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Full Length Research Paper

Biodegradation of *Panicum repens* residues by *Pleurotus ostreatus* for its use as a non conventional feedstuff in diets of *Oreochromis niloticus*

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Biological degradation of torpedo grass, *Panicum repens* L. residues by *Pleurotus ostreatus* and suitability of using the highly digestible, protein-enriched, as a non conventional feedstuff in diets of Nile tilapia, *Oreochromis niloticus*, fingerlings were investigated. The optimal pH and temperature for growth *Pleurotus ostreatus* and its cellulase production were 6 and 25°C, respectively. The cellulase was induced in submerged culture with presence of the carboxymethyl cellulose and Torpedo grass residues in MSL, while the presence of additional carbon sources such as glucose, dextrose or a complex media (Potato Dextrose) suppressed enzyme production. The amount of reducing sugar present in the biodegraded biomass by cellulase after 30 min incubation time was 2.5 (U/ml/min) but was 2.1 (U/ml/min) by *Pleurotus ostreatus* after 14 days incubation time under optimum growth conditions. The solid substrate fermentation (SSF) was carried out at pH 6 and 25°C for 32 days. Protein contents of the biodegraded biomass increased from 7.52 to 8.91% and crude fiber contents decreased from 23.27 to 11.28. This biodegraded biomass was used as non conventional feedstuff in diets of *Oreochromis niloticus* fingerlings. The results showed that Nile tilapia fingerlings received diets containing 25% treated torpedo grass showed the best results in growth parameters, feed efficiency and economic efficiency.

Key words: Biodegradation, *Pleurotus ostreatus*, torpedo grass, *Panicum repens* L., Nile tilapia, growth parameters.

INTRODUCTION

Aquaculture is the fastest growing animal production sector in the world since 1984. Today, aquaculture production accounts for over a 61.5% of total fish production. Egypt, production from cultured tilapia had increased from 9,000 ton in 1980 to 595,030 ton in 2006 (FAO, 2006). To sustain the high rates of increase in aquaculture production, there should be a matching increase in the levels of production of fish feed. As the aquaculture industry grows, the need for specialized feeds designed for particular production situations is increasing. To date, nutritionists and feed manufactures have concentrated their efforts on determining which of the wide variety of feedstuffs available to the feed industry may be used to produce lower cost aquaculture feeds.

Using the non-conventional sources is an adventive, rhizomatous grass species that has become an invasive weed of terrestrial, wetland, and aquatic environments in tropical and subtropical regions worldwide an obligatory partial solution to minimize the feed shortage gap. *Panicum repens* L. is a perennial grass that frequently forms dense colonies and has long, creeping rhizomes. Brecke et al. (2001) state: "*P. repens* is a perennial weed that can be found along ditch banks, around ponds, along roadsides, and in managed turfgrass areas, including golf courses (McCarty et al., 1993). David (1999) reported that, "*P. repens* formed dense monotypic stands in response to increased hydroperiod (depth and duration of

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flooding)." Brecke et al. (2001) states that in Florida, "*P. repens* is very competitive and has reduced common Bermuda grass (*Cynodon dactylon*) Growth by nearly 40% after 2 year. Smith et al. (2004) state that, "On Lake Okeechobee the ability of *P. repens* to disperse and become established at different water depths was evaluated in a series of experimental pond studies. Hossain et al. (2001) state that, "*P. repens* is also recognized as a pasture grass, and it could be harvested five to seven times a year in tropical and subtropical areas. A higher amount of rhizomes and roots makes a loose mat-like structure in soil up to 50 cm in depth, and indicates that this species could be used for soil erosion control.

In Northern Vietnam, plant leaves and on-farm wastes, derived from cultivated crops or native plants, are the main feed input to the fish ponds. In order to gain nutrients from a plant, an animal must first render it suitable for digestion by breaking or grinding it into smaller pieces, ultimately breaking or opening the cells (Vincent, 1991). In Cyprinids the fifth branchial arches are modified into pharyngeal jaws at the entrance to the esophagus. These are especially well developed in herbivorous cyprinids (Sibbing, 1991), thus the fish is able to gain direct access to the cell content of the plant by mechanical means (Vincent and Sibbing, 1992). The digestibility of feedstuffs might also depend on the digestive physiology/enzyme profile of the fish concerned (Smith, 1989; De Silva and Anderson, 1995). Some herbivorous fish such as grass carp have ability to use, indirectly (via microorganisms) or directly, cellulase, which can aid this process (Das and Tripathi, 1991). In Son La province the farmers normally feed typical crop residues such as leaves of banana (*Musa nana*), cassava (*Manihot esculenta*), bamboo (*Bambusa vulgaris*) and to a lower extent mixture of *Lemna* sp. and *Azolla* sp., leaves of plants such as, maize (*Zea mays*), sweet potato (*Ipomoea batatas*), mulberry (*Morus*) and peanut (*Arachis hypogaea*); weeds such as barnyard grass (*Echinochloa crusgalli*), shingle flatsedge (*Cyperus imbricatus*); mixed weeds collected from paddy fields. In addition plant materials such as Napier grass (*Pennistum purpureum*), Cassava tubercles and peels, and rice bran are also occasionally fed to the fish. The application of feed in the ponds is often based on the availability of feed ingredients, the current farming activities (e.g. weeding) and the availability of labour time. On the other hand, the ever increasing human population has resulted in a demand for protein food, particularly in the Third World countries. Conventional and traditional methods have proved insufficient to meet the rising demand, resulting in the need to explore areas for low cost production of unconventional protein-rich food and feed.

Various abundantly available lignocellulosic wastes may be used as cheap substrates for production of protein-enriched food and feed (Reid, 1989; Mukherjee and Nandi, 2004). Hence, partial delignification of lignocellulosic feeds may promote feed intake and animal productivity. This method has now become popular for improving the nutritional qualities of ruminant feed especially proteins and sugars as well as its digestibility, thereby upgrading the economic value of lignocellulosic waste. White rot fungi, which are capable of degrading lignin preferentially along with cellulose and hemicellulose, are widely used to increase the digestibility of lignocellulosic biomass (Dhanda et al., 1994).

Many authors have shown that some fungi, particularly some species of *Pleurotus* are able to colonize different types of lignocellulsic wastes, increasing their digestibility (Platt et al., 1984; Commanday and Macy, 1985; Rajarathnam and Bano, 1989; Villas-Boas et al., 2002; Zhang et al., 2002; Mukherjee and Nandi, 2004; Salmones et al., 2005). Previous studies have shown the feasibility of using these kinds of wastes to produce animal feed (Calzada et al., 1987; Adamovic et al., 1998), and as substrate for mushroom production (Breene, 1990; Sermanni et al., 1994; Kakkar and Danad, 1998; Dhanda et al., 1996; Yildiz et al., 2002).

To our knowledge, little work has been conducted to assess the nutritional potential of the plant feed ingredients currently used as feed for stomach less fish in Egypt. Torpedo grass, *P. repens* L., a widely prevalent enormously fast-growing aquatic weed, can be an inexpensive source of protein-enriched, as a non conventional feedstuff in diets of Nile tilapia, *Oreochromis niloticus*, fingerlings, although it is used in the feeding of ruminants, and it presents a very low protein content and low digestibility.

The objective of the present study aimed to evaluate the effect of replacing yellow corn in fish feed by biodegraded torpedo grass, *P. repens* L. with white-rot fungus, *Pleurotus ostreatus* on growth performance, feed utilization, body composition, hematology and preliminary economical evaluation. The study is expected to provide important inputs for developing feeding strategies for forage-eating fish such as Nile tilapia.

MATERIALS AND METHODS

This study has been carried out at Agricultural Microbiology branch, Agricultural Botany Department and the Wet Fish Laboratory, Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University.

Media

Minimal medium as mineral salt medium (MSL) was used through this study as described by (Drews, 1968) as well as Potato glucose Agar (PGA) and Potato Dextrose Agar (PDA) were used also as complex media in the present study.

Microorganisms and degradation of torpedo grass, *Panicum repens* L. by the microorganisms via measuring of clear zone

Agaricus bisporus was obtained from China General Microbiological culture collection center, Institute of Microbiology, Chinese Academy of Sciences, China and *P. ostreatus* was obtained from

Table 1. Chemical analysis of untreated or biodegraded torpedo Grass, (TG) *Panicum repens* L. (% on DM basis).

Item	*Panicum repens* (%)	
	Untreated	Treated
Dry matter (DM)	92.94	93.64
Crude protein (CP)	7.52	8.91
Ether extract (EE)	1.50	1.68
Crude fiber (CF)	23.27	11.28
Ash	13.46	14.01
Nitrogen free extract (NFE)*	54.25	64.12
Energy kcal GE/kg**	3635.2	3678.2

*Calculated by differences, ** Estimated (5.65, 9.45, 4.0 and 4.0 kcal GE/g dry matter for CP, EE, CF and NFE, respectively, (Jobling, 1983).

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The both strains were then tested for their ability to grow and degrade torpedo grass, *P. repens* L. in MSL medium. The dried torpedo grass, *P. repens* L. was milled. 100 ml MSL medium containing 10 g/L from dried-milled torpedo grass, *P. repens* L. was inoculated by 3 ml from each fungal suspension at 10^6 cfu/ml. One treatment contained the medium and carboxymethyl cellulose and the other contained the medium without carboxymethyl cellulose and the strain (control).

The cultures were shaken at 150 rpm and 25°C for 14 days. All assays were carried out from cultures supernatant as extracellular cellulase source after removing the growth by using sterile membrane filter (0.2 m). 50 l of culture supernatant was added in wells (5 mm in diameter) of MSA (containing carboxymethyl cellulose 10 g/L as substrate). The plates were treated and the clear zone was measured according to the method described by (Teather and Wood, 1982; Bradner et al., 1999; Peciulyte 2007; Belal, 2008).

Effect of pH and temperature on growth of *P. ostreatus* and its cellulase (CMCase) production

One hundred ml MS- medium supplemented with carboxymethyl cellulose (10 g/L) as a sole source of carbon were used to determine the effect of pH and temperature on growth of *P. ostreatus* and its cellulase production. The medium was inoculated by 3 ml (10^6 cfu/ml) of culture of *P. ostreatus* strain. The experiments were carried out at pH 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 and the culture was incubated at 25°C with shaking (150 rpm) for 14 days. To determine the optimum temperature, MSL medium at pH6 was incubated at 20, 25, 30, 35 and 40°C with shaking (150 rpm) for 14 days. The activity of *P. ostreatus* cellulase was determined by measuring of clear zone as described above. The growth was determined as mycelial dry weight of biomass (g) after 14 days as described by Belal (2003).

Effect of different carbon source on *Pleurotus ostreatus* growth and enzyme induction

One hundred ml MSL in conical flasks (250 ml) containing 10 g/L from (dried- milled torpedo grass, *P. repens* L.) or glucose and 20 g/L from dextrose was inoculated by 3 ml from fungal suspension at 10^6 cfu/ml (one-week-old colonies of fungi grown at 25°C on PDA plates). Potato dextrose (PD) was used as complex medium and it was carried at the same conditions. Cultures were incubated in shaker incubator for 14 days at 25°C and 150 rpm. After 14 days of cultivation, culture aliquots were centrifuged at 5000 rpm to remove solids. The supernatants were assayed for their enzymatic activity by measuring of clear zone as described above. The growth was determined as mycelial dry weight of biomass (g) after 14 days as described by Belal (2003).

Enzyme assay and saccharification of dried-milled torpedo grass, *Panicum repens* L. by the *Pleurotus ostreatus* cellulase

Cellulase activity was determined by incubating 0.5 ml of the supernatant (at a concentration of 250 g/ml, while the enzyme concentration was determined according to Lowry et al. (1951) with 0.5 ml of an amount 10 g/L of dried-milled torpedo grass, *P. repens* L. in 0.05 M citrate buffer (pH 4.8) at 50°C for 30 min. After incubation, the reaction was terminated by adding 3 ml of 1% 3,5-dinitrosalicylic acid (DNS) reagent to 1 ml of the reaction mixture and heated for 10 min. In these tests, reducing sugars were estimated calorimetrically after Miller (1959), using glucose as standards. One unit of cellulase activity is defined as the amount of enzyme that releases 1 µmol reducing sugars (measured as glucose) per ml per min.

Improvement of the nutritive value dried-milled torpedo grass, *Panicum repens L.* by treatment with *Pleurotous ostreatus* using solid state fermentation technique

Dried-milled torpedo grass, *P. repens* L.) (30 g) was placed in 500 ml Erlenmeyer flasks, moistened with distilled water (65%) and autoclaved at 121°C for 20 min, cooled overnight, and inoculated with 10 agar disks (5 mm diameter) from culture *Pleurotous ostreatus* from a (7 day old). After incubation for 32 d at 25 ±1°C, the sterilized biodegraded substrates were used as inoculum for autoclaved dried milled biomass with rate 10% as follow: Dried-milled torpedo grass, *Panicum repens* L.) (30 g) was placed in 500 ml Erlenmeyer flasks, moistened with distilled water (65%) and autoclaved at 121°C for 20 min, cooled overnight, and inoculated with rate 10% from the described biodegraded substrate (10^6 cfu/gm), mixed well and after that was incubated for 32 days at 25 ±1°C (Mukherjee and Nandi 2004; Belal, 2008; Das and Ghosh, 2009).

Experimental fish

Nile tilapia (*O. niloticus)* fingerlings were brought from a fresh water commercial farm in Motobas, Kafr El-Sheikh governorate. Prior to the start of the experiment, fingerlings were placed in a fiberglass tank and randomly distributed into glass aquaria to be adapted to the experimental condition until starting the experiment. Fish were fed on the control diet for two weeks, during this period healthy fish at the same weight replaced died ones. All the experimental treatments were conduced under an artificial photo period equal to natural light/darkness period (12 h light: 12 h darkness).

Experimental diets

Five experiment diets were formulated to contain treated torpedo grass, *P. repens* L. to substitute 0, 25, 50, 75 and 100% of the diet yellow corn, and biologically evaluate through 12 weeks of experimental period. The chemical analysis of untreated or biodegraded Torpedo Grass, (TG) *Panicum repens* L. (Table 1). The basal and tested diets were formulated from the commercial

Table 2. Feed ingredients (%) of the experimental diets.

Feed ingredient	Diet No.				
	D1 Control	D2 25% TG	D3 50%TG	D4 75%TG	D5 100%TG
Herring fish meal	12	12	12	12	12
Soybean meal	32	32	32	32	32
Yellow corn	36	27	18	9	0
Wheat bran	15	15	15	15	15
Sunflower oil	3	3	3	3	3
Vitamins and minerals premix[1]	2	2	2	2	2
Torpedo Grass, *Panicum repens*	0	9	18	27	36
Total	100	100	100	100	100

[1]Vitamins and minerals premix at 2 % of the diet supplies the following per kg of the diet: 75000 IU Vit.A; 9000 IU Vit. D3; 150 mg Vit. E; 30 mg Vit. K3; 26.7 mg Vit. B1; 30 mg Vit. B2; 24.7 mg Vit. B 6; 75 mg Vit.B12; 225 mg Nicotinic acid; 69 mg Pantothenic acid; 7.5 mg Folic acid; 150 mg vit. C; 150 mg Biotien; 500 mg Choline chlorid 300 mg DL-methionine; 93 mg Fe; 11.25 mg Cu; 210 mg Zn; 204 mg Mn; 5 mg Se and Co 5 mg (Local market).

feed ingredients. The dry ingredients were grounded through a feed grinder to very small size (0.15 mm). Experimental diets were formulated (Table 2) to be isocaloric and isonitrogenous (about 29.32% crude protein and about 438.56 kcal GE/100 g diet.

The ingredients were weighted and mixed by a dough mixer for 20 min to homogeneity of the ingredients. The estimated amount of oil components (sunflower oil) was gradually added (few drops gradually) and the mixing operation was continued for 20 min. The diets were pelleted through fodder machine and the pellets were dried under room temperature. The diets were collected, and stored in plastic bags in refrigerator at 4° during the experimental period to avoid the deterioration of nutrients.

Experimental design of rearing fish

A total of 150 Nile tilapia, *O. niloticus* fingerlings with an average initial body weight about 10.16 g ± 0.09 were randomly divided into five treatment groups and stocked into 15 glass aquaria (70 liter each). Three aquaria were assigned for each treatment.

Fresh tap water was stored in fiberglass tanks for 24 h under aeration for dechlorination. One third of all aquaria were replaced daily. Five air stones were used for aerating the aquaria water. Water temperature ranged between 26 - 27°C. Fish feces and feed residues were removed daily by siphoning. Fish from each replicate were weighted at the start of each experiment and hencefore counted and weighted every two weeks through out the experimental period (12 weeks).

Fish in all treatment were daily fed the experimental diets at a level of 5, 4 and 3% of the body weight daily for the 1-4, 5-8 and 9-12 week, respectively. The feed amount was given three times daily (9 00, 1200 and 1500) in equal proportions, six days a week for 12 weeks. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new weight.

Chemical analysis

The chemical analysis of ingredients, diets and fish samples were analyzed according to AOAC (1990) for dry matter, crude protein, ether extract, crude fiber and ash. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993) .

Measurements of water parameters

Water samples were taken each week for ammonia and pH analysis. Analytical methods were done according to the American Public Health Association (APHA) (1985). The pH values were determined by (A digital pH-meter). Water temperature and oxygen level were measured daily at 8 o clock by (Oxygen meter model 9070).

Blood parameters

Blood samples were collected at the end of experiment, fish in each aquarium were weighted and 5 fish were taken randomly for blood sampling. The blood was collected using heparinized syringes from the caudal vein. Blood samples were centrifuged at 4000 rpm for 20 min to allow separation of plasma which was subjected to determine plasma total protein (Tietz, 1990). Blood plasma total lipids were determined according to the method of McGowan et al. (1983). Glucose concentration was determined according to Trinder (1969). Alanine aminotransferase (ALT) and activity of aspartate aminotransferase (AST) were determined by the methods of Young (1990).

Preliminary economical efficiency

Preliminary economical evaluation of the experimental diets has been calculated based on the cost of one kg fish weight gain produced (in LE), using feed conversion rate and the price of feed ingredients in local markets during July, 2008. The prices were 10, 2.5, 1.25, 2.00, 7.00 and 8.00 LE/kg, for fish meal, soybean meal, wheat bran, yellow corn, sunflower oil and vitamins and minerals premix, respectively, while treated torpedo grass costs about 0.5 LE/kg for cutting and collected.

Statistical analysis

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F-test was significant, least significant difference was calculated according to

Table 3. Degradation of torpedo grass, (TG) *Panicum repens* L. materials by the microorganisms via measuring clear zone.

Microorganism	Diameter of clear zone (mm)	
	Carboxymethyl cellulose	*Panicum repens L.*
Pleurotous ostreatus	48	34
Agaricus bisporus	40	25

Duncan (1955).

RESULTS AND DISCUSSION

Two mushroom strains comprising *Pleurotous ostreatus* and *Agaricus bisporus* were tested for cellulase production on MSA (mineral salt agar) supplemented with carboxymethylcellulose as substrate by using clear zone formation on agar plates. After that, the both strains were then tested for their ability to grow and degrade torpedo grass; *P. repens* L. in MSL medium. Fungi are well-known agents of decomposition of organic matter in general and cellulose substrates in particular (Lynd et al., 2002).

Results in Table 3 shows that the strains were tested for their growth ability on MSL supplemented with the torpedo grass as a sole source of carbon. The general trend of biodegradability with the two strains of carboxymethyl cellulose was > torpedo grass, (TG) *P. repens* L. Carboxymethyl cellulose exhibited the highest degree of bioconversion followed by torpedo grass, (TG) *P. repens* L. because the diameter of clear zone value was wider than torpedo grass, (TG) *P. repens* L. Carboxymethyl cellulose was a more favourable carbon source for screening the cellulolytic microorganisms. On the other hand, torpedo grass, (TG) *P. repens* L. exhibited the lowest degree of bioconversion by the both strains and this may be depending on cellulose type (amorphous or crystalline) acting on the organism (Ortega et al., 2001).

Among two mushroom strain, one strain was identified as *Pleurotous ostreatus* exhibited relative high clearing of plates which was supplemented with carboxymethyl cellulose as substrate for cellulase. This indicates that this strain is the highest degradability for the tested torpedo Grass, (TG) *P. repens* L. than the *Agaricus bisporus*. The obtained results were compared with the growth of the isolates in MSL (no torpedo grass, (TG) *P. repens* L.). *Pleurotous ostreatus* is known as a very good producer of cellulases, perhaps due to the different adaptability of fungi to the anthropogenic substrates and different resistance to the factors affecting fungal populations during the recycling procedures. Our results are in agreement with previous findings reported by (Teather and Wood, 1982; Bradner et al., 1999; Peciulyte, 2007; Belal, 2008).

Therefore, *Pleurotous ostreatus* as efficient for productivity of extracellular cellulase was selected for the further experiments.

Effect of pH and temperature on growth of *Pleurotous ostreatus* and its cellulase (CMCase) production

Environmental factors do not only influence the torpedo grass to be degraded, they also have a crucial influence on the microbial population and on the activity of the different microorganisms themselves and also the amount of the enzyme production depends on the biomass and so these conditions must be considered when the biodegradability of torpedo grass is tested. Belal (2008) reported previously that, factors such as temperature and pH, have important effects on the microbial degradation of wastepapers by *Trichoderma viride*. Karpouzas and Walker (2000) reported that the degradation of ethoprophos by *Pseudomonas putida* strains epI and II affected by pH and temperature. The question is now, what are the optimal conditions (pH and temperature) for the growth of *Pleurotous ostreatus* and its cellulase (CMCase) production? To determine the optimal growth conditions, carboxymethyl cellulose was used as a sole source of carbon.

Optimum pH

The influence of pH on biomass yield of *Pleurotous ostreatus* and its cellulase (CMCase) production is shown in Figure 1. Generally, the optimum pH was 6 for *Pleurotous ostreatus*. The maximum mycelial dry weight for *Pleurotous ostreatus* and its cellulase (CMCase) production were recorded at pH6. *Pleurotous ostreatus* grew at quite wide pH range (from 4 to 8). This variation is very useful to use these isolates in degradation test in different environments at different pH. Therefore, it can expect that these isolates can tolerate the pH change during the degradation process thereby increase the degradation potential for these isolates.

Optimum temperature

The effect of different temperatures on growth of *Pleurotous ostreatus* and its cellulase (CMCase) production is shown in Figure 2, respectively. A temperature 25°C appears to be the optimum for growth of *Pleurotous ostreatus* and its cellulase (CMCase) production. *Pleurotous ostreatus* and its cellulase (CMCase) production exhibited growth and cellulase CMCase) production at different temperatures but *Pleurotous ostreatus* did not grow at 40°C. Therefore, this (strain

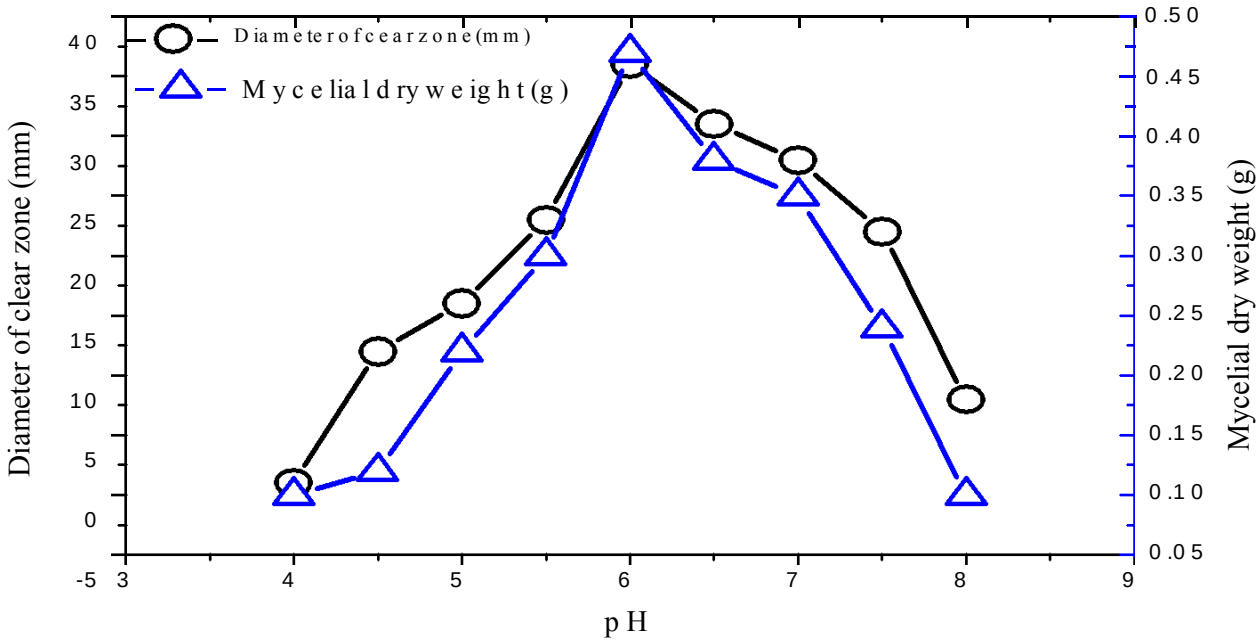

Figure 1. Effect of pH on growth of *Pleurotous ostreatus* and its cellulase (CMCase) production by measuring clear zone.

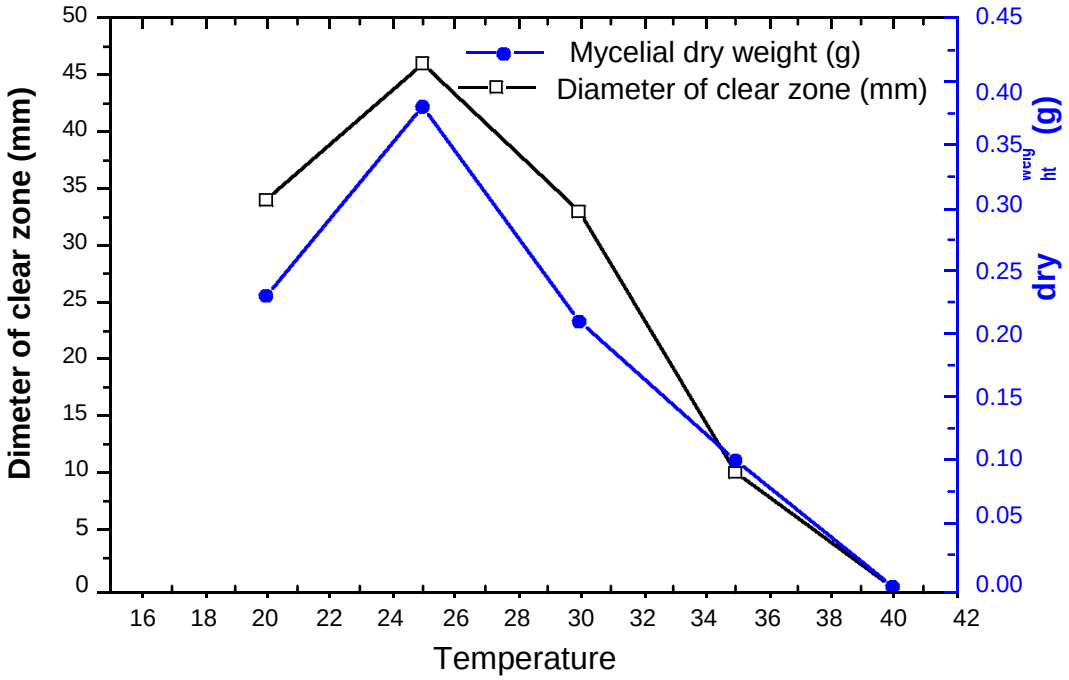

Figure 2. Effect of temperature (°C) on growth of *Pleurotous ostreatus* and its cellulase (CMCase) production by measuring clear zone.

was used for further studies under the optimum growth conditions with the aim of the effect of different carbon source on growth *Pleurotous ostreatus* and enzyme induction as well as determination of the degradation potential for the wastepaper material under solid state fermentation.

Regulation of enzyme production (constitutive or inductive enzyme)

According to Schlegel (1992) most enzymes systems involved in substrate degradation are inductive enzymes. Therefore it is of interest to know, if the torpedo grass,

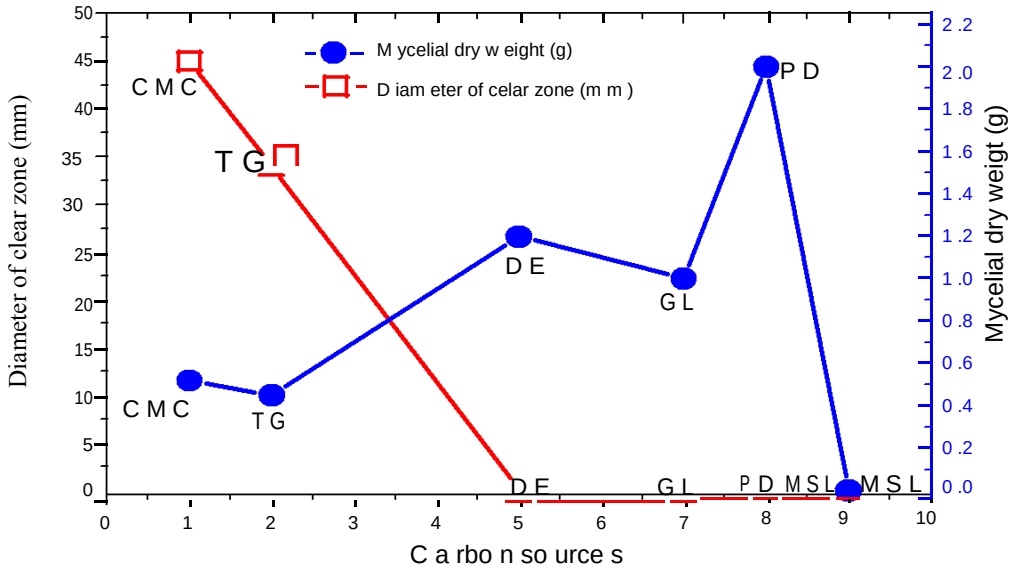

Figure 3. Effect of different carbon sources on growth of *Pleurotous ostreatus* and its cellulase (CMCase) induction, where: CMC - MSL medium + Carboxymethyl cellulose, TG - MSL medium + Torpedo Grass, (TG) *Panicum repens L.*, DE - MSL medium + Dextrose, GL – MSL medium + Glucose, PD – Potato dextrose liquid medium as complex medium and MSL medium plus *Pleurotous ostreatus* without any carbon source

(TG) *P. repens* L. degrading enzyme system is constitutively secreted or induced by the presence of torpedo grass, (TG) *P. repens* L. or other carbon source. Furthermore it is of interest, if the enzyme is inducible, what are the substances inducing the enzyme activity? Figure 3 shows that the fungal growth (determined as mycelial dry weight) was high on PD, followed by MSL + dextrose, MSL + glucose, MSL + carboxymethyl cellulose, MSL + torpedo grass, (TG) *P. repens* L. and latter on MSL without carbon source.

Figure 3 indicates that the extracellular cellulase was produced only during growth of *Pleurotous ostreatus* on carboxymethyl cellulose or torpedo grass, (TG) *P. repens* L. in MSL medium as a sole of carbon source. The results demonstrated that a maximum cellulase activity was obtained when carboxymethyl cellulose followed by torpedo grass, (TG) *P. repens* L. was used as substrate. The low enzyme activities measured with torpedo grass, (TG) *P. repens* L. On the other hand in PD as a complex medium or in MSL + dextrose and MSL + glucose, enzyme secretion is not induced despite the media generated a good cell growth. This results are in agreement with my previous findings and other investigators while secretion of (PCL- hydrolase was only induced in the culture supernatant with PCL as aliphatic homopolyester or BTA 45:55 (Ecoflex) as copolyester as substrates but was not induced on glucose or GYM as complex medium (Lin and Kolattukudy, 1978; Murphy et al., 1996; Belal, 2003) and cellulase was only induced in the culture supernatant with wastepaper materials and carboxy methylcellulose as substrates but was not induced on glucose or PD as complex medium (Belal, 2008).

Saccharification of torpedo grass, (TG) *Panicum repens* L. by the *Pleurotous ostreatus* cellulase

Aside from the traditional methods of waste management, biowaste has been used in the production of clean energy where it replaces coal, oil or natural gases to generate electricity through combustion. The conversion process of wastes to energy has been proved to be safe, environmental friendly and reduces the incoming volume of waste to a great extent. An alternative to the combustion of biowaste could be through the fermentation of saccharified waste cellulose into bioproducts.

An initial increasing trend of sugar formation was observed when more of torpedo grass, (TG) *P. repens* L. substrate was degraded with a fixed enzyme concentration (Table 4). Carboxymethyl cellulose showed more bioconversion than the torpedo grass, (TG) *P. repens* L. The trend of biodegradability with *Pleurotous ostreatus* cellulase of carboxymethyl cellulose was > torpedo grass, (TG) *P. repens* L. Due to the structural composition of *P. repens* L. it can be biodegraded into fermentable sugars. The variation in torpedo grass and carboxymethyl celluloase bioconversion by *Pleurotous ostreatus* cellulase could be due to the composition of the enzyme system as well as the structure of cellulose, this consists of a crystalline section, which is difficult to hydrolyze, and an amorphous section that is more susceptible to cellulase attack (Van Wyk and Mohulatsi, 2003). The present study showed also that, the trend of biodegradability of torpedo grass with cellulase was more than the trend of biodegradability of torpedo grass, (TG) *P. repens* L. by *Pleurotous ostreatus* because after 14 days of cultivation

Table 4. Degradation of torpedo grass, (TG) *P. repens* L. by *P. ostreatus* cellulose.

Treatment	Activity of cellulase (U/ml/min) after the incubation period (at 50°C for 30 min)	Activity of *P. ostreatus* cellulase (U/ml/min) after the incubation period (14 days at 25°C and 150 rpm)
Carboxymethyl cellulose	3.4	3.1
P. repens L.	2.5	2.1

Table 5. Chemical analysis (%) of the experimental diets.

Ingredient	Treatment[4] (On DM basis, %)				
	T1 Control	T2 25% TTG	T3 50%TTG	T4 75%TTG	T5 100%TTG
Dry matter	90.07	90.24	90.40	90.55	91.10
Crude protein	29.53	29.35	29.30	29.26	29.18
Ether extract	6.37	5.90	5.36	4.88	4.36
Crude fiber	4.73	5.22	5.86	6.54	7.26
Total ash	4.66	5.62	6.73	7.83	8.92
Nitrogen free extract	54.71	53.91	52.75	51.49	50.28
Calculated energy value					
GE (kcal/kg)[1]	4579	4491	4388	4287	4183
DE (kcal/kg)[2]	3434	3368	3291	3215	3137
P/E, mg/kcal[3]	85.99	87.14	89.03	91.01	93.02

[1]GE (Gross energy) was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 K cal per gram of protein, lipid and carbohydrate, respectively .
[2]DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher et al. (1983).
[3]P/E (protein energy ratio) = crude protein x 10000 / digestible energy, according to Hepher et al. (1983).
[4]Treatments: T1 (control): 0% treated Torpedo grass, T2: 25% treated Torpedo grass, T3: 50% treated Torpedo grass, T4: 75% treated Torpedo grass, T5: 100% treated Torpedo grass.

Pleurotous ostreatus, the production of reducing sugar was almost (enzymatic activity measured-by the production of reducing sugars end group, which is taken to be an indication of cleavage of cellulose molecules) low to which produced with cellulase after the incubation period (at 50°C for 30 min).

It is of interest that isolation and purification as well as characterization of *Pleurotous ostreatus* cellulase in the next study and using it in different industrial purposes as well as in bioconversion other cellulolytic materials such as agricultural wastes.

In most investigations, members of the fungal genus *Trichoderma* have been extensively studied due to their ability to secrete cellulose-degrading enzymes. Most of the works have been carried out on *T. aureoviride* Rifai, *T. viride* Pers., *T. reesei* E. G. Simmons, *T. harzianum* Rifai strains and their mutants evaluating their ability to produce extracellular cellulolytic enzymes (endoglucanases, exoglucanases and cellobiase) which act synergistically in the conversion of cellulose to glucose. The cellulases secreted by *Trichoderma* have received widespread industrial interest leading to commercial applications (Olson and Hahn-Hagerdahl, 1997; Oksanan et al., 2000; Mach and Zeilinger, 2003; Cavaco-Paulo and Gübitz, 2003; Nierstrasz and Warmoeskerken, 2003; Van Wyk and Mohulatsi, 2003; Penttila et al., 2004; Belal, 2008).

It is of interest to degrade of dried –milled of torpedo grass, (TG) *P. repens* L. by *Pleurotous ostreatus* in solid state fermentation under 25°C for 32 days. *Pleurotous ostreatus* showed good mycelial growth on, torpedo grass, (TG) *P. repens* L. biomass at end of the incubation time. The produced biomass (bioprocessed materials) contained high numbers of from *P. ostreatus* which contained (10^5 cfu/ml).

It was of a particular interest to use the produced biomass (bioprocessed materials) as a non conventional feedstuff in diets of Nile tilapia, *O. niloticus* fingerlings.

Chemical composition of diets

Experimental diets in Table 5 contain nearly similar levels of DM, CP, EE, CF, Ash, NFE, GE, DE and P/E ratio. The CP and GE content of experimental diets were around 29.32% and 4.39 kcal/g, respectively. These values were

Table 6. Growth performance parameters of Nile tilapia (*O. niloticus*) fed on the experimental diets.

Item	Treated torpedo grass (%)					SE*
	Control (T_1)	25 (T_2)	50 (T_3)	75 (T_4)	100 (T_5)	
Initial weight, g/fish	10.11	10.20	10.17	10.15	10.19	0.12
Final weight, g/fish	47.91[a]	46.32[a]	42.09[ab]	38.71[b]	37.07[b]	0.80
Average total gain[1], g/fish	37.80[a]	36.12[a]	31.92[b]	28.56[b]	26.88[c]	1.10
Average daily gain[2], g/fish/day	0.45[a]	0.43[a]	0.38[ab]	0.34[b]	0.32[b]	0.07
Specific growth rate[3] (SGR % /day)	1.85[a]	1.80[a]	1.69[ab]	1.59[b]	1.54[c]	0.11
Survival rate[4], %	100	100	95	90	90	0.02
Feed intake (FI), g/fish	50.51[a]	49.73[a]	48.61[a]	44.82[b]	43.62[b]	0.54
Feed conversion ratio[5] (FCR)	1.20[c]	1.24[bc]	1.38[b]	1.42[ab]	1.48[a]	0.15
Protein efficiency ratio[6] (PER)	2.81[a]	2.74[ab]	2.48[b]	2.40[bc]	2.32[c]	0.14
Protein productive value[7] (PPV, %)	50.11[a]	48.14[a]	41.46[b]	40.15[b]	36.90[c]	1.38
Energy retention[8] (ER, %)	28.56[a]	27.66[a]	24.94[b]	24.91[b]	24.13[b]	1.54

a, b and c means in the same rows bearing different letters differ significantly at 0.05 level.
* Standard error of the mean derived from the analysis of variance.
1. ATG (g/fish) =Average final weight (g) – Average initial weight (g). 2. ADG (g/fish/day) = [ATG (g)/experimental period (d)]. 3. SGR (%/day) = 100(Ln final weight–Ln initial weight)/experimental period (d). 4. SR =100[Total No of fish at the end of the experimental/Total No of fish at the start of the experiment]. 5. FCR = DM Feed Intake (g)/Live weight gain (g). 6. PER = Live weight gain (g)/ Protein intake (g). 7. PPV (%) =100 [Final fish body protein (g)–Initial fish body protein (g)]/crude protein intake (g). 8. ER % = 100 [gross energy gain / gross energy intake]

within the range suggested for tilapia by (Jauncey and Ross, 1982; NRC, 1993).

Water quality

Results showed that, the average values of water quality parameters were 26-27°C, 7.8- 8.1, 5.85-6.40, 0.12- 0.15 mg/L, for water temperature, pH, dissolved oxygen and water ammonia, respectively.

It is well known that fish are cold- blooded animals; therefore, environment has more effect on fish compared to other livestock animals. The averages of water temperature, pH, dissolved oxygen and water ammonia content were suitable for growth of tilapia, *O. niloticus*. In this respect, Degani et al. (1988) observed that, the optimum water temperature for *Oreochromis aureus* was ranged between 24 and 31°C. Reite et al. (1974) indicated that tilapia tolerated a range of pH between 5 – 11 which had no ill effect in 24 h test period, while, at pH lower than 3-5 or above 12 caused 100% mortality within 2-6 h for brackish water. Kamal et al. (2004) reported that, levels of dissolved oxygen above 4 ppm are considered a limiting value for a suitable feeding and growth of fish. European Inland Fisheries Advisory Commission (1973) reported that the toxic level of ammonia to fish is 2 mg/L.

Growth performance and survival rate

Data in Table 6 show the growth performance and nutrient efficiencies on Nile tilapia fingerlings fed diets containing different levels of treated torpedo grass, *P. repens L*.

Results showed that, average initial live body weight of Nile tilapia among the different experimental treatments ranged between 10.11 and 10.20 g. Statistical analysis showed that no significant differences (P>0.05) in initial body weight were found among the different experimental treatments, indicating the accuracy of randomization process between the experimental treatments.

It is clearly shown (Table 6) that although averages of initial weight of fish for the different experimental group were the same, however. All the tested growth parameters (gain, ADG and SGR) showed that, the group fed diet containing 25% substitution of yellow corn by treated torpedo grass (T_2) surpassed all other groups fed treated torpedo grass (T_3, T_4 and T_5). On the other hand, the group of fish fed diet containing 100% treated torpedo grass substitution of yellow corn (T_5) exhibited the lowest final body weight. Statistical analysis showed that, the group of fish fed diet containing 25% substitution of yellow corn by treated torpedo grass (T_2) had significantly ($P<0.05$) higher value than those of 75 and 100% levels of substitution, but not significantly higher than 50% level of substitution (T_3) . Also no significant differences was observed between fish group fed diet containing 100% yellow corn (T_1) and group of 25% treated torpedo grass (T_2).

The results obtained from the present study showed that partial replacement (about 25%) of the yellow corn could be replaced by treated torpedo grass, without deleterious effect on growth, and when the level of substitution was increased to 50, 75 or 100% there was a reduction in fish growth. Survival rate of the experimental

fish groups was within the normal range. It recorded 100% for fish fed diets 1 (control) and 2 (25% treated torpedo grass), but fish fed diet 3 (50% treated torpedo grass) which gave 95% survival rate, also fish groups fed diets containing 75 and 100% treated torpedo grass gave 90% survival rate (Table 6).

Results of feed intake and nutrient utilization in terms of feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) are illustrated in Table 6. Replacing yellow corn by treated torpedo grass at different levels showed that, the group of fish fed diet containing 100% yellow corn (T_1) exhibited significantly ($P<0.05$) better FCR (1.20) than those of other treatments 50, 75 and 100% levels of substitution (1.38, 1.42 and 1.48, respectively) but not significantly better than the groups of 25% level of substitution (1.29). There were no significant differences ($P>0.05$) among T_2 and control (T_1). The same trend was observed for protein efficiency ratio, protein productive value and energy retention.

Many measurements of anti-nutrients reported before for the tropical plant materials have mostly been done in sun-dried (or oven-dried) plant material samples. The content and activity of antinutrients in those materials might have been considerably altered during sun and oven drying. Growth reduction caused by supplementation with synthetic antinutrients (or with known amounts of naturally occurring antinutritional substances) have confirmed their harmful effects to fish (Johnson et al., 1986; Bureau et al., 1998; Shimoyamada et al., 1998; Makkar and Becker, 1998; Becker and Makkar, 1999; Dongmeza et al., 2006).

Tan (1970) fed several types of vegetation to grass carp in ponds and found Hydrilla verticillata to be an excellent feed. Fish fed Napier grass (*Penisetum porporem*) and cassava leaves grew slower than those fed hydrilla. The superiority of hydrilla as food was attributed in that study to the soft nature of the plant (low fibre content) and high ash (mineral) content. It has been found that the concentration of minerals was proportional to the ash content in plant materials (aquatic weeds, sweet potato and cassava leaves, guinea and napier grasses); especially those samples that were used as supplementary feed for the grass carp by Tan (1970).

Dongmeza et al. (2009) studied the quality of two groups of plant residues used as fish feed. The first group was constituted of residues commonly fed to fish, such as cassava (*M. esculenta*), banana (*Musa nana*), and bamboo (*B. vulgaris*) leaves, and the second group included residues occasionally fed to fish by farmers, such as barnyard grass (*Echinochloa erusgalli*), mixed weeds from paddy fields, Elephant grass (*Pennisetum purpureum*), mulberry (*Morus*), maize (*Zea mays*), sweet potato (*Ipomoea batatas*), peanut (*A. hypogaea*). Results of proximate analysis indicated the high potential of some of these plant materials such as cassava and mulberry leaves as fish feed because of their higher protein and energy content. However, the protein and energy content of these leaves were generally very low when compared to that of the common standard fish feed. Thus, these plant feedstuffs alone may not be sufficient to cover the requirements for rapid growth in cultured grass carp. The data presented here could be used for formulating cost effective and balanced animal feeds for the use of small-scale farmers in rural areas in Northern Vietnam. Many plant leaves among those analyzed in this study can be used as fish feed, especially for grass carp. Cassava and mulberry leaves have high potential as they showed high CP and lower NDF content. However, because of the presence of antinutrients in the cassava leaves, it might need to be detoxified before being fed to fish. Practical methods have been developed for reducing the cyanide content of the cassava leaves (Ng and Wee, 1989): the cassava leaves could be soaked before being fed to fish or soaked and sun-dried and incorporated in pelleted diet for fish. Wood ash, easily available in rural areas, was found to be an efficient cost- effective way to deactivate tannins (phenolic compounds) in plant leaves (Ben Salem et al., 2005a). Simple physical techniques such as chopping, water soaking or storage under anaerobic conditions were also found to be efficient in reducing the tannin content of foliage (Ben Salem et al., 2005b). Organic solvent extraction could considerably remove the antinutrients (phenolic and saponin) and improve the palatability of plant leaf materials, leading to a better feed intake and growth (Wee and Wang, 1987; Ng and Wee, 1989; Makkar and Becker, 1996; Francis et al., 2001; Siddhuraju and Becker, 2002, 2003; Afuang et al., 2003; Dongmeza et al., 2006).

Biochemical blood parameters

Results in Table 7 shows that blood plasma glucose, total protein and total lipids were not significantly affected ($P>0.05$) by the different levels of substitution yellow corn by treated torpedo grass. It was clear that, increasing of treated torpedo grass levels in tilapia diets caused a slight decrease in plasma glucose, total protein and total lipid.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed insignificantly ($P>0.05$) activity in fish fed diets containing different levels of treated torpedo grass. However, it was slight decreased in control diet (T_1). These results suggested that, treated torpedo grass may influence through its component to unaffected on liver function. Also all values of pervious blood parameters were within the normal range reported by (Abd Elmonem et al., 2002; Shalaby, 2004; El-Dakar 2004) in Nile tilapia.

Body composition

Body chemical composition of Nile tilapia fish fed varied levels of treated torpedo grass is shown in Table 8. No significant differences ($P>0.05$) were observed for dry

Table 7. Blood plasma parameters of Nile tilapia fed different levels treated Torpedo grass.

Item	Treated Torpedo Grass (%)					SE*
	Control (T_1)	25 (T_2)	50 (T_3)	75 (T_4)	100 (T_5)	
Plasma glucose, mg/dl	58.11	57.22	55.80	54.22	54.47	0.45
Plasma total protein, g/dl	5.33	5.28	4.86	4.56	4.42	0.15
Plasma total lipid, g/dl	4.55	4.37	4.39	4.34	4.52	0.12
AST, U/dl	130	132	135	133	137	3.45
ALT, U/dl	50	53	54	56	55	1.58

*Standard error of the mean derived from the analysis of variance.

Table 8. Effect of treated Torpedo grass (%) on Nile tilapia body composition (%, on DM basis).

Item	Initial fish	Treated torpedo grass (%)					SE*
		Control (T_1)	25 (T_2)	50 (T_3)	75 (T_4)	100 (T_5)	
Dry matter, %	22.82	27.66	27.35	26.68	26.98	26.24	0.35
Crude protein, %	56.16	60.58	60.35	59.11	58.08	57.48	1.25
Ether extract, %	14.45	18.59	17.52	17.63	17.26	18.08	0.87
Ash, %	13.15	15.48	15.26	14.89	15.18	14.65	0.12
Energy, Kcal/100g	522	540	536	536	531	537	1.10

*Means of the standard error derived from the analysis of variance.

Table 9. Preliminary economic efficiency for production of 1 kg gain of Nile tilapia fed the different treatments.

Item	Treated torpedo grass (%)				
	Control (T_1)	25 (T_2)	50 (T_3)	75 (T_4)	100 (T_5)
Cost* of one ton of feed (L.E)	3317.5	3191.5	3065.5	2939.5	2813.5
Reduction in feed cost, %	0.00	126	252	378	504
Feed intake g/fish	50.51	49.73	48.61	44.82	43.62
Total gain g/fish	37.80	36.12	31.92	28.56	26.88
Cost of 1 Kg fish gain (L.E)	4.43	4.39	4.67	4.61	4.57
Cost of 1 Kg fish gain relative to control	100	99.10	105.42	104.06	103.16

*Costs were as common commercial feeds in local markets during 2008/2009. Costs of 1 kg of fish meal, soybean meal, wheat bran, yellow corn, sunflower oil, vitamins and minerals premix and Torpedo Grass were 10, 2.5, 1.25, 2, 7and 10 LE, respectively, while treated Torpedo Grass costs about 0.5 LE/kg for cutting and collected.

matter, crude protein, ether extract, ash and energy content. Also, fish at the start of the experiment had lower dry matter, crude protein, ether extract, ash and energy contents.

Preliminary economical efficiency

Successful and sustainable aquaculture depends on economically viable and environmental friendly feeds. Feed is the major operational cost involving 50 to 60% of the total production costs in intensive farming (Collins and Delmendo, 1979). Under the present experimental condition all other costs are constant, accordingly, the feeding costs to produce one kilogram of fish body weight gain could be used as a comparison parameter between treatments.

The cost of producing one ton of mixed feed and the cost of producing one kg fish gain in LE from each diet are presented in Table 9. The calculated figures in this experiment showed that, the inclusion of treated torpedo grass in fish diets reduced the cost of producing one ton mixed feed. This reduction is dependent on the replacement level of treated torpedo grass. The results obtained from the present study showed that, the cheapest diets for producing one kg fish gain was T_2 (25% level of replacement), which was 4.39 LE while, the control diet (100% yellow corn) was 4.43 LE. The highest feed cost

to produce one kg fish gain was T_3 (50% level of replacement), which was 4.67 LE.

Conclusion

From the results of the present study**,** fish fed diet containing 25% treated torpedo grass substitution of yellow corn (T_2) gave a positive response to growth performance, feed conversion, nutrient utilization, protein efficiency and economical efficiency.

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