and can trap heavy metals in the cloud clusters while reducing thus the effect of global warming. At the same time, nitrate (NO<sub>3</sub>) and carbonate (CO<sub>3</sub>) are formed to improve soil fertility and natural plants. Furthermore, they are used to purify the environment. Enzymes contained in the solution neutralize toxins and other pollutants from rivers, soils and atmospheres. Eco-enzymes are also used to disinfect water on farms; as food supplements in animals and to reduce odors from farms.

International aqua feed (2012) reports that such enzymes have the ability to stabilize the soil organic matter and can be effectively used to ensure the quality of soil and farming conditions of aquatic species. The mixture that contains the variety of enzymes can be effective means for bioremediation in aquaculture. Eco-enzymes have been used to accelerate the degradation of organic matter (feces, uneaten feed and dead algae), destroy the deposition of particles and reduce deposit accumulation, reduce the content of solids, decompose plant debris; reduce anaerobic conditions depths of the pond, promote the degradation of some complex nutrients and facilitate high nutrient digestibility.

The expansion of global aquaculture production increased the demand for aquaculture feed. Fishmeal is the main and important ingredient in the production of aquaculture feed. The rising cost of fishmeal made manufacturers find sources of protein such as low-cost vegetable protein. However, the acceptability of many plants is poor; anti-nutrients are most concerned in the complete replacement of fishmeal in feed formulations. Indeed, the anti-nutritional factors have negative impact on the digestion of food and efficiency. There are several types of anti-nutritional factors associated with the increased use of vegetable such as trypsin inhibitory protein, glucosinolates and phytate.

Enzymes provide action that can inactivate anti-nutritional factors and enhance the nutritional value of plant proteins in diets. They provide a natural way to transform complex food compounds into absorbable nutrients. Endogenous enzymes found in the digestive system of fish help the catalysis of large organic molecules such as starch, cellulose and protein in simple substances. The addition of enzymes in food can improve the utilization of nutrients, reducing then the feed cost and excretion of nutrients in the environment (Felix et al., 2004).

As a matter of fact, the objective of the study was to evaluate the nutritional value of diets supplemented with garbage enzymes with respect to weight gain, survival and carcass composition in a feeding trial with Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758).

## Materials and Methods

### Ingredients used to produce Eco-enzymes

Molasses is a byproduct from the processing of sugar cane or beet; it contains 35% of sucrose and 20% of various sugars. It is very rich in minerals, especially potassium, which is the main limiting factor at a maximum rate of 10 to 15% (Ngom, 2004). It was obtained through the ANA (National Aquaculture Agency); the plant residues consist primarily of fresh organic matter. The water used does not contain any chlorine.

### Production of eco-enzymes

Various ingredients, above mentioned, are weighed into the following proportions: 1 molasses, 3 for plant residues, and 10 to water. It involves four steps. Eco-enzymes were made through the mixture of plant

residues already rinsed with dissolve molasses in the plastic bottle filled with water, then homogenized. Finally the sealed bottle was kept for at least 3 months for fermentation, and from time to time the seal was

opened to release gazes. After three months, the solution was filtered and kept at room temperature before usage.

Table 1: composition of main ingredients used in this experiment

Ingredients	Dry matter	Protein	Lipid	Fiber	Ash
Fish meal	93.2	56	10.5	0.5	0.8
Sorghum meal	87.9	9.3	2.7	2.5	1.6
Maize meal	67.3	10.4	4.5	2.3	1.5

### Diets preparation

To evaluate the effects of eco enzymes on the growth of fry tilapia (*O. niloticus*) five iso-protein experimental diets (30%), which differ in the degree of incorporation of eco-enzymes (A: 0%, B: 2.5%, C: 5%, D: 7.5% and E: 10%) were formulated (Table 1). Mineral and vitamin premix were purchased from Aquavet feed Company, Thiès, Sénégal. After all,

ingredients were thoroughly mixed and an appropriate quantity of water provided (30% for 100 g of mixed ingredients), accordingly. Diets were supplemented with 5% of mixture of fish oil (FO) (Table 2). Dough was passed through an extruder to produce spaghetti and dried at 37°C for two days. So, the concerned dried diet was packaged into plastic bag and stored frozen until its usage.

Table 2: composition of experimental diets for *O. niloticus*

Ingredients	Treatments				
	A	B	C	D	E
Sorghum meal	20	20	20	20	20
Fish Oil	5	5	5	5	5
Carboxyméthyl cellulose	1	1	1	1	1
Vitmix <sup>a</sup>	2	2	2	2	2
Min mix <sup>b</sup>	2	2	2	2	2
Fishmeal	46	46	46	46	46
Maize meal	24	24	24	24	24
Additive garbage enzymes	0	2.5	5	7.5	10

<sup>a</sup>= vit A 250000 UI; vit D3 250000UI; vit E 5000mg ; vit B1 100mg ; vit B2 400mg ; vit B3(pp) 1000mg ; vit B5 pantode Ca2000mg ; vit B6 300mg ; vit K3 1000g ; vit C 5000mg ; H biotin 15mg ; choline 100g ; anti-oxydant (BHT), crushed and calcined attapulgateqs 1000mg;;

<sup>b</sup>= phosphorus 7% ; calcium 17% ; sodium 1,5% ; potassium 4,6% ; magnesium 7,5% ; manganese 738mg ; zinc 3000mg ; iron 4000mg ; copper 750mg ; iodine 5mg ; cobalt 208mg ; calcined and ground attapulgateqs 1000g; fluorine 1.5% (approximately).

### Culture conditions

Tilapia fingerlings (*O. niloticus*) male and female of the age of two months with an initial mean weight of  $4.54 \pm 0.3$  g were supplied from the Fish Culture Station Richard Toll, Saint-Louis Senegal. Fish were acclimated to experimental conditions in a FRP tank which capacity is estimated at 800L (200cm x 80cm x 50cm) for a two weeks period. During this specific period, they were fed with commercial diet imported from China.

At the beginning of the experiment, 100 tilapia fry were randomly divided into five different groups with two replicates containing 10 fish/each. Fish were kept in 10 glass tank (50 x 40 x 30 cm) containers (50 L). Each aquarium was part of a closed re-circulating system maintained at  $28 \pm 1^\circ\text{C}$ . An air stone continuously aerated each aquarium. All aquaria were cleaned every day in mornings and afternoons by siphoning off accumulated waste materials.

Fish were then fed with 10% of body weight per day and gradually decreased to 4% per day. Each diet was fed twice a day at 08:00 (a.m.) and 5:00 (p.m.) for 42 days to duplicate groups of fish. On the other hand, each group of such fish was weighed in the beginning and every two weeks and the amount of diet fed was adjusted, accordingly. A photoperiod of 12 h light, 12 h dark (08:00 (a.m.)-08:00 (p.m)) was used, while fluorescent ceiling lights supplied the illumination. After 8 weeks of feeding, fish were taken out from each treatment; the dorsal muscle tissue of each was dissected and used for carcass composition analysis purposes.

### Proximate analysis of diet and dorsal muscle

The experimental diets and samples of the dorsal muscle were analyzed for proximate composition in the laboratory of Food Science Department of Animal Production of the following ENSA, Thiès based on AOAC (1984) methods.

### Fatty Acids analysis

Lipid was extracted from feed samples by homogenization in chloroform / methanol (2:1, v/v) containing 0.01% butylatedhydroxytoluene (BHT) as antioxidant, according to the methods of Folch et al. (1957). Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids (Shantha and Ackman 1990). The FA composition was analyzed by a gas chromatograph (Auto System XL Perkin Elmer) using a 30 x 0.25 mm capillary column (FID detector CP- 2380 Supelco, Bellefonte, USA). The conditions of the method were: carrier gas, helium; flame ionization detection temperature, 260°C; split rate: 1 / 50, oven temperature programmed to rise from 120°C / 2 min to 220°C / 15 min at a rate of 5°C /min; injector temperature, 240°C. The identification of the individual methyl esters was achieved by comparison of their retention times with commercial standards (Sigma, St. Louis, MO, USA).

### Amino acids analysis

The amino acid compositions of experimental diets were analyzed following acid hydrolysis using an automatic amino acid analyzer (Hitachi 835-50, Tokyo, Japan) equipped with a column for

physiological fluid analysis by a professional laboratory.

### Growth Parameters

Growth response parameters were calculated as follows: Weight gain (%) =  $100 * ((\text{final mean body weight} - \text{initial mean body weight}) / \text{initial mean body weight})$ ; Specific Growth Rate (SGR, % /day) =  $100 * ((\text{In Wt} - \text{In Wi}) / T)$ , where Wt is the weight of fish at time t, Wi is the weight of fish at time 0 and T is the rearing period in days; Feed Conversion Rate (FCR) = total dry feed fed g/ fish / total wet weight gain g/ fish. Survival rate (%) =  $100 * (\text{number of fish which survived} / \text{initial number of fish})$ .

### Water Quality Measurement

Water temperature and dissolved oxygen were measured each following day using YSI Model 58 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA).

### Statistical Analysis

Data were analyzed using the following statistic system (SAS-PC) (Joyner, 1985) and subjected to one-way analysis of variance (ANOVA). Treatment effects were considered significant at  $P < 0.05$ ; Duncan's test was used to compare significant difference among treatments.

## Results

### Water quality parameters

During the experiment, the mean values of the temperature, dissolved oxygen and pH were 29.06, 7.58 and 7.5 respectively.

### Feed analysis

The results presented in Table 3 show that the proximate composition of the diets is marginally the same proportion in the different compositions. The crude protein level is 30.49%, while the lipid content 11.54% remains high as well.

**Table 3:** Proximate analysis of experimental diets fed tilapia *O. niloticus*

Composition	Treatments				
	A	B	C	D	E
Dry matter (%)	90.90	90.90	90.90	90.90	90.90
Ash (%)	1.96	1.96	1.96	1.96	1.96
Gross energy (MJ/kg)	5.63	5.63	5.63	5.63	5.63
Digestible energy (MJ/kg)	3.70	3.70	3.70	3.70	3.70
Crude protein (%)	30.49	30.49	30.49	30.49	30.49
Digestible protein (%)	1.20	1.20	1.20	1.20	1.20
Lipid (%)	11.54	11.54	11.54	11.54	11.54
Fiber (%)	1.42	1.42	1.42	1.42	1.42

Data presented in Table 4 show the same proportion of fatty acids in different diets. Polyunsaturated fatty acid in the proportions of n-3 and n-6 are respectively 9.2% and 4.8%; and the ratio n3:n6 is 19.2. The proportion of n-3 is higher than that of n-6; therefore

polyunsaturated n-3 fatty acids are richer in diets. In addition, LA (18: 2n-6), EPA (20: 5n-3) and DHA (22: 6n-3) are more important in diets in respective proportions of 4.5, 3.8 and 4.9%.

**Table 4:** Fatty acids profile of the different diets fed to tilapia *O. niloticus*

Fatty acids	A	B	C	D	E
LA (18:2n-6) (%)	4.5	4.5	4.5	4.5	4.5
ALA (18:3n-3) (%)	0.6	0.6	0.6	0.6	0.6
ARA (20:4n-6) (%)	0.3	0.3	0.3	0.3	0.3
EPA (20:5n-3) (%)	3.8	3.8	3.8	3.8	3.8
DHA (22:6n-3) (%)	4.9	4.9	4.9	4.9	4.9
Total n-3 (%)	9.2	9.2	9.2	9.2	9.2
Total n-6 (%)	4.8	4.8	4.8	4.8	4.8
n3:n6	19.2	19.2	19.2	19.2	19.2
Total phospholipide (%)	7.4	7.4	7.4	7.4	7.4
Cholesterol (%)	0.3	0.3	0.3	0.3	0.3

**Table 5:** Amino acids profile of the different diets fed to tilapia *O. niloticus*

Amino acids	A	B	C	D	E	NRC, 2011
Arginine (%)	2.1	2.1	2.1	2.1	2.1	1.2
Histidine (%)	0.5	0.5	0.5	0.5	0.5	1.0
Isoleucine (%)	1.5	1.5	1.5	1.5	1.5	1.0
Leucine (%)	4.2	4.2	4.2	4.2	4.2	1.9
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.6
Methionine (%)	0.5	0.5	0.5	0.5	0.5	0.7
Met+Cys (%)	1.0	1.0	1.0	1.0	1.0	1.0
Phenylalanine (%)	2.0	2.0	2.0	2.0	2.0	1.1
Phe+Tyr (%)	3.4	3.4	3.4	3.4	3.4	1.6
Threonine (%)	1.3	1.3	1.3	1.3	1.3	1.1
Tryptophane (%)	0.4	0.4	0.4	0.4	0.4	0.3
Valine (%)	2.0	2.0	2.0	2.0	2.0	1.5

The results of the proximate analysis of diets amino acids are shown in Table 5. The amino acid profile shows that diets register a fall in histidine, lysine and

methionine 0.5; 0.4 and 0.2% respectively when compared with the NRC (2011) which represents the minimum values of the needs of essential amino acids recommended for Nile tilapia.

**Table 5:** Amino acids profile of the different diets fed to tilapia *O. niloticus*

Amino acids	A	B	C	D	E	NRC, 2011
Arginine (%)	2.1	2.1	2.1	2.1	2.1	1.2
Histidine (%)	0.5	0.5	0.5	0.5	0.5	1.0
Isoleucine (%)	1.5	1.5	1.5	1.5	1.5	1.0
Leucine (%)	4.2	4.2	4.2	4.2	4.2	1.9
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.6
Methionine (%)	0.5	0.5	0.5	0.5	0.5	0.7
Met+Cys (%)	1.0	1.0	1.0	1.0	1.0	1.0
Phenylalanine (%)	2.0	2.0	2.0	2.0	2.0	1.1
Phe+Tyr (%)	3.4	3.4	3.4	3.4	3.4	1.6
Threonine (%)	1.3	1.3	1.3	1.3	1.3	1.1
Tryptophane (%)	0.4	0.4	0.4	0.4	0.4	0.3
Valine (%)	2.0	2.0	2.0	2.0	2.0	1.5

### Growth parameters and survival

**Table 6:** initial and final mean weight, mean weight gain, SGR, FCR and survival

Composition	Treatments				
	A	B	C	D	E
Initial mean weight gain (g/fish)	4.54	4.55	4.56	4.53	4.53
Final mean weight gain (g/fish)	10.34±0.95	9.71±0.41	11.44±1.62	10.92±0.83	9.64±0.94
Mean weight gain (g/fish)	5.80±0.94	5.16±0.41	6.88±1.62	6.39±0.83	5.11±0.94
Mean Weight gain (%)	127.75±20.32	113.46±9.01	151.23±35.31	140.95±18.30	112.71±20.80
SGR	1.50±0.16	1.38±0.07	1.67±0.25	1.60±0.14	1.37±0.18
FCR	4.12	4.37	3.68	4.02	4.90
Survival (%)	45	25	60	90	70

### The mean weight gain and specific growth rate

During eight (8) weeks, the results reported in Table 6 showed that the best mean weight gain 151.23 % was obtained in fish fed with diet C followed by fish fed with diet D amounting to 140.95%; compared with those fed with diet E which recorded the lowest mean weight gain with 112.71%. On the other hand, the best SGR 1.67 was also recorded in diet C and lowest in fish fed with diet E.

### Feed conversion ratio

The results reported in Table 6 have enabled us to identify the most efficient diet that has recorded the lowest FCR. Indeed, diet C had the lowest FCR with 3.68, while the highest value was obtained in fish fed with diet E 4.90.

### Survival

The different survival rates obtained from different batches of fish are shown in Table 6. The better survival rate of fish fed with diet D was 90%. Diets E, C, A and B be 70, 60, 45 and 25% survival rate, respectively.

**Table 7:** carcass composition of tilapia *O. niloticus*

Composition	Initial fish	Treatments				
		A	B	C	D	E
Dry matter (%)	89.14	92.35	92.76	96.02	93.80	94.05
Protein (%)	86.15	80.53	75.87	78.45	80.65	82.30
Lipid (%)	12.56	12.50	8.80	8.90	8.90	10.14

The results of the proximate analysis of fish tissue shown in Table 7 revealed information on specific components.

The dry matter content of the carcass of initial fish (89.14%) is lower than that of fish fed with different diets A (92.35%), B (92.76%), C (96.02%), D (93.80%) and S (94.05%). The composition of the carcass of fish treated with diet C is the highest.

The carcass protein content of initial fish is significantly higher (86.15%) compared with the carcass of fish subject to the test diets A (80.53%), B (75.87%), C (78.45%), D (80.65%) and E (82.30%). Initial fish presented higher body lipid content (12.56%) in comparison with diets A (12.50%), B (8.80%), C (8.90 %), D (8.90%) and E (10.14%).

## Discussion

### Water quality parameters

Water quality parameters, namely temperature, dissolved oxygen and pH were measured during the experiment. The mean temperature obtained in this study is  $29.06 \pm 0.3$  ° C. This value is within the optimum temperature range for the growth and survival of the species (28 to 31 ° C) reported by Boyd (1982) and Hossain et al. (2004).

The mean value of dissolved oxygen obtained in the experiment is  $7.58 \pm 0.1$  mg / L. This is in line with the results of Boyd and Lichtkoppler (1979) who argued that dissolved oxygen concentrations greater than 5.0 mg / L are desirable for fish survival.

The mean value of pH recorded in this study was  $7.5 \pm 0.2$ . Based on Santhosh and Singh (2007) the ideal pH range for fish is between 7.5 and 8.5. Higher or lower values than the optimal pH can cause stress of fish. According to observed results, this means that the addition of eco-enzymes in diets improve the existing water quality. Such observations were confirmed by Tang and Tong (2011), Nazim and Meera (2013) using eco-enzymes in the water treatment.

### Growth and survival

The incorporation of eco-enzymes in different diets tested in tilapia fingerlings (*O. niloticus*) during the experiment showed good growth performance and survival compared with control.

The present study has shown that the best mean weight gain was obtained with the diet containing 5% eco-enzymes (151.23%) in comparison with control diets (127.75%). These results are higher than those reported by Niang (2013 personal communication) that incorporated eco-enzymes at dilution of 1/500 in diets of tilapia fingerlings that had a mean weight gain of 54.15%, while Olusola and Nwanna (2014) obtained a weight gain of 84.95% by incorporating phytase to a quantity of 8.000 units of phytase / kg in the Nile tilapia diet. Our findings are supported by those of Tudkaew et al. (2008), which supplemented with phytase Ronozyme P in red tilapia diet obtaining 312.74% weight gain. Olusola and Nwanna (2014) suggest that the inclusion of exogenous enzymes in food tilapia can positively influence on growth.

A certain number of studies have reported the positive use of enzymes in the bioavailability of nutrients and minerals, protein digestibility and amino acids, growth performance and reduction of anti-nutritional factors: Niang (2013, personal communication) reported that the use of eco-enzymes in feed with a 1/500 dilution of tilapia fingerlings (*Oreochromis niloticus*) had effects on growth performance as well as the usage of feed and survival rate. Indeed, the results showed a significant increase in weight gain with 54.15% in the group fed with diet containing eco-enzymes against 20% with the control. Also, diet containing eco-enzymes enabled a better feed conversion ratio and survival, respectively 0.23 against 1.08 when controlled and 90% against 80% with the control.

The addition of commercial enzyme Pescazyme TM 5602 in a different soy diet fishmeal shows an equivalent performance diet containing 10 or 12% of fishmeal in carp and tilapia (Viola. 1994; Feord . 1996).

Liebert and Portz (2005) reported that the optimum growth of Nile tilapia was achieved when adding 750 to 1250 units of phytase / kg of food, while Cao et al. (2008) found that 1.000 units phytase / kg diet give better growth performance and feed conversion among the same species. Vielma et al. (2004) reported an increase in weight gain of 243-459% in the rainbow trout fed with soybean meal with phytase and phosphorus supplementation.

For specific growth rate (SGR), the analysis of statistics shows that there is no significant difference between the different regimes. However, the best SGR were observed in fish subjected to diet containing 5% eco-enzyme with 1.67% / day against 1.50% / day in control. Our results are superior to those obtained by (Niang 2013) with a SGR of 1.44% / day through the incorporation of eco-enzymes in food tilapia. These results are also higher than those reported by Olusola and Nwanna (2014) who noted a SGR of 0.42% / day by inclusion of phytase in tilapia diets. In contrast, our results were lower than those obtained by Tudkaew et al. (2008) which recorded a SGR of 2.53% / day in tilapia fed on phytase.

Moreover, our results recorded high feed conversion ratio (FCR) in each diet. The best FCR was obtained through the regime C with FCR 3.68 against 4.12 with control. The results of the present study remain lower than those obtained by Al Dilaimi (2009), which get FCR 6.96; 10.92 and 6.82 in tilapia fingerlings (*O. niloticus*) fingerlings fed with diets containing 6%, 9% and 15% lipid. Therefore, our results are superior to those of Niang (2013), earning 0.23 FCR in tilapia subject to the system of eco-containing enzymes. According to Philippart et al. (1979) and O'Connor et al. (1985), plus the value of the FCR is reduced more food is used and converted. The high levels of FCR recorded in this study could be explained by mortality influence in different batches of fish and the quality of food.

The addition of phytase to feeds showed good ingestion, growth and better FCR compared with the control diet in channel catfish (*Ictalurus punctatus*), both reducing phosphorus load excreted (Jackson et al., 1996).

A feeding trial conducted on tilapia fingerlings (*O. niloticus*) in Brazil shows the importance of phytase in diets based on vegetable protein. So, feed was added to the commercial enzyme phytase "Natuphas" at 0. 500, 1.500 and 3.000 units / kg of feed. Fish fed 500 units show a higher weight gain and

improved FCR of 1.80. Also, the addition of protease as an additive in fish food equal the performance of milk protein (24% protein) and reached higher rate (28% protein) of food (Feord 1996).

The results on fish survival showed a better survival rate among those fish fed with diet D and E, respectively providing 90% and 70% against 45% when controlled. They are similar to those reported in (NIANG 2013) with 90% survival in fish fed on eco-enzymes and Tudkaew et al. (2008) achieving better survival of tilapia with different treatments, giving then 93.33% during the control treatment, 95% treatment containing di-calcium phosphate, 91.67% in the treatment containing phytase and 96.67% treatment containing both di-calcium phosphate and phytase. A survival rate higher than 80% constitutes an excellent nursery (Sumi et al. 2011). Note that the low survival recorded control of 45% could be justified by a marked aggressiveness observed in fish.

For the different results observed, we can confirm that eco-enzymes could have positive effects on the growth of tilapia and improve their digestibility. This is in line with the results of Davis et al. (1998) who note better growth and better digestibility in shrimp subjected to diet containing protease.

### **Bromatological diets analysis**

Results of the proximate analysis of studied diets showed high proportion of polyunsaturated fatty acids of n-3 (9.2%) compared with polyunsaturated fatty acids of the n-6 (4.8%). In general, n-3 fatty acids are not required for warm water fish, but for the membrane structure at least small amount of these acids may be necessary (Stickney and Hardy. 1989). Warm water fish have needs of polyunsaturated n-6 fatty acids or a mixture of fatty acids of n-3 and n-6, while cold-water species require fatty acids of the n-3 series (Webster and Lim. 2002). Various tilapia species require almost 1% n-6 fatty acids in their diet (Teshima et al. 1985). Our results revealed an important proportion of LA fatty acid (18: 2n-6) assessed at 4.5% covering the need in tilapia. Takeuchi et al. (1983) showed that in tilapia fatty acid needs of the n-6 series (linoleic acid 18: 2n-6) are most important. They assess the need 0.5% in the diet. Similarly, Kaushik et al. (1993) reported the same value.



## Carcass composition

At the beginning and the end of the experiment, carcass composition (dry matter contents, proteins and lipids) is used to determine the influence of diets on the body composition of fish. According to Hopher (1988), endogenous factors (size, sex and stage of the life cycle) and exogenous factors (diet composition, feed rate and temperature) affect the body composition of fish.

The results of the present survey work showed that the protein content of the initial fish flesh (86.15%) is higher than that of the various schemes A (80.53%), B (75.87%), C (78.45 %), D (80.65%) and S (82.30%). Based on Médale and Kaushik (2009), fish use part of dietary protein for energy supply. Moreover, this fall could be explained by the lack of essential amino acids such as histidine, lysine and methionine in food. Médale and Kaushik (2009) argue that protein sources of food must provide amino acids in appropriate amounts for optimal use of protein intake. They shall contain essential amino acids (IAA) to cover the needs of fish.

The results showed that the body lipid content of initial fish (15.56%) is high compared with other diets A (12.50%), B (8.80%), C (8.90%), D (8.90%) and E (10.14%). Our results match those of Niang (2013), which recorded high in initial flesh lipids (4.72%) compared with the flesh of fish (2.76%) subject to the system containing eco-enzymes. According to Aksnes et al. (1986), during sexual maturation, a portion of body fat is used for the production of gametes, particularly among females. A strong mobilization of body fat of the carcass and viscera is observed in rainbow trout, rainbow female for egg formation (Nassour and Leger, 1989). The results of this study indicate that the eco-enzymes do not affect the body composition of fish.

## Conclusion

The use of exogenous enzymes, such as eco-enzymes, in the manufacture process of feed for fish farming, plays a key role in fish, including digestibility and growth. Also, profitable effects of eco-enzymes to improve the water quality of the trays play a leading role in the development of the aquaculture industry. Furthermore, the use of eco-enzymes in aquaculture highly contributes to the enhancement/development of plant residues that might have positive impact on the environment.

At the end of our study, results obtained accordingly enabled us to understand the possible impact performance of eco-enzymes for growth and survival of Nile tilapia. In fact, results first revealed that food contains 50% C eco-enzymes showing the best growth performance and then it (food) contains 75% D eco-enzymes exhibited the best performance of survival in Nile tilapia.

Diets developed in our study are formulated based on local products, which have the advantage of being available and locally accessible (financially: cost) for farmers.

To sum up, the information from this study allow us to state that objectives set for the valuation of plant residues, quality and cost of food, growth performance and survival of species have been achieved and eco-enzymes can be used as complements in food components for tilapia.

## Acknowledgments

We would like to express our thanks to the National Agency of Aquaculture (NAA) for the supply of fish, vitamin and mineral premix. We would highly appreciate the assistance of staff members of ENSA (Ecole Nationale des Sciences Agronomiques) laboratory. We highly appreciate M. Rene Ndiero FALL for his critical review on this manuscript.

## References

- Ai Q., Mai K., Zhang W., Xu W., Tan B., Zhang C., Li H. 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese sea bass, *Lateolabrax japonicus*, Comparative Biochemistry and Physiology. 147:502-508.
- Akhtar S. and Husain Q. 2006. Potential applications of immobilized bitter melon (*Momordica charantia*) peroxidase in the removal of phenols from polluted water. Chemosphere. 65:1228-1235.
- Aksnes A., Gjerde B., Roald S.O. 1986. Biological, chemical and organoleptic changes during maturation of farmed atlantic salmon. *Salmosalar*. Aquaculture. 53:7-20.
- Al Dilaimi A. 2009. Détermination de la ration lipidique alimentaire optimale chez les alevins du tilapia du Nil (*Oreochromis niloticus*). Mémoire de fin d'étude. 78p.

- AOAC, 1984, Official Methods of Analysis, 14 the edition, OAC Arlington, VA, 1 141p.
- Balarin J.D. and Hatton J.D. 1979. Tilapia: A guide to their biology and culture in Africa, Unit of Aquatic Pathobiology, Stirling University. 174 p.
- Bénech V. and Dansoko D.F. 1994. Reproduction des espèces d'intérêt halieutique. In Quensière J. (éd.): La pêche dans le delta central du Niger : approche pluridisciplinaire d'un système de production halieutique, Paris, IER, Orstom, Karthala, 213-228.
- Biswas A.K. Kaku H. Ji S.C. Seoka M. Takii K. 2007. Use of soybean meal and phytase for partial replacement of fishmeal in the diet of red sea bream *Pagrus major*. Aquaculture. 267:284-291.
- Boyd C.E. and Lichtkoppler F., 1979. Water quality management in fish ponds, Research and Development Series N° 22 International Center for Aquaculture (J.C.A.A) Experimental Station Auburn University, Alabama, P 45-47.
- Boyd C.E. 1982. Water quality management for pond fish culture. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York, 318p.
- Cao L., Yang Y., Wang W.M., Yakupitiyage A., Yuan D.R., Diana J.S. 2008. Effect of pretreatment with microbial phytase on phosphorus utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition. 14:99-109.
- Carter C.G. and Hauler R.C. 1998. Fish meal replacement in aquaculture feeds for Atlantic Salmon. 23-45.
- Chowdhury K.M.A.. 2014. Enzymes in Shrimp Nutrition – Is there the Future? The Aquaculture Roundtable Series. 0900 – 0930, Consultation 27/08/2014 à 15:28.
- Christian V., Shrivastava R., Shukla D., Modi H., Rajiv B., Vyas M. 2005. Mediator role of veratryl alcohol in lignin peroxidase-catalyzed oxidative decolorization of Remazol Brilliant Blue R. Enzyme Microb, Technol. 36: 426-431.
- Davis D.A., Johnston W.L. and Arnold C.R.. 1998. The use of enzyme supplements in shrimp diets. Symposium publication IV International Symposium on Aquatic Nutrition. La Paz. B.C.S. Mexico. 18p.
- Derouiche E., Azaza M.S., and Kraiem M.M. 2009. Essai d'acclimatation du tilapia du Nil *Oreochromis niloticus* dans la retenue du barrage Lebna (cap bon. Tunisie). Bull. Inst. Natn. Scien. Tech. Mer de Salammbô. Vol 36.
- Felix F. and Selvaraj S. 2004. Enzymes for sustainable aquaculture. Aquaculture Asia. 9:5-6.
- Feord J.C. 1996. Exogenous enzymes improve performance of carp and tilapia when fed diets contain high levels of soybean meal. VII International Symposium on Nutrition and Feeding of Fish.
- Forster I., Higgs D.A., Dosanjh B.S., Rowshandeli M. and Parr J. 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout *Oncorhynchus mykiss* held in 11°C freshwater. Aquaculture. 179:109-125.
- George T.T. 1976. Observation on the growth of *Tilapia nilotica* in tropical freshwater fish ponds treated with different fertilizers. Paper presented at the F.A.O./C.I.F.A. Symposium on Aquaculture in Africa, 30 Sept.- 2 Oct. 1975, Accra, Ghana, C.I.F.A/75/SE 11, Rome, 16p.
- Hepher B. 1988. Nutrition of pond fishes. Cambridge, Cambridge University Press, 388p.
- Hossain M.A., Roy R., Rahmatullah S.M. and Kohinoor A.H.M., 2004. Effect of stocking density on the growth and survival of GIFT tilapia (*Oreochromis niloticus*) fed with formulated diet, J. Agric. Rural Dev, 2:127-133.
- Jackson L.S., Li M.H. and Robinson E.H., 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve the utilisation of phytate phosphorus. Journal of the World Aquaculture Society, 27:309-313.
- Jeganathan J., Bassi A. and Nakhla G., 2006. Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydrolyzation, Hazard Mater J., 137:121-128.
- Johnson D.R., Park J.H., Kakor J.J., Abriola L.M., 2006. Effect of carbon starvation on toluene degradation activity by toluene monooxygenase-expressing bacteria. Biodegradation, 17:437-45.
- Kestemont P., Micha J.C. and Falter U., 1989. Les méthodes de la production d'alevins de *Tilapia nilotica*, FAO/PNUD-Programme de mise en valeur de coordination de l'aquaculture, ADCP/REP/89/46, 131p.
- Khan A.A. and Husain Q., 2007. Potential of plant polyphenoloxidases in the decolorization process and removal of textile and non-textile dyes, J. Env. Sci., 19:396-402.
- Khan M.S., Zaidi A. and Wani P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. Agron. Sustain. Dev., 27:29-43.
- Kulshrestha Y., Husain Q., 2007. Decolorization and degradation of acid dyes mediated through salt fractionated turnip (*Brassica rapa*) peroxidase.

- Toxicology and Environmental Chemistry.89: 255-267.
- Lei Y., Mulchandani P., Chen W. and Mulchandani A. 2005. Direct determination of p-nitrophenyl substituent organophosphorus nerve agents using a recombinant *Pseudomonas putida* JS444-modified Clark oxygen electrode. J. Agric. Food Chem., 53:524-527.
- Lemal D. 1989. Recueil des méthodes et techniques utilisées pour l'expérimentation en alimentation animale au DPA de l'INDR, Thiès: INDR, 46p.
- Liebert F., Portz L., 2005. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based on low phosphorus diets supplemented with graded levels from different sources of microbial phytase. Aquaculture, 248:111-119.
- Li M.H., Manning B.B., Robinson E.H., 2004. Summary of phytase studies for channel catfish. Mississippi Agricultural and Forestry Experimental Station Research report, 23:1-5.
- Magid A. and Babiker M.M., 1975. Oxygen consumption and respiratory behaviour of tree Nil fish. Hydrobiologia, 46:359-367.
- Mansee A.H., Chen W. and Mulchandani A., 2005. Detoxification of the organophosphate nerve agent coumaphos using organophosphorushydrolase immobilized on cellulose materials, J. Ind. Microbiol, Biotechnol., 32:554-560.
- Matto M. and Husain Q., 2007. Decolorization of direct dyes by salt fractionated turnip proteins enhanced in the presence of hydrogen peroxide and redox mediators. Chemo, 69:388-345.
- Médale F., Kaushik S., 2009. Les sources protéiques dans les aliments pour les poissons d'élevage. Cah Agric. 18:2-3.
- Mélard Ch., Philippart J.C., 1981a. Pisciculture intensive du tilapia *Sarotherodon niloticus* dans les effluents thermiques d'une centrale nucléaire en Belgique. In proceedings word symposium on aquaculture in heated effluents and recirculation systems. Stavanger, 1:637-658.
- Mélard Ch. and Philippart J.C., 1981b. La production de tilapia de consommation dans les rejets industriels d'eau chaude en Belgique. Cahiers d'Éthologie appliquée; collection Enquêtes et dossiers: 2, vol. 1, suppl. 2, Institut de Zoologie de l'Université de Liège, 122p.
- Moriarty C.M. and Moriarty D.J.W., 1973a. Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis rubripinnis* in Lake George. Uganda, J. Zool. Land., 171:15-24.
- Moriarty D.J.W., 1973. The physiology of digestion of blue-green algae In the Cichlid fish, *Tilapia nilotica*, J. Zool. Land., 171:25-39.
- Nassour L., Léger C.L., 1989. Deposition and mobilization of body fat during sexual maturation in female trout (*Salmo gairdneri* Richardson), Aquat. Living Resource, 2:153-159.
- National Research Council (NRC), 2011. Nutrient requirements of fish and shrimp, National Academy Press, Washington. DC, 55p.
- Nazim F., and Meera V., 2013. Treatment of synthetic greywater using 5% and 10% garbage enzyme solution; Bonfring International Journal of Industrial Engineering and Management Science, Vol. 3, No. 4.
- Ngom S., 2004. Ebauche d'un référentiel sur la composition chimique et valeur nutritive des matières premières utilisables en alimentation des volailles au Sénégal. Thèse de doctorat de troisième cycle de chimie et biochimie des produits naturels, 158p.
- Niang T., 2013. Utilisation des microorganismes effectifs et des éco-enzymes dans la croissance du tilapia du Nil (*Oreochromis niloticus*). Mémoire de fin d'étude de technicien supérieur, 22p.
- O'Connor T.P., Roebuck B.D., Peterson F. et Campbell T.C., 1985. Effect of dietary intake of fish oil and fish protein on the development of L-azaserine-induced preneoplastic lesions in the rat pancreas; J. Nat Cancer Inst, 75:959-62.
- Ouédraogo S., 2000. Biologie de reproduction du tilapia : *Oreochromis niloticus* du lac de barrage de la Comoé; Mémoire de fin d'étude, 77p.
- Philippart J.C., Mélard Ch. and Ruwet J.C., 1979. La pisciculture dans les effluents thermiques industriels. Bilan et perspectives d'une année de recherche à la centrale nucléaire de Tihange sur la Meuse. In : Calembert L. (Ed). Problématique et gestion des eaux intérieures, Ed. Derovaux, Liège, 779-791.
- Philippart J.C. and Ruwet J.C., 1982. Ecology and distribution of tilapias. In: The biology and culture of tilapias (Pullinet Lowe Mc Connell. Eds.); ICLARM Conference Proceedings, 7, Manila, Philippines, 15-59.
- Robaina L.E. 1998. Utilización nutritiva de fuentes de proteína alternativa a la harina de pescado en dietas de encorde para dorada (*Sparus aurata*), n°4. 195p.
- Santhosh B. and Singh N.P., 2007. Guidelines for water quality management for fish culture in Tripura. ICAR Research Complex for NEH Region, Tripura Center; Publication N°29.

- Shimazu M., Mulchandani A., Chen W., 2001. Simultaneous degradation of organophosphorus pesticides and *p*-nitrophenol by genetically engineered *Moraxella* sp. with surface-expressed organophosphorushydrolase. *Biotechnol, Bioeng.*, 76: 318-324.
- Sugiura S.H., Gabaudan J., Dong F.M., Hardy R.W., 2001. Dietary microbial phytase supplementation and the utilization of phosphorus; trace minerals and protein by rainbow trout *Oncorhynchus mykiss* (Walbaum) fed on soybean meal-based diets. *Aquaculture Research*, 32:583-92.
- Sumi K.R., Das M., Siddika I., 2011. Effect of different protein levels of fry feed on the production of quality tilapia (*Oreochromis niloticus*) fry. *Journal Bangladesh Agriculture University*, 9:365-374.
- Tang F.E. and Tong C.W., 2011. A study of garbage enzyme's effects in domestic wastewater. *World Academy of Science, Engineering and Technology*, 5:12-27.
- Thomas G.M.H., Cunningham E., Fenssome A., Ball A., Totty N.F., Truong O., Hsuan J.J. and Cockcroft S., 1993. An essential role for phosphatidylinositol transfer protein in phospholipase C-mediated inositol lipid signaling; *Cell*.74:919-928.
- Vielma J., Ruohonen K., Gabaudan J., Vogel K., 2004. Top-spraying soybean meal-based diets with phytase improve protein and mineral digestibilities but not lysine utilization in rainbow trout. *Oncorhynchus mykiss*(Walbaum). *Aquaculture Research*, 35:955-964.
- Viola S., Angeoni H. and Lahav E., 1994. Present limits of protein sparing by aminoacid supplementation of practical carp and tilapia feeds. *Israeli Journal of Aquaculture*, 46:212-222.
- Yan W., Reigh R.C., 2002. Effects of fungal phytase on utilization of dietary protein and minerals and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant protein diet. *Journal of the World Aquaculture Society*, 33:10-22.
- Yeager C.M., Arthure K.M., Bottomley P.J. and Apr D.J.. 2004. Trichloroethylene degradation by toluene-oxidizing bacteria grown on non-aromatic substrates. *Biodegradation*. 15:19-28.