Egyptian J. Nutrition and Feeds (2010) 13 (1) :177-191

USE OF THE BIOPROCESSED TORPEDO GRASS *PANICUM REPENS L.* AS A NON CONVENTIONAL FEEDSTUFF IN DIETS OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* FINGERLINGS.

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(Received 5/1/2010, Accepted 27/3/2010)

SUMMARY

Biological degradation of lignocellulosic biomass of Torpedo grass, *Panicum repens L.* and suitability of using the highly digestible, protein-enriched, as a non conventional feedstuff in diets of Nile tilapia, *Oreochromis niloticus*, fingerlings were investigated. Using a method based on clear zone formation on agar plates, two strains from white-rot fungi *Pleurotus ostreatus* and *Agaricus bisporus* were tested for cellulose production using carboxymethyl cellulose as substrate. *Pleurotus ostreatus* exhibited wider clear zone than *Agaricus bisporus*. *Pleurotus ostreatus* was selected as a potential cellulase producer. The solid state fermentation (SSF) of Torpedo grass, *Panicum repens L.* with *Pleurotus ostreatus*, was carried at the optimized cultural conditions (pH 6 and 25°C for 32 days). Protein contents of the biodegraded Torpedo grass, *Panicum repens L.* after fungal growth increased from 7.52 to 8.91 % and crude fiber contents decreased from 23.27 to 11.28. This degraded biomass was used as a non conventional feedstuff in diets of Nile tilapia, *Oreochromis niloticus* fingerlings

Five experimental diets were formulated to contain biodegraded Torpedo grass, *Panicum repens L.* to substitute 0, 25, 50, 75 and 100 % of the diet yellow corn, and biologically evaluated through 12 weeks experimental period. All formulated diets were isocaloric (about 4385 kcal/kg DM) and isonitrogenous (about 29.32% CP). A total number of 150 Nile tilapia fingerlings with average initial weight of 10.16±0.09 g/fish were randomly distributed into five treatment groups and stocked in 15 glass aquaria (70 liter each). The results showed that tilapia fingerlings received diets containing 25% treated Torpedo grass showed the best results in growth parameters, feed efficiency and economic efficiency. Also no significant differences (P>0.05) were observed among all fish groups fed the experimental and control diets in chemical composition of the whole body fish in dry matter, crude protein, ether extract, ash and energy contents and blood plasma content. It could be concluded that replacing 25% of yellow corn by Torpedo grass in diet of Nile tilapia showed no adverse effect on growth performance.

Keywords: Nile tilapia, Torpedo grass, Pleurotus ostreatus,, Panicum repens L., growth parameters, biodegradation

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 9000 ton in 1980 to 595030 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.

The use of non-conventional sources as adventives, like rhizomatous grass species that has become an invasive weed of terrestrial, wetland and aquatic environments in tropical and subtropical regions worldwide can help in part to minimize the feed shortage gap. *Panicum repens L.* is a perennial grass that frequently forms dense colonies and has long, creeping rhizomes.

Various abundantly available lignocellulosic wastes may be used as cheap substrates for production of protein-enriched food and feed. Many authors have shown that some fungi, particularly some species of *Pleurotus* are able to colonize different types of lignocellulsic wastes, which increase their digestibility (Mukherjee and Nandi, 2004 and Salmones et al. 2005).

Carboxymethylcellulose showed more bioconversion than the Torpedo Grass, (TG) *Panicum repens L.*. Due to the structural composition of Torpedo Grass, (TG) *Panicum repens L.* it can be biodegraded into fermentable sugars. The variation in Torpedo Grass, (TG) *Panicum repens L.* and carboxymethylcelluloase 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, (TG) *Panicum repens L.* with cellulase was more than the trend of biodegradability of Torpedo Grass, (TG) *Panicum repens L.* by *Pleurotous ostreatus* because after 14 days of cultivation *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 cellulose after the incubation period (at 50°C for 30min).

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 (Penttila *et al.*, (2004) and Belal (2008).

Upto our knowledge, little work has been conducted to assess the nutritional potential of the plant feed ingredients currently used as feed for stomachless fish in Egypt. Torpedo grass, *Panicum repens L.*, a widely prevalent enormously fast-growing aquatic weed, and it can be a non conventional feedstuff in diets of *Oreochromis niloticus*, fingerlings, although it is used in feeding 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 diet by biodegraded Torpedo Grass, *Panicum repens L.* with white-rot fungus, *(Pleurotus ostreatus)* on growth performance, feed utilization, body composition, hematological parameters and preliminary economical evaluation.

MATERIALS AND METHODS

This study has been carried out at the Wet Fish Laboratory, Department of Animal Production and the Agricultural Microbiology branch, Agricultural Botany Department, 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 *Pleurotus ostreatus* was obtained from Faculty of Agriculture, Ain Shams University, Egypt .Both strains were tested for their ability to grow and degrade Torpedo grass, *Panicum repens L.* in MSL medium. The dried Torpedo grass, *Panicum repens L.* was milled. 100ml MSL medium containing 10g/L from dried-milled Torpedo grass, *Panicum 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 isolate (control).

The cultures were shaken at 150rpm 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 (0.2μm). 50μl of culture supernatant was added in wells (5mm 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 Peciulyte (2007) and Belal (2008).

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 μg/ml, while the enzyme concentration was determined according to

Lowry *et al.,* (1951) with 0.5 ml of an amount 10g/L of dried-milled Torpedo grass, *Panicum repens L.* in 0.05 M citrate buffer (pH4.8) at 50°C for 30min. 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 of reducing sugars (measured as glucose) per ml per min.

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

Dried-milled Torpedo grass, *Panicum repens L.*) (30g) 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 diam.) from culture *Pleurotous ostreatus* (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 at rate 10% as follow: Dried-milled torpedo grass, *Panicum repens L.*) (30g) was placed in 500 ml Erlenmeyer flasks, moistened with distilled water (65%) and autoclaved at 121°C for 20 min, cooled overnight, and inoculated at the rate of 10% from the described biodegraded substrate (10^6 cfu/gm), mixed well and incubated for 32 days at 25 ±1°C. (Belal 2008 and Das and Ghosh, 2009).

Experimental fish:

Nile tilapia (*Oreochromis 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 (12h light: 12h darkness).

Experimental Diets:

Five experimental diets were formulated to contain treated Torpedo grass, *Panicum repens L.* to substitute 0, 25, 50, 75 and 100 % of the diet yellow corn, and biologically evaluate through 12 weeks experimental period.

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

The ingredients were weighted and mixed by a dough mixer for 20 minutes to homogeneity of the ingredients. The estimated amount of oil components (sunflower oil) was gradually added and the mixing operation was continued for 20 minutes. 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 C° during the experimental period to avoid the deterioration of nutrients.

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

Item	Untreated *Panicum repens*, %	Treated *Panicum repens*, %
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 difference, ** Estimated (5.65, 9.45, 4.0 and 4.0 kcal GE/g dry matter for CP, EE, CF and NFE, respectively, (NRC, 1993).

Experimental design of rearing fish:

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

Fresh tap water was stored in fiberglass tanks for 24h 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 hence fore counted and weighted every two weeks throughout the experimental period (12 weeks).

Fish in all treatment were daily fed the experimental diets at a rate of 3% of live body weight per day. The feed amount was given three times daily (9:00, 12:00 and 15:00) 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 A.O.A.C. (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 two days 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 08:00 by an oxygen meter model 9070. Results showed that, the average values of water quality parameters were 26-27°C, 7.8- 8.1, 5.85-6.40 mg/L, 0.12-0.15 mg/L, for water temperature, pH, dissolved oxygen and water ammonia, respectively.

Table (2): Composition and proximate analysis of the experimental diets.

Item	Diet[5] No (On DM basis, %)				
	D1 Control	D2 25% TG	D3 50%TG	D4 75%TG	D5 100%TG
Feed ingredients					
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
Chemical composition %					
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)[2]	4579	4491	4388	4287	4183
DE (kcal/kg)[3]	3434	3368	3291	3215	3137
P/E,mg/kcal[4]	85.99	87.14	89.03	91.01	93.02

[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).

[2]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 .

[3]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).

[4]P/E (protein energy ratio) = crude protein x 10000 / digestible energy, according to Hepher et al., (1983).

[5]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.

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 minutes 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 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 a substrate using clear zone formation on agar plates. Both strains were then tested for their ability to grow and degrade Torpedo grass; *Panicum 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) showed the strains that were tested for their growth ability on MSL supplemented with Torpedo Grass, (TG) *Panicum repens L.* as a sole source of carbon. The general trend of biodegradability of the two strains with carboxymethylcellulose was higher than that with Torpedo Grass, (TG) *Panicum repens L.*. carboxymethylcellulose exhibited the highest degree of bioconversion followed by Torpedo Grass, (TG) *Panicum repens L.* because the diameter of clear zone value was wider than Torpedo Grass, (TG) *Panicum repens L.*. Carboxymethylcellulose was a more favourable carbon source for screening the cellulolytic microorganisms. On the other hand, Torpedo Grass, (TG) *Panicum repens L.* exhibited the lowest degree of bioconversion by both strains and this may be depending on cellulose type (amorphous or crystalline) acting on the organism (Ortega *et al.*, 2001).

Among the two mushroom strains, one strain was identified as *Pleurotous ostreatus* exhibited relative high clearing of plates which was supplemented with

carboxymethylcellulose as substrate for cellulase. This indicated that this strain had the highest degradability for the tested Torpedo Grass, (TG) *Panicum repens L.* than the *Agaricus bisporus*. The obtained results were compared with the growth of the isolates in MSL (no Torpedo Grass, (TG) *Panicum 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 the findings reported by Peciulyte (2007) and Belal (2008).

Besed on the previous results, *Pleurotous ostreatus* as an efficient extracellular cellulose provider was selected as a feedstuff in diets of Nile tilapia.

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

Microorganisms	Diameter of clear zone (mm)	
	Carboxymethyl cellulose	Torpedo Grass, (TG) *Panicum repens L.*
Pleurotous ostreatus	48	34
Agaricus bisporus	40	25

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) *Panicum repens L.* substrate was degraded with a fixed enzyme concentration (Table 4).

Table (4): Degradation of Torpedo Grass, (TG) *Panicum repens L.* by *Pleurotous ostreatus* cellulase

Treatments	Activity of cellulase (U/ml/min) after the incubation period (at 50°C for 30min)	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
Torpedo Grass, (TG) *Panicum repens L.*	2.5	2.1

It is of interest to degrade of dried –milled of Torpedo Grass, (TG) *Panicum repens L.* by *Pleurotous ostreatus* in solid state fermentation under 25°C for 32days. *Pleurotous ostreatus* showed good mycelial growth on, Torpedo Grass, (TG) *Panicum repens L.*

biomass at end of the incubation time. The produced biomass (bioprocessed materials) contained high numbers of from *Pleurotous ostreatus* which contained (10^5cfu/ml).

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

Chemical composition of diets:

Experimental diets in Table 2. contained 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 within the range suggested for tilapia by Jauncey and Ross (1982) and NRC (1993).

Growth performance and survival rate:

Data in Table (5) show the growth performance and nutrient efficiencies on Nile tilapia fingerlings fed diets containing different levels of treated Torpedo grass, *Panicum repens L.*

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) (Table 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 5).

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 (5). 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 antinutrients (saponins, glucosinolates, s-methylcysteine sulphoxide and erucic acid) 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 (Becker and Makkar, 1999 and Dongmeza et al., 2006).

Table (5): Growth performance parameters of Nile tilapia (*O. niloticus*) fed on the experimental diets.

Items	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

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]

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 (*Manihot esculenta*), banana (*Musa nana*), and bamboo (*Bambusa 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 (*Arachis 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. Many plant leaves among those analyzed in this study can be used as fish feed, especially for grass carp.

Biochemical blood parameters:

Results in Table (6) showed 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 treated Torpedo grass level in tilapia diets caused a slight decrease in plasma glucose, total protein and total lipids.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed insignificant (P>0.05) activity in fish fed diets containing different levels of treated Torpedo grass. However, there was a slight decrease in control diet (T_1). These results suggested that, feeding treated Torpedo grassdid not affect liver function. Also all values of pervious blood parameters were within the normal range reported by El-Dakar (2004) in Nile tilapia. .

Table (6): Blood plasma parameters of Nile tilapia fed different levels treated Torpedo grass.

Items	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.

Body composition:

Body chemical composition of Nile tilapia fish fed different levels of treated Torpedo grass is shown in Table (7). No significant differences (P>0.05) were observed for dry 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.

Table (7): Effect of treated Torpedo grass (%) on Nile tilapia body composition (%, on DM basis).

Items	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 (8): Preliminary economic efficiency for production of one 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 relative to control (absolute values L.E.)	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 one Kg fish gain (L.E)	4.43	4.39	4.67	4.61	4.57
Cost of one 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. 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.

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 T2 (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 T3 (50% level of replacement), which was 4.67 LE..

CONCLUSION

Results of the present study showed that fish fed diet containing 25% treated Torpedo grass with substitution of yellow corn (T2) gave comparable results to diet containing 100 % yellow corn concerning growth performance, feed conversion, nutrient utilization, protein efficiency but better economical efficiency.

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استعمال حشيشة الأمشوط المدورة بيولوجيا كمادة غذائية غير تقليدية في علائق أصبعيات البلطي النيلي

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تم دراسة التكسير الحيوي للكتلة الحيوية الليجنوسيليلوزية لحشيشة الامشوط (النسيلة) ومدى الاستفادة منها باستخدامها كمادة غذائية غير تقليدية في علائق أصبعيات البلطي النيلي. باستعمال طريقة الهالة الشفافة بالإطباق ، تم تقييم سلالة من *كل من Pleurotus ostreatus و Agaricus bisporus* باستعمال مادة كربوكسي ميثيل سيليلولوز كمادة تفاعل لإنزيم السيليلوليز. تم اختيار سلالة *Pleurotus ostreatus* والتي أعطت أكبر هالة شفافة عن السلالة *Agaricus bisporus* الأخرى. وقد أوضحت النتائج إن رقم الحموضة 6 وأيضا درجة الحرارة ٢٥° مئوية هما المثلى لنمو فطر *Pleurotus ostreatus* وأيضا إنتاجية إنزيم السيليلوليز. وقد أظهرت الدراسة أن فطر *Pleurotus ostreatus* له قدرة تحليلية لحشيشة الامشوط (النسيلة) مستخدما تكنيك تخمر المواد الصلبة تحت الظروف المثلى للنمو (رقم الحموضة 6 وأيضا درجة الحرارة ٢٥° مئوية وفترة تحضين لمدة ٣٢ يوم). حيث زاد محتوى البروتين للكتلة المتحللة من حشيشة الامشوط (النسيلة) من ٧,٥٢ إلى ٨,٩١% بينما انخفض محتوى الألياف الخام للكتلة المتحللة من حشيشة الامشوط (النسيلة) من ٢٣,٢٧ إلى ١١,٢٨%. وتم استخدام هذه الخام لكتلة المتحللة من حشيشة الامشوط (النسيلة) كمادة غذائية غير تقليدية في علائق أصبعيات البلطي النيلي.

وقد أجريت تجربة لبحث تأثير إستبدال الذرة الصفراء بحشيشة الامشوط (النسيلة) المعامل بيولوجيا على أداء النمو والاستفادة من الغذاء والعناصر الغذائية وكذلك التركيب الكيماوي لجسم الأسماك في نهاية التجربة وتقدير الكفاءة الاقتصادية الأوليه للمعاملات على اسماك البلطي النيلي . تم تركيب خمسة علائق تجريبية متزنة في نسبه البروتين (٢٩,٣٢%) والطاقة (٤٣٨٥ ك كالورى/كجم مادة جافة) لاستبدال صفر و ٢٥ و ٥٠ و ٧٥ و ١٠٠ % من الذرة الصفراء والذى يمثل ٣٦% من العليقة وتم عمل تقييما حيويا خلال فترة التجربة والتى امتدت لمدة ١٢ أسبوع . وقد تم استخدم خمسة عشر أحواض زجاجيه سعة كل منها ٧٠ لتر بواقع ثلاثة أحواض لكل معامله . وتم تخزين عشرة اسماك (بمتوسط وزن ابتدائي ١٠,١٦ جم/سمكه) في كل حوض .

وأظهرت نتائج التجربة أن أصبعيات البلطي النيلي التى تغذت على عليقة تم أستبدال ٢٥% من الذرة الصفراء بحشيشة الامشوط (النسيلة) المعاملة بيولوجيا أعطت أحسن النتائج فى قياسات النمو وكفاءة الاستفادة من الغذاء والكفاءة الاقتصادية مقارنة بالمعاملات الأخرى. كما أظهرت النتائج عدم وجود أي اختلافات معنوية بين جميع الأسماك بالمجموعات المختلفة ومجموعة المقارنة وذلك بالنسبة لمحتوى الجسم من المادة الجافة والبروتين والدهون والرماد والطاقة وكذلك مكونات بلازما الدم .

ومن النتائج السابقة فانه يوصى باستبدال ٢٥% من الذرة الصفراء بنبات الامشوط المعامل بيولوجيا في علائق البلطي النيلي .