BROODSTOCK DIETS WITH ADDED CRUDE PALM OIL RESULTED IN IMPROVED REPRODUCTIVE PERFORMANCE, EGG HATCHABILITY AND LARVAL QUALITY OF NILE TILAPIA *Oreochromis niloticus*

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ABSTRACT

The intensive farming of tilapia is rapidly expanding and the need to produce sufficient quantities of quality fry is becoming crucial to meet increasing global demands for stocking tilapia farms. Furthermore, it is increasingly important to produce high quality tilapia fry due to the low fecundity of broodfish. Tilapias of the *Oreochromis* genus, the major farmed species, are female mouth-brooders and exhibit high parental care with relatively low number of eggs produced in each clutch. The problem in the mass production of tilapia seed is further exacerbated due to the low degree of female spawning synchrony and reduction in spawning rigor with time. Broodstock nutrition is recognized as a major factor that can influence fish reproduction and subsequent larval quality of many fish species. The development of cost-effective and nutrient optimized broodstock feeds for tilapia is both pertinent and crucial.

The present study was conducted to evaluate the effects of dietary lipid source on the reproductive performance of tilapia broodfish. Four isonitrogenous (35% protein) and isolipidic (10%) casein-based diets were formulated with added fish oil (FO), FO and crude palm oil (FO+CPO; 1:1), CPO or linseed oil (LSO) as the lipid source, respectively. Pre-spawning female Nile tilapia (*Oreochromis niloticus*, GIFT strain) was individually color-tagged, and six females and two males were stocked into a one-tonne breeding tank. Each diet was fed to two tanks of broodfish and the reproductive performance of 12 individual female fish was monitored over 25 weeks. Female broodfish fed the two CPO-based diets showed significantly ($P < 0.05$) larger gonad sizes and lower intraperitoneal fat compared to fish fed the FO or LSO diets. First spawning occurred earliest in broodfish fed the CPO diet at 30.8 ± 9.9 days compared to 44.1, 45.5 or 76.3 days for fish fed the FO+CPO, FO or LSO diet, respectively. The highest number of actively spawning tilapia was observed in fish fed the FO+CPO diet, followed by fish fed the CPO, FO or LSO diet, respectively. At the end of 25 weeks, tilapia fed the two CPO-based diets produced the highest total number of eggs per fish due to the shorter inter spawning interval and higher spawning frequency. Mean diameter, volume and weight of eggs did not vary among dietary treatments. Egg hatchability was significantly higher in broodfish fed the CPO-based diets. The fatty acid composition of the muscle, gonad, egg and newly hatched larvae was influenced by dietary lipid source. However, evidence of preferential fatty acid conservation, conversion and utilization was also observed in these tissues. The fatty acid composition of tilapia eggs did not vary over four consecutive spawns. The gonads, eggs and larvae of tilapia fed the CPO diet contained the highest relative concentration of saturates, monoenes, arachidonic acid and n-6/n-3 ratio. The high total n-3 PUFA concentration observed in the gonads of fish fed the LSO diet, and to a lesser degree the FO diet, seemed to be detrimental to the reproductive performance of tilapia.

In conclusion, the ability to replace expensive dietary FO with CPO will augur well in reducing the costs of broodstock diets as well as contributing to the environmental sustainability of wild fishing stocks from which FO is derived. The beneficial impact of dietary CPO on female tilapia reproductive performance included larger gonad sizes, earlier first spawning activity, shorter inter-spawning interval, a longer period of broodfish fertility, higher overall total egg production, higher egg hatching rates and lower incidence of larval deformities as compared to broodfish fed a FO-based diet.

DISTILLERS CRIED GRAINS WITH SOLUBLES AS ALTERNTIVE PROTEIN SOURCES IN DIETS OF TILAPIA, *Oreochromis niloticus*

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ABSTRACT

Research efforts by nutritionist to reduce feed costs have resulted in increased use of lower cost alternative plant proteins in fish feed formulations as replacements of fish meal and other more expensive protein sources. Distillers dried grains with solubles (DDGS), a dried residue that remains after the fermentation of grain mash by selected yeasts and enzymes to produce ethanol and carbon dioxide, is currently readily available and less expensive than other conventional protein sources on a per unit protein basis. The nutrient content of DDGS varies with the source and quality of grain as well as between and within ethanol plants due to fermentation time and efficiency, the drying process and the quantity of distiller's solubles added. Relative to the grain sources, nutrient concentrations in DDGS approximately triple due to the utilization of starch during fermentation process. Generally, corn and wheat DDGS are deficient in lysine and methionine for most fish species, with lysine being the most limiting, but do not content antinutritional factors. Research evaluating the nutritional value of DDGS in fish diets has shown that DDGS derived from corn and wheat are promising protein sources in fish diets, particularly the omnivorous species such as tilapia. Results of several studies showed that, depending on the composition and nutrient concentrations of the basal diets, 20 to 30% corn or wheat DDGS can be included in tilapia diets without requiring lysine supplementation. With supplementation of lysine, DDGS at levels of 40% or higher can be used without affecting growth performance and feed utilization efficiency. DDGS also contains yeast, a rich source of beta glucan and nucleotides that have been reported to enhance immunity and disease resistance in fish. Corn DDGS, due to its high oil content that is rich in linoleic acid, is an excellent source of essential fatty acid for tilapia. High concentrations of xanthophylls present on corn DDGS may impart yellow pigment in fish flesh if included at high levels. Taking into consideration various factors affecting the nutritional value of diets and the quality of pellet and fish product, 15 to 20% DDGS appears to be optimum in diets of tilapia.

ECONOMICALLY FEASIBLE FISH FEED FOR GIFT TILAPIA (*Oreochromis niloticus*) FOOD FISH CULTURE IN SRI LANKA

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Abstract

Tilapia is an unique fish in the inland fish production of Sri Lanka with maximum contribution coming reservoir fisheries catches. There is a possibility to enhance tilapia aquaculture further through other systems of aquaculture practices. However, feed cost is the highest operating cost in the aquaculture practices and an economically and efficient feed would play major role in stimulating feed based aquaculture of tilapia. Keeping this in view, a trial was carried out in cages ($1m^3$) that were installed in abandoned clay pits. Nine cages were used and the advance fingerlings of Tilapia (GIFT strain) (mean length= 9.7**±** 2.08 cm and mean weight = 18.7**±**12.0 g) were stocked at a stocking density of 100 fingerlings m^{-3}. Two aqua feeds, namely, Feed-A and Feed-B were prepared by using locally available ingredients. The protein percentage in Feed-A and Feed-B was adjusted to 20% while another poultry feed with the same level of protein was used as Feed-C. In feed A , protein was contributed mainly by fish meal , but in feed B , fishmeal and Soybean meal were used as the major protein sources. The cost for feed-A, Feed-B and Feed-C were US$ 0.59, 0.48 and 0.61 per kg respectively. Feed-A and Feed-B were tested by using Feed-C as the control diet and all treatments had triplicates. Feed was provided twice daily at 5% of body weight. The trial lasted for 125 days. The pH, Temperature, DO and Toxic Ammonia were measured in clay pit and they were in acceptable ranges. The final mean weight and specific Growth Rate (SGR-W) of the fish fed on Feed-A, Feed-B and Feed-C were 36.57±14.49, 48.67±17.80,35.58±16.11 g, and 1.0634±0.1017,1.2929±0.0905, 1.0410±0.1094, respectively. As such the Average Daily Growth (ADG) of the fish fed on Feed-A, Feed-B and Feed-C were 0.2155±0.0374, 0.3123±0.0450, 0.2077±0.0374 g day^{-1} respectively and significantly different($p<0.05$) in Feed-B than Feed-A and Feed-C. The mean survival and condition factor(CF) of the fish fed with Feed-A, Feed-B and Feed-C were 98.67±1.15, 81.00±14.18, 97.00±4.58 and 1.8617± 0.0679, 1.9373±0.0599, 2.1077±0.3531 and not were significantly different respectively ($p>0.05$). Feed Conversion Ratio (FCR) of Feed-A, Feed-B and Feed-C were s2.03, 1.69 and 1.99. Accordingly, Feed-B could be recommended as suitable economically feasible feed for tilapia (GIFT strain) food fish culture.

Key words: GIFT tilapia, food fish culture, aquafeed, poultry feed

INTRODUCTION

Tilapia is a unique fish in the inland fish production of Sri Lanka with maximum contribution coming from reservoir fisheries catches. There is a possibility to enhance tilapia aquaculture further through other systems of aquaculture practices. According to (Pullin,1985),Tilapias are widely recognized as one of the most important fish species for freshwater aquaculture in a wide range of farming systems from simple small-scale water-fed fish ponds to intensive culture systems. As the growth rates of the Genetically Improved Farmed Tilapia (GIFT) strain were superior to those of local strains of the Nile Tilapia (Eknath *et al.* 1993; Bentsen *et al.* 1998; Guptha and Acosta 2004 and Ridha 2006) this variety should be popularised in Sri Lanka. As such, thousands of abandoned clay pits (in Gampaha and Hambantota Districts) and abandoned shrimp ponds (in Northwestern province) are the available resources to produce Tilapia for world market. However, as feed cost is the highest operating expense in the semi-intensive aquaculture practices, an economically as well as efficient feed would play a major role in stimulating feed based aquaculture of tilapia. Currently farmers used different food items and poultry feed is much more popular among farmers as the cost is lower than commercially available fish feed. The aim of this research is to evaluate the growth performance of GIFT Tilapia with two formulated feed and with poultry feed as control feed.

MATERIAL AND METHODS

The feeding trial was conducted in cages installed in abandoned clay pits in Gampaha District. The area of the abandoned clay pit was 200m^2 with 10-20m water depth. Nine plastic net cages with the capacity of 1m^3 (1x1x1m in each cage) were used in this trial. Two experimental feed (Feed-A and Feed-B) were formulated using locally available raw materials such as rice bran, coconut meal, extracted soybean meal and fishmeal (Malaysian). These feed ingredients were purchased from an urban market in Colombo. Feed-A was prepared with fishmeal as the major source of protein. Feed-B was prepared with fishmeal and soybean meal as protein sources. Feed-A and Feed-B were formulated with the percentage (%) protein as 20% according to the Pearson's square method and estimated for crude protein % prior to the formulation of feeds. The % protein (N x 6.25) of both fishmeal and soybean meal were determined by semi-micro Kjeldahl digestion, distillation and titration described in APHA (1985). Commercially available poultry feed (control) was used as the control feed and treatments were tested in triplicate. Ingredient compositions of these three feeds are shown in Table 2. The ingredients for two feed types were measured according to the ingredient composition of the respective formulae and mixed together using a laboratory electrical mixer (Sherry). The required amount of feed was adjusted as 5% of the body weight throughout the culture period according to the total biomass in respective cages. The total biomass of fish in respective cages was determined through the mean weight of fish that was obtained through the sampling in each cage and assuming no mortality had occurred in cages. Sampling was carried out monthly from the beginning until the trial was ended. Fish in the sample were observed externally for the fish disease particularly external worms. The water temperature and the pH of the tanks were measured using glass mercury thermometer and the pH meter (Model: GENWAY-3051) in each sampling day around 0900 -1000 hrs. The feed was divided into two portions and kept in polythene bags to hand it over to the farmer.

Table 1. Ingredient composition and cost of the feeds

Feed-A	Feed-B	Feed-C (Poultry feed)
Coconut meal	Coconut meal	Coconut meal
Rice bran	Rice bran	Rice bran
Fish meal (Malaysian)	Fish meal (Malaysian)	Fish meal (Brasil-999)
	soybean meal	soybean meal
Vitamin premix	Vitamin premix	Vitamin .premix
Wheat flour as Binder	Wheat flour as Binder	Raw rice
		Maize
		Shells
20% protein	20% protein	18-20% protein
US$ 0.59/kg	**US$0.47/kg**	**US$0.61/kg**

Advanced fingerlings of GIFT Tilapia (Mean length=9.7±2.08 cm and Mean weight= 18.7±12.0 g) were obtained from Aquaculture Development Centre in Dambulla and stocked in these cages according to the stocking density of 100 fingerlings m^{-3}.

Two farmers involved in the preparation of feed dough in situ adding warm water to the premix ingredients and homogenized until a dough-like paste was formed and feeding fish twice per day once in the morning (0830 hrs) and once in the evening (1530 hrs). The feed dough was provided to the middle of the top cover of the cage which was 15 cm submerged in the water. This trial was lasted 125 days.

Specific growth rate (SGR), Average Daily Growth (ADG), Condition factor (CF) Weight gain (WG), % survival and Food Conversion Ratio (FCR) of the fish for each cage with different feed types were calculated using the following equations.

$$WG = \text{Mean weight} \times \text{No.of survived fish}$$

$$SGR\text{-}W = \frac{Ln_{\text{Final weight}} - Ln_{\text{Initial weight}}}{\text{Experimental duration}} \times 100 \quad \text{Ricker, 1979}$$

$$ADG = \frac{\text{Final weight of fish- Initial weight of fish}}{\text{Days of rearing}}$$

$$FCR = \frac{\text{Weight gained by fish (g)}}{\text{Weight of feed consumed}} \quad \text{Helper, 1988.}$$

$$CF = \frac{\text{Weight of fish(W)}}{\text{Total length of fish}(L)^3} \times 100 \quad \text{Ricker, 1975}$$

$$\%\ \text{Survival} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

In order to detect statistically significant differences, experimental values were compared using a One-way analysis of variance (ANOVA), and the significance of mean differences tested using a Tukey's multiple range test. The significance level was set at $p<0.05$.

RESULT AND DISCUSSIONS

The crude protein level of fish meal (Malaysia) was 50.14±1.21 and soybean meal was 34.19±2.25. These two components were used in a 1:1 ratio in Feed-B. There were not significant differences ($p>0.05$) in the survival rate of fish fed on Feed-A, Feed-B and Feed-C (Table 2). As such, the condition factor (CF) of the fish that fed on these 3 feed types was not significantly different ($p>0.05$) too. However, further growth performance of fish should be considered to select one or more feed types for the culture of GIFT Tilapia.

Table 2. Growth performance of Tilapia (GIFT strain) food fish with two different aquafeed (Feed-A & Feed-B) and with poultry feed (control feed) within 125 days culture period.

Growth Indices	Feed-A	Feed-B	Feed-C
MW_{final}	36.57^{a} ±4.6758	48.67^{b} ±5.6255	35.74^{a} ±4.8569
Weight Gain (g)	2.6547^{a} ±0.4373	3.9147^{b} ±0.5594	2.5177^{a} ±0.4698
CF_{final}	1.8617^{a} ±0.679	1.9373^{a} ±0.0597	2.1077^{a} ±0.3531
% Survival	98.67^{a} ±1.15	81.00^{a} ±14.18	97.00^{a} ±4.58
$SGR\text{-}_{final}$	1.06^{a} ±0.10	1.29^{b} ±0.09	1.04^{a} ±0.11
ADG_{final}	0.2155^{a} ±0.0374	0.3123^{b} ±0.0450	0.2077^{a} ±0.0374
FCR	2.03	1.695	1.988

Figures in the same row having similar superscripts are not significantly different at $p>0.05$

Accordingly, these three feed types help to keep the health and well-being of the fish and provide more or less similar survival. Accordingly these three feed types could be considered in GIFT Tilapia food fish culture. The weight gain of the fish fed on Feed-B has shown the significantly highest value while Feed-C has shown the poorest value. As such, the ADG of the fish fed on Feed-B was significantly higher and different from the ADG of the fish fed on Feed-A and the control feed, Feed-C. As such the higher ADG of the fish fed on Feed-B could be seen through out the culture period (Figure 1).

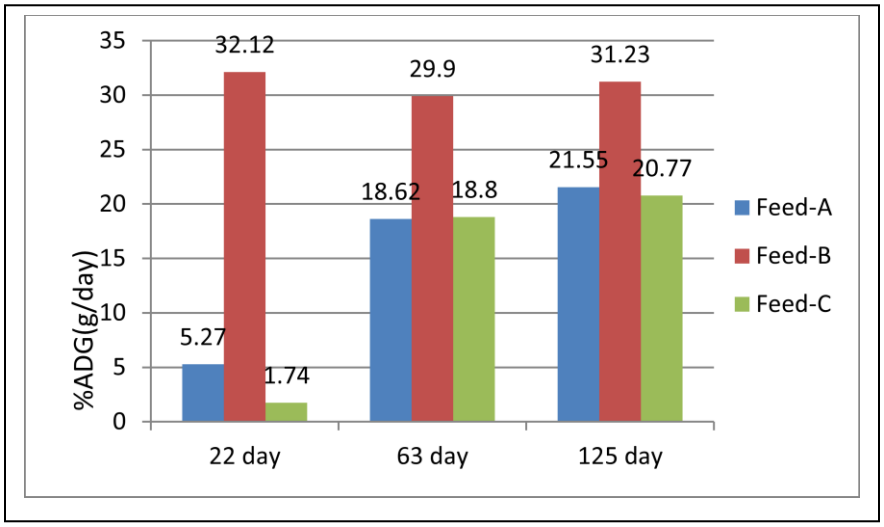

Figure 1. Average Daily Growth (% ADG) of GIFT Tilapia in day 22, day 63 and 125 day in the culture period

Accordingly it was revealed that the growth of fish enhances with Feed-B than Feed-A and Feed-C. As such the final mean body weight of the fish fed on Feed-B was significantly different ($p<0.05$) and higher than the fish fed on Feed-A and Feed-C (Table 3). It also revealed that the importance of Feed-B in GIFT tilapia food fish culture than Feed-A and Feed-C.

Furthermore the SGR of the fish fed on Feed-B has shown significantly higher value (1.29±0.09) than Feed-A (1.06±0.1) and Feed-C (1.04±0.11) Table 3). The higher value of SGR could be seen in the fish feed on Feed-B through out the culture period than the fish fed on Feed-A and Feed-C (Figure 2).

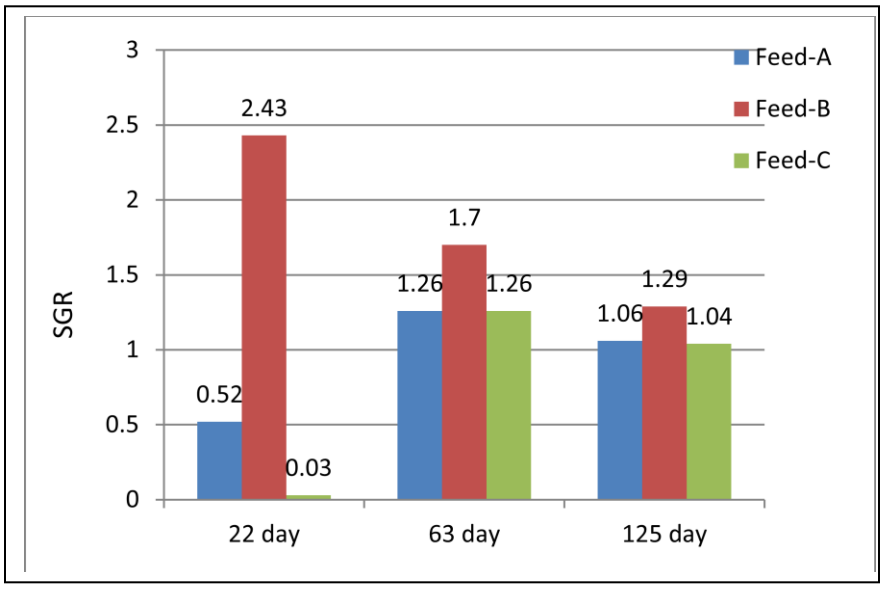

Figure 2. The Specific Growth Rate (SGR) of GIFT Tilapia in day 22, day 63 and 125 day in the culture period

Wannigama *et al*. (1985) have observed no significant difference in the growth of Tilapia when fed 29% protein diet or a 19% protein diet in the cages in perennial reservoirs in Sri Lanka. These three feed types were in 20% protein and have shown significantly difference ADG in Feed-B than Feed-A and Feed-C. It may be due to other reason such as (i) palatability,

(ii)digestibility or (iii) more or less acceptability of the Feed-A. The remaining big grain particles in Feed-C could be observed in the top cover of the cage where feed provided but it was not observed in Feed-A and Feed-B provided cages as the ingredients were used in fine powder form. Accordingly the better ADG, SGR and Weight gain of the fish with Feed-B could be happen due to the presence of soybean meal with fish meal together as protein provider.

Then according to the growth performance (ADG, SGR, CF, Weight gain) of the fish, Feed-B could be recommended for GIFT Tilapia food fish culture in Sri Lanka. However the FCR and production cost of the three feed types should be considered.

The better FCR has observed in Feed-B=1.695, then in Feed-C=1.9886 and finally in Feed-A=2.03. Accordingly, it has shown the efficiency of Feed-B is higher than the other 2 feed types used. As such the cost of feed should be considered as we need the economically feasible feed. The production cost of Feed-B was lesser than the production cost of Feed-A (Table 1). It was due to the replacement of the part of the fishmeal (the expensive component of fish feed) through soybean meal.

Considering all the facts shown above, Feed-B could be considered as economically feasible feed for GIFT Tilapia food fish culture in Sri Lanka. Further research is needed to improve this feed as commercial feed. De Silva (1989) has shown, in developing countries where labour costs are comparatively low, a significant saving in feed costs can be made by feeding diets with a lower protein content than that which is thought to be the optimal dietary protein requirement, without significant loss in growth or yield. In this study it has clearly shown this less protein amount could be provided through the mixture of fishmeal and soybean meal.

Recommendations

- Promotion of oil extracted soybean meal instead of import it as already produce big quantities of soybean seeds in dry zone in Sri Lanka.
- Production of fishmeal component through the minor cyprinid fauna, the unexploited fishery resources in reservoirs to reduce the production cost of Feed-B further more (future research are needed).
- Feed-B should be developed up to commercial level to promote Tilapia Aquaculture in the country (future research are needed).

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SUPPLEMENTAL FEEDING OF NILE TILAPIA (*Oreochromis niloticus* L.) IN FERTILIZED PONDS USING COMBINED FEED REDUCTION STRATEGIES

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Abstract

The study was conducted in nine 500-m^2 earthen ponds at the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, to determine the effect of using combined feed reduction strategies on the grow-out culture of Nile tilapia in fertilized earthen ponds. There were three treatments with three replicates: (I) 67% daily feeding until harvest; (II) 67% daily feeding for 60 days, 50% daily feeding until harvest; (III) 67% daily feeding for 60 days, 100% alternate day feeding until harvest. Ponds were stocked with sex-reversed GIFT tilapia fingerlings at 4 fish m^{-2}.

The study showed that Nile tilapia cultured in fertilized earthen ponds using different combined feed reduction strategy had no significant difference in terms of growth performance. Final mean weight and length of Nile tilapia in Treatment I were 183.1 $\pm$ 77.1 g and 20.1 $\pm$ 2.9 cm, Treatment II had 168.5 $\pm$ 39.9 g and 19.9 $\pm$ 1.4 cm and Treatment III had 183.1 $\pm$ 16.0 g and 20.5 $\pm$ 0.6 cm. Yield after harvest in Treatments I, II and III were 2,968.7 $\pm$ 439.6, 1,980.7 $\pm$ 541.8 and 2,024.7 $\pm$ 329.0 kg ha^{-1}, respectively. Net tilapia yield in Treatment I was significantly higher compared to the other treatments considering the higher survival of the treatment.

Treatment I gave the highest net return among treatments with a mean value of US$705.90 followed by Treatment III with a mean value of US$6.41 then Treatment II with a mean value of US$-36.12. Net return was low among treatments because of the low survival after the study. Numerically, Treatment I showed the most profitable reduction strategy with the obtained survival, however, analysis of variance showed no significant differences in net return among treatments.

With this result, Treatment I seemed to have the best result for tilapia culture, however, previous studies also shows feasibililty of the use of other feed reduction strategies if more viable survival is attained leading to better FCR and net return.

Introduction

Grow-out culture of tilapia has been modified with several technologies including feeding option that promotes cost-saving strategies; however, the determination of feeding strategies based on mathematical and economic models can be rather complex (Cacho, 1993). It is not known whether the reduction of food costs without a net reduction in fish yield is a result of more efficient food consumption (i.e. lack of waste), better food utilization (increased food conversion ratio) or both.

Previous Aquafish CRSP studies introduced the different feeding strategies with the aim of reducing the total cost of tilapia production that can increase the profit of the farmers while limiting the degradation of the environment with lesser nutrient load given to the fish. The strategies include, alternate-day feeding strategy, 45 and 75 day delayed feeding, 67% sub-satiation feeding (Brown, *et al.* 2004) and other modified feeding strategies like the 50% reduction of daily feed ration (Bolivar, *et al.* 2010) and the use of combined feed reduction (Borski, *et al.* 2010) which were generally developed to reduce the cost of tilapia production.

The objective of this study was to determine the effect of using combined feed reduction strategies on the grow-out culture of Nile tilapia in fertilized earthen ponds.

Materials and Methods

Nine 500-m^2 earthen ponds were stocked with sex-reversed fingerlings of size #20 (0.36 g) at a density of 4 pcs-m^{-2} with 3 replicates per group. The fish stocks in all treatments were fed first with pre-starter feeds with 34% crude protein (CP) for the first month and starter feeds with 34% CP on the second month then grower feeds with 31% (CP) on the 3rd month until harvest. Feeding adjustment was done every two weeks based on a feeding rate from 20% down to 2% of the average body weight. The amount of feeds used per treatment was recorded daily. Fish sampling was done every two weeks by getting the bulk weight of 100 fish samples. Individual weight and length of 100 fish samples were measured on the initial and final sampling.

Water temperature and dissolved oxygen were measured weekly at 9 o'clock in the morning and 3 o'clock in the afternoon using dissolved oxygen meter (YSI model 55). Hydrogen ion concentration (pH) and Secchi disc visibility depth (SDVD) reading was also measured weekly. Determinations of the other water quality parameters (total ammonia nitrogen and nitrite-nitrogen level) were measured using freshwater test kit (Lamotte Model AQ2). Weekly fertilization of the experimental ponds was adjusted depending on the SDVD of the pond water. Inorganic fertilizers such as Urea (46-0-0) and ammonium phosphate (16-20-0) were used as inorganic fertilizers at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$.

The following treatments based on combined feed reduction strategies were used in this study:

Treatment I - 67% daily feeding until harvest

Treatment II – 67% daily feeding for 60 days, 50% daily feeding until harvest

Treatment III – 67% daily feeding for 60 days, 100% alternate day feeding until harvest

Differences in growth performance, survival rate and feed consumption were statistically analyzed by analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used for the comparison of treatment means. Feed cost kg^{-1} of body weight were estimated to assess economic feasibility of these combined feed reduction strategies.

Results and Discussions

The feed reduction strategies as a means to reduce cost in the grow-out of tilapia in fertilized ponds were previously developed and with this experiment, further development can be obtained in lowering the cost of production of tilapia in ponds.

Figure 1 shows the average growth trend of stocks after 120 days of culture period in fertilized ponds. Analysis of variance showed that Nile tilapia cultured using different combined feed reduction strategy were not significant in terms of growth performance. The graph shows the comparable growth per treatment from the start up to the end of the study.

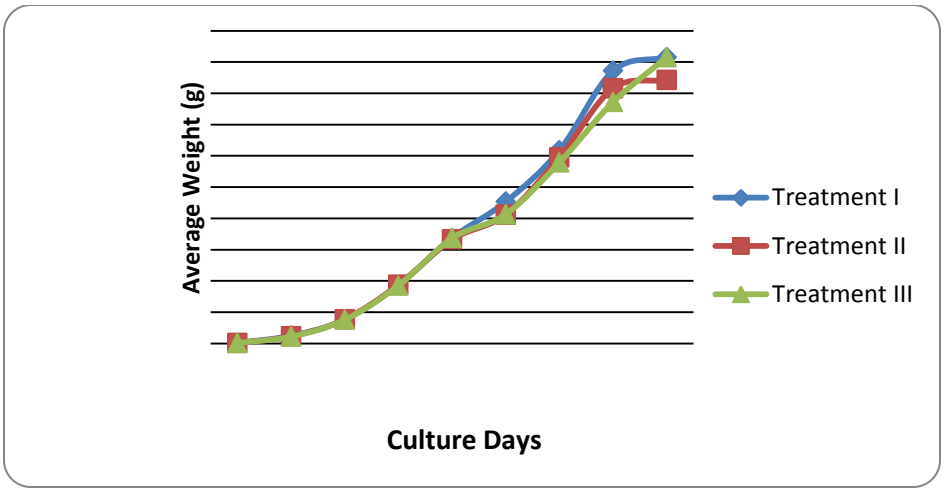

Figure 1. Average weight of Nile tilapia after 120 days of culture period.

In terms of fish yield, the highest yield per hectare was observed in Treatment I followed by Treatment III then Treatment II with a mean values of 2,968.7, 2,024.7 and 1,980.7 kgs hectare^{-1}, respectively. Having the greatest yield, analysis showed that Treatment I was significantly different compared from Treatments II and III.

For the feed conversion ratio (FCR), Treatment I with 1.8 had the best FCR compared to Treatments II and III with mean FCR values of 2.0, however, there was no significant difference among treatments at 5% level of significance.

Feed consumption per hectare of Treatment I was the highest followed by Treatment III then Treatment II with a mean values of 5,201.1, 4,045.3 and 3,965.2 kgs hectare^{-1}, respectively. Treatment I had varying volume of feed consumed, but based on the analysis of variance, no significant differences were found on the feed consumed hectare^{-1}. Table 1 shows the summary of growth performance, survival, feed consumption and yield of Nile tilapia cultured in earthen ponds using combined feed reduction strategies.

Table 1. Growth performance of Nile tilapia in ponds using combined feed reduction strategies

Parameters	Treatments		
	67% daily feeding until harvest	67% daily feeding for 60 days, 50% daily feeding until harvest	67% daily feeding for 60 days, 100% alternate day feeding until harvest
Initial weight (g)	0.36 [a]	0.36 [a]	0.36 [a]
Final average weight (g)	183.1 ± 77.1 [a]	168.5 ± 39.9 [a]	183.1 ± 16.0 [a]
Initial length (cm)	2.8 [a]	2.8 [a]	2.8 [a]
Final average length (cm)	20.1± 2.9 [a]	19.9 ± 1.4 [a]	20.5 ± 0.6 [a]
Gain in weight (g)	182.7 ± 77.1 [a]	168.1 ± 39.9 [a]	182.7 ± 16.0 [a]
Daily gain in weight (g)	1.5 ± 0.6 [a]	1.4 ± 0.3 [a]	1.5 ± 0.1 [a]
Gain in length (cm)	17.3 ± 2.9 [a]	17.1 ± 1.4 [a]	17.7 ± 0.6 [a]
Daily gain in length (cm)	0.14 ± 0.02 [a]	0.14 ± 0.01 [a]	0.15 ± 0.00 [a]
Feed Conversion Ratio	1.8 ± 0.3 [a]	2.0 ± 0.1 [a]	2.0 ± 0.2 [a]
Yield per hectare (kg ha^{-1})	2968.7 ± 439.6 [a]	1980.7 ± 541.8 [b]	2024.7 ± 329.0 [b]
Feed consumed per hectare (kg ha^{-1})	5201.1 ± 1238 [a]	3965.2 ± 1037 [a]	4045.3 ± 1104 [a]
Survival (%)	46.9 ± 24.1 [a]	29.3 ± 4.7 [a]	27.7 ± 4.1 [a]

Means with the same letter superscipt are not significantly different ($P<0.05$).

The highest survival was found in Treatment I with 46.9% followed by Treatment II with 29.3% and Treatment III with 27.7%. Generally, the survival obtained after the experiment was low due to observed mortality during the third and fourth month of the study. Recorded high water temperatures during the afternoon could have caused stress which affected growth and survival even with replenishment of water. Analysis of variance did not indicate significant differences among treatments in terms of survival rate.

The cost and return analysis of Nile tilapia cultured in ponds using combined feed reduction strategy was shown in Table 2. Treatment I gave the highest net return with a mean value of US$705.90 followed by Treatment III with a mean value of US$6.41 then Treatment II with a mean value of US$-36.12. The net return per treatment numerically differs with Treatment I as the most profitable among treatments, however, analysis of variance showed no significant difference at 5% level of significance.

Table 2. Cost and return analysis of Nile tilapia in ponds using combined feed reduction strategies per hectare in US dollar.

	67% daily feeding until harvest	67% daily feeding for 60 days, 50% daily feeding until harvest	67% daily feeding for 60 days, 100% alternate day feeding until harvest
GROSS INCOME	4,487.52	2,994.03	3,060.54
Costs			
Fingerlings	400.00	400.00	400.00
Feeds	3,343.42	2,572.84	2,620.71
Fertilizers	38.20	57.30	33.42
Total Costs	3,781.62	3,030.15	3,054.13
NET RETURN	705.90	-36.12	6.41

Assumptions:

Price of tilapia fingerlings: US$ 0.01 piece^{-1}
Price of commercial feeds:
Pre-starter: US$ 0.81 kg^{-1}
Starter: US$ 0.66 kg^{-1}
Grower: US$ 0.62 kg^{-1}
Price of inorganic fertilizers:
Ammonium phosphate (16-20-0): US$ 0.36 kg^{-1}
Urea (46-0-0): US$ 0.35 kg^{-1}
Price of marketable tilapia: US$ 1.51 kg^{-1}

Results on average minimum and maximum reading for water quality parameters during the 120-day culture period are summarized in Table 3. Generally, the dissolved oxygen readings during the morning ranged from 0.98 and 7.78 mg-L^{-1} for Treatment I, 1.20 and 3.89 mg-L^{-1} for Treatment II and 1.39 and 6.64 mg-L^{-1} for Treatment III. Afternoon dissolved oxygen readings for Treatments I, II and III were 3.64 and 12.87, 4.67 and 11.41 and 5.36 and 13.67 mg-L^{-1}, respectively. Dissolved oxygen measured during the study remained in the favourable range for tilapia (Boyd, 1990). The result supports the findings of Liti *et al*. (2002) that Nile tilapia can tolerate low DO levels.

Table 3. Average minimum and maximum reading for water quality parameters during the experimental period (120 days).

Parameters	67% daily feeding until harvest		67% daily feeding for 60 days, 50% daily feeding until harvest		67% daily feeding for 60 days, 100% alternate day feeding until harvest	
	Min	Max	Min	Max	Min	Max
Dissolve Oxygen (9AM) ($mg\text{-}L^{-1}$)	0.98	7.78	1.20	3.89	1.39	6.64
Dissolve Oxygen (3PM) ($mg\text{-}L^{-1}$)	3.64	12.87	4.67	11.41	5.36	13.67
Water Temperature (9AM) (^oC)	28.43	31.90	28.53	31.77	28.50	31.97
Water Temperature (3PM) (^oC)	31.70	36.37	31.57	36.27	32.00	37.50
Hydrogen-Ion (pH)	7.07	8.37	6.97	8.20	6.93	8.27
Total Ammonia Nitrogen ($mg\text{-}L^{-1}$)	0.017	1.090	0.018	1.456	0.022	0.942
Nitrite-Nitrogen ($mg\text{-}L^{-1}$)	0.067	0.075	0.067	0.075	0.075	0.075
Secchi Disc Visibility (cm)	23.3	72.7	22.3	78.3	24.3	57.7

The average water temperature readings in the morning range between 28.43 and 31.90 ^oC for Treatment I, 28.53 and 31.77 ^oC for Treatment II and 28.50 and 31.97 ^oC for Treatment III. While afternoon water temperature readings for Treatments I, II and III were 31.70 and 36.37, 31.57 and 36.27 and 32.00 and 37.50 ^oC, respectively. Preffered water temperatures for tilapia growth are approximately 28.0-32.0 ^oC, but range varies depending on what species of tilapia is being cultured. Tilapias reportedly tolerate temperatures up to 40 ^oC, but stress-induced disease and mortality are problematic when temperatures are around 37.0 or 38.0 ^oC (Teichert-Coddington, *et al.,* 1997). Fluctuation of the water temperature also affects growth due to the rise and fall of temperature, energy required for maintenance increases rapidly, thus decreasing the energy available for growth (Soderberg, 1997).

Average pH ranged between 7.07 and 8.37, 6.97 and 8.20 and 6.93 and 8.27 for Treatments I, II and III, respectively. Boyd (1998) reported that waters with a pH range of 6.5 – 9 are the most suitable for fish production. Readings for total ammonia nitrogen and nitrite levels during the experiment were in the desirable range for all the treatments. The European Inland Fisheries Advisory Commission (1993) reported that the toxic level of NH_4 to fish is 2 mg/L. The average value of secchi disc reading were 23.3 and 72.7, 22.3 and 78.3 and 24.3 and 57.7 cm for Treatments I, II and III, respectively. Water quality parameters showed no significant difference among treatments at 5% level of significance.

Conclusion

Results in this study showed that there were no significant differences observed on the growth performance and survival of Nile tilapia after 120 days of culture period; however, significant difference was observed on the fish yield with Treatment I having the highest yield among treatments.

The cost and return analysis showed that Treatment I had highest net profit among the treatments due to higher fish yield and a negative income in Treatment II having low survival after the experiment. Statistically, data showed that profit was not significantly different from the other combined feed reduction strategies. With this result, Treatment I seemed to have the best result for tilapia culture, however, previous studies also shows feasibililty of the use of the other feed reduction strategies like alternate-day feeding if more viable survival is attained leading to better FCR and net return.

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THE USE OF ROASTED COFFEE PULP AS A FEED SUPPLEMENT IN PRACTICAL DIETS FOR NILE TILAPIA, *Oreochromis niloticus* (L.)

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ABSTRACT

The present study was undertaken to evaluate the use of ground roasted coffee (*Coffee Arabica*; GRC) as a natural feed additive in practical fish diets and its impact on growth, feed utilization, biochemical variables, and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Ground roasted coffee was added to the ingredients of tested diets to represent 0.0 (control), 0.5, 1.0, 2.0, or 5.0 g/kg diet. Fish (1.9 ± 0.03 g) were distributed to various treatments at a rate of 20 fish per 80-L aquarium and fed one of the experimental diets for 10 weeks. No growth-promoting influences of GRC were observed; however, the optimum fish growth and feed utilization were obtained at 0.0 – 1.0 g GRC/kg diet. The inclusion of GRC in fish diet over 1.0 g/kg diet reduced fish growth, feed consumption, and the protein contents in fish body. The highest lipids and ash contents were obtained at 5.0 g GRC/kg diet. Glucose, plasma protein, and plasma lipids decreased significantly, meanwhile aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine increased significantly in fish fed 5.0 g GRC/kg diet. Fish survival (93.3 – 97.8%) was not affect by GRC inclusion in fish diets. These results indicate that GRC supplement is not a promising growth stimulant for Nile tilapia.

Keywords: Nile tilapia, ground roasted coffee, *Coffee Arabica*, fish growth, feed utilization, body composition, biochemical variables, fish health.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (L.) is one of the most popular species in Egypt and worldwide (El-Sayed, 2006). As the regular use of antibiotics and chemicals as preventative and curative measures for disease leads to drug-resistant bacteria and harmful effects on the environment (Teuber, 2001; Bachère, 2003; Hermann et al., 2003), alternatives to antibiotics and chemicals to improve the quality and sustainability of aquaculture production have been seen as desirable (Meunpol et al., 2003; Vaseeharan and Ramasamy, 2003; Li et al., 2006).

Medicinal plants have been used as immune-stimulants for human in China and old civilization for thousands years (Tan and Vanitha, 2004). These plants contain many types of active components such as polysaccharides, alkaloids, or flavonoids that have immuno-stimulating activities in mice, chickens, or human cell lines (Cao and Lin, 2003; Lin and Zhang, 2004). The use of medicinal plants as immuno-stimulants in fish diets has been considered (Abdel-Tawwab et al., 2010; Ahmad and Abdel-Tawwab 2011; Ahmad et al.; in press).

Many studies have been conducted on using coffee pulp in fish diets and they found adverse effects of coffee pulp on fish growth and feed utilization (Fagbenro and Arowosoge, 1991; Moreau et al., 2003; Ulloa and Verreth, 2003; Chatzifotis et al., 2008). Some other studies reported that coffee shows an antioxidant activity because it contains many substances like caffeine, cafestol, kahweol, and chlorogenic acids (Pellegrini et al., 2003; Vinson et al., 2005). Due to the abundance of antioxidant compounds in coffee, these agents must be seriously considered when elucidating potential pharmacological effects of coffee intake. Therefore, the present research aims to evaluate the effect of ground roasted coffee (GRC) supplementation on growth, feed efficiency, feed consumption, biochemical variables, and proximate composition of Nile tilapia, *O. niloticus*.

MATERIALS AND METHODS

Fish culture and feeding regime - Ground roasted coffee (*Coffee Arabica*; GRC) was obtained from the local market. Five different diets containing 0.0, 0.5, 1.0, 2.0 and 5.0 g GRC/kg diet were formulated. The dietary ingredients were thoroughly mixed and moistened by the addition of 100 ml warm water per kg diet and then made into pellets by a mincing machine. The pellets were cut into shape manually, dried in an oven at 55 ^{o}C till constant weight was obtained and stored in a freezer at -2 ^{o}C until use.

Nile tilapia, *O. niloticus* were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, abo-Hammad, Sharqia, Egypt. Before starting the experiment, fish were acclimated and hand-fed to apparent satiation twice a day for 2 weeks. For the experiment, 15 80-L aquaria were used and oxygenated to saturation by air pumps. In each aquarium, 20 randomly distributed fish (1.9 ± 0.03 g) were stocked. The tested diets were administered to five fish groups with three replicates per each. Fish were hand-fed for satiation thrice daily 5 days a week for 10 weeks. Settled fish wastes along with three-quarter of aquarium's water were siphoned daily. Siphoned water was replaced by clean and aerated water from a storage tank. Average weight per aquarium was assessed every 2 weeks by group-weighing all fish. Fish were starved for a day before weighing.

Fish growth and feed utilization - At the end of the experiment, fish per each aquarium were harvested, counted, and weighed. Fish growth and feed utilization variables were calculated as follows:

Weight gain (g) = final weight – initial weight;
Specific growth rate (SGR; %/day) = 100 (Ln final weight – Ln initial weight) / days;
Feed conversion ratio (FCR) = feed intake (g) / weight gain (g);
Protein efficiency ratio (PER) = weight gain (g) / protein intake (g);
Fat efficiency ratio (FER) = weight gain (g) / fat intake (g);
Energy utilization (EU; %) = 100 x (energy gain / energy intake).

Chemical analysis of diets and fish - The proximate chemical analyses of the tested diets and fish samples were done for moisture, crude protein, total lipids, and total ash according to the standard methods of AOAC (1990). Moisture content was estimated by drying the samples to constant weight at 95 ^{o}C in drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 ^{o}C for 6 hours.

Biochemical measurements - At the end of the 10-week feeding trial, feed was withhold 24 hour immediately prior to sampling and five fish per aquaria were randomly chosen and anesthetized with tricaine methanesulfate (20 mg/L). Blood samples were collected from the caudal vessel and the extracted blood was collected in Eppendorf tubes contained 500 U sodium heparinate/mL; used as an anticoagulant. The collected plasma was stored at –20 ^{o}C for further assays. Blood glucose, plasma total protein, plasma total lipids, and plasma creatinine were calorimetrically determined according to Trinder (1969), Henry (1964), Joseph et al. (1972), and Henry (1974), respectively. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma were determined colorimetrically according to Reitman and Frankel (1957).

Statistical analysis - The obtained data were subjected to one-way ANOVA to evaluate the effect of GRC supplementation. Differences between means were tested at the 5% probability

level using Duncan test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

RESULTS

In the present study, fish grow gradually by time in all treatments (Figure 1). Final fish weight, weight gain, and specific growth rate were not significantly ($P < 0.05$) affected with the increase in GRC levels up to 1.0 g/kg after which growth declined (Table 1). The lowest fish growth was obtained at 2.0 – 5.0 g GRC/kg diet. Moreover, fish fed on diets containing 2.0 and 5.0 g GRC/kg consumed less diet than the other treatments giving the highest FCR (1.4 and 1.5, respectively). Meanwhile, fish fed on 0.0 – 1.0 GRC/kg diet consumed approximately the same feed amount giving the same FCR (1.3; Table 2).

Table 1. Growth performance and survival of Nile tilapia fed different levels of ground roasted coffee (GRC) for 10 weeks.

GRC levels (g/kg diet)	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (%/day)	Fish survival (%)
0.0	1.9±0.03	14.5±0.35 a	12.6±0.38 a	2.90±0.059 a	95.6±4.43
0.5	1.9±0.01	14.5±0.55 a	12.6±0.55 a	2.90±0.052 a	95.5±2.23
1.0	1.9±0.01	14.0±0.58 ab	12.1±0.58 ab	2.85±0.058 ab	97.8±2.23
2.0	1.9±0.03	12.5±0.55 bc	10.6±0.52 bc	2.69±0.043 bc	93.3±3.84
5.0	1.9±0.03	11.2±0.36 c	9.3±0.38 c	2.53±0.066 c	95.6±4.43

Means having the same letter in the same column are significantly differed at $P < 0.05$.

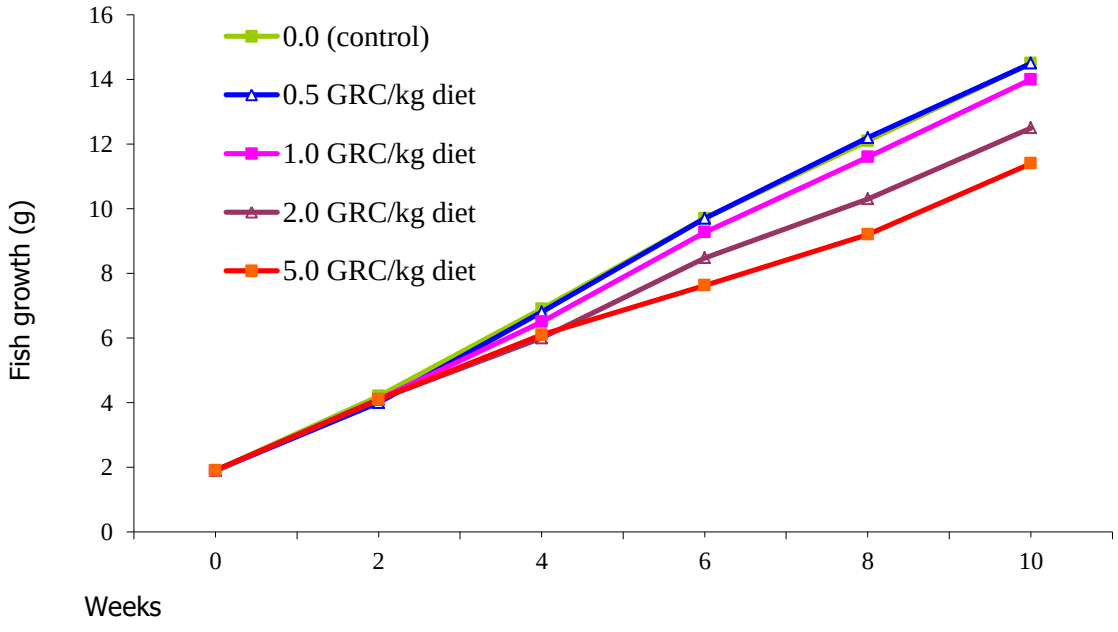

Figure 1. The weight of Nile tilapia (g) fed different levels of ground roasted coffee (GRC) for 10 weeks.

Furthermore, no significant differences were observed in fat efficiency ratio, protein efficiency ratio, and energy utilization at 0.0 – 1.0 GRC/kg diet levels and the lowest values of these parameters were obtained when fish fed 2.0 – 5.0 g GRC/kg diet (Table 2). On the other hand, fish survival range was 93.3 – 97.8% with no significant difference ($P > 0.05$) among the different treatments.

Table 2. Feed utilization by Nile tilapia fed different levels of ground roasted coffee (GRC) for 10 weeks.

GRC levels (g/kg diet)	Feed intake (g feed/fish)	FCR	Fat efficiency ratio	Protein efficiency ratio	Energy utilization (%)
0.0	16.0±0.88 a	1.3±0.033 b	10.50±0.876 a	2.86±0.238 a	32.0±1.271 ab
0.5	16.0±0.44 a	1.3±0.058 b	10.08±0.123 a	2.86±0.033 a	32.8±2.119 a
1.0	16.1±0.44 a	1.3±0.033 b	9.45±0.568 ab	2.74±0.154 ab	31.4±2.227 ab
2.0	14.7±0.78 b	1.4±0.033 ab	9.22±0.108 b	2.62±0.027 b	30.1±1.266 bc
5.0	14.0±0.58 b	1.5±0.058 a	8.38±0.390 c	2.39±0.106 c	28.5±0.203 c

Means having the same letter in the same column are significantly differed at $P < 0.05$.

The GRC supplementation in the present study significantly affected the whole-fish body constituents except moisture content, which did not vary significantly ($P > 0.05$; Table 3). The protein content decreased significantly, meanwhile lipid and ash contents increased significantly by increasing GRC levels. The lowest protein (15.1%), the highest lipids (9.7%) and the highest ash (3.8%) contents were obtained at 5.0 GRC/kg diets. In addition, fish fed the control diet exhibited the highest protein (61.4%) and the lowest lipid (25.5%) contents (Table 3).

Table 3. Proximate composition of whole-body (%; on fresh weight basis) of Nile tilapia fed different levels of ground roasted coffee (GRC) for 10 weeks.

GRC levels (g/kg diet)	Moisture	Crude protein	Total lipid	Total ash
0.0	72.3±0.31	17.2±0.29 a	7.1±0.03 c	3.2±0.09 b
0.5	71.8±0.28	16.9±0.17 a	7.7±0.19 bc	3.2±0.07 b
1.0	72.0±0.27	16.4±0.18 a	8.0±0.16 b	3.3±0.13 b
2.0	72.1±0.87	16.5±0.53 a	8.1±0.26 b	3.2±0.17 b
5.0	71.7±0.41	15.1±0.30 b	9.7±0.15 a	3.8±0.21 a

Means having the same letter in the same column are significantly differed at $P < 0.05$.

The biochemical variables were significantly affected by GRC supplementation ($P < 0.05$; Tables 4 and 5). The inclusion of 0.5 – 5.0 g/kg diet of dietary GRC resulted in significant decreases in glucose, plasma protein and plasma lipids, whereas the highest values of above parameters were obtained with fish fed the control diet (Table 4). Contrarily, AST, ALT, and creatinine values increased significantly with increasing GRC levels and the highest values of these parameters were obtained with fish fed 5.0 g GRC/kg (Table 5). Fish fed on the control diets exhibited the lowest values.

Table 4. Changes in glucose, plasma protein, and plasma lipids in Nile tilapia fed different levels of ground roasted coffee (GRC) for 10 weeks.

GRC levels (g/kg diet)	Glucose (mg/dL)	Protein (g/dL)	Lipids (g/dL)
0.0	67.53±1.362 a	1.77±0.057 a	2.69±0.167 a
0.5	55.23±1.468 b	1.63±0.064 b	1.61±0.067 b
1.0	55.42±2.669 b	1.60±0.061 b	1.57±0.083 b
2.0	52.63±4.435 b	1.51±0.021 b	1.53±0.035 b
5.0	50.23±1.386 b	1.37±0.056 c	1.42±0.059 c

Means having the same letter in the same column are significantly differed at $P < 0.05$.

Table 5. Changes in AST, ALT, and creatinine in plasma of Nile tilapia fed different levels of ground roasted coffee (GRC) for 10 weeks.

GRC levels (g/kg diet)	AST (mg/dL)	ALT (mg/dL)	Creatinine (mg/dL)
0.0	52.57±2.919 d	22.60±2.023 d	0.252±0.0147 d
0.5	63.60±2.386 c	37.23±3.187 c	0.328±0.0117 c
1.0	76.90±2.312 b	45.20±4.046 bc	0.386±0.0684 b
2.0	80.13±2.440 b	48.46±5.017 b	0.393±0.0392 b
5.0	97.10±5.103 a	59.30±1.350 a	0.467±0.0304 a

Means having the same letter in the same column are significantly differed at $P < 0.05$.

DISCUSSION

The present study showed that GRC adversely affected Nile tilapia growth at a concentration higher than 1.0 g/kg diet. These results are in concomitant with Fagbenro and Arowosoge (1991), Moreau et al. (2003), and Ulloa and Verreth (2003) who found adverse effects of coffee-containing diets on fish growth. Similarly, Chatzifotis et al. (2008) reported that sea bream, *Sparus aurata* did not accept the caffeine-containing diet at a 10 g/kg dose but at doses at or lower to 5 g/kg caffeine appeared not to have a deterrent effect. They also stated that the negative effect of caffeine on sea bream growth can be traced in its increased FCR. Throughout the feeding period the fish in all experimental groups were in good health and dose-related mortalities were not observed, indicating that Nile tilapia can tolerate GRC levels (up to 5 g/kg diet) albeit with reduced growth rate and increased feed conversion ratio.

It is worth mentioning that 2 - 5 g GRC/kg diet caused a significant decrease in feed consumption and a significant increase in FCR. These results suggested that GRC did influence the diet palatability, implying that the growth retardation at 2 - 5 g GRC/kg diet may be due to the low diet utilization. It has been inferred that caffeine in GRC, together with polyphenols and tannins can deter feed consumption in fish (Ulloa and Verreth, 2003); possibly because of its bitter taste usually perceived by animals (Mazzafera, 2002; Frank et al., 2004). Furthermore, Kasumyan and Døving (2003) reported that caffeine inhibited the feeding behavior of turbot, *Psetta maxima*.

The proximate composition of whole-fish body was significantly affected by GRC inclusion (Table 3). However, protein content decreased, meanwhile lipids contents decreased by increasing GRC levels. These results disagree with Kobayashi-Hattori et al. (2005) who reported

that caffeine induced lipolysis and thereby reduce the body fat mass and body fat percentage in Sprague–Dawley rats fed on a high fat diet. Chatzifotis et al. (2008) found that caffeine cannot reduce the lipid content of white muscle and liver in heterotherm sea bream when reared in low winter temperatures. These changes in protein and lipid contents in fish body herein could be linked with changes in their synthesis and/or deposition rate in fish body (Abdel-Tawwab et al., 2006).

Glucose, serum protein, and serum lipids decreased significantly, meanwhile AST, ALT, and creatinine increased significantly in fish fed 5.0 g GRC/kg diet. In this regard, Gagne et al. (2006) stated that in rainbow trout, *Oncorhynchus mykiss*, long-term exposure to caffeine could lead to lipid peroxidation. Furthermore, caffeine is an inhibitor of glycogen phosphorylase in the mantle tissue of mussel (*Mytilus galloprovincialis*; Serrano et al., 1995) and of lactate dehydrogenase in the muscle of rabbit (Gardiner and Whiteley, 1985). The increase in AST and ALT activities is an indicative to liver dysfunction and the increase in creatinine is an indicative to kidney dysfunction. These results suggest that GRC may contain compounds that caused some kind of stress on fish affecting these biochemical variables. Corradetti et al. (1986) found a chronic-caffeine effect on rats.

These results indicate that GRC supplement is not a promising growth stimulant for Nile tilapia and in some cases GRC should not exceed 1.0%. Further work is needed to explore the role of GRC in enhancing antioxidant activity and/or the anti-toxicity effect against water pollutants

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PARTIAL AND TOTAL REPLACEMENT OF FISHMEAL WITH CHEESE PROCESSING BY-PRODUCT MEAL IN PRACTICAL DIETS FOR NILE TILAPIA, *Oreochromis niloticus* (L.): A PRELIMINARY STUDY

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Abstract

Aquaculture is the fastest expanding food production system in the world. This rapid development largely depends upon the increased production of aqua-feeds, which traditionally rely on fishmeal (FM) as the main protein source. The increasing demand for FM use in animal and fish diets has resulted in FM becoming difficult to obtain and more expensive. Therefore, this study was conducted as a trial to use cheese processing by-product meal (CPBM) as a substitute for FM in practical diet for Nile tilapia, *Oreochromis niloticus* (L.). Triplicate fish groups were fed on one of five isonitrogenous (30.0%) and isolipidic (7.5%) diets. The control diet (D1) used FM as the sole protein source. In the other four diets (D2 – D5), FM protein was substituted by 25, 50, 75, or 100% CPBM. Fish (3.5 ± 0.1 g) were stocked at a rate of 20 fish per 100-L aquarium and fed one of the tested diets for satiation twice daily, 6 days a week for 12 weeks. Fish growth, feed utilization, protein efficiency ratio, apparent protein utilization, and energy utilization for fish fed CPBM diets up to 75% of FM (D2 – D4) were all higher, but not significantly, than those for fish fed D1. No significant changes were found in whole-body moisture, crude protein, total lipid, and total ash contents. Cost–benefit analysis of the test diets herein indicated that CPBM was economically superior to FM. This study concluded that the optimal replacement level of FM by CPBM was 75%.

Keywords: Nile tilapia, fishmeal, cheese processing byproduct meal, fish growth, feed utilization, whole-body composition.

INTRODUCTION

Nowadays, aquaculture industry accounts for a massive 68% of global fishmeal (FM) consumption (Naylor et al., 2009); however, FM is a major conventional ingredient in many aqua-feeds (El-Sayed, 2004). FM is the single most expensive macro-feed ingredient and is highly sought after by other livestock industries (Tacon et al., 2000). With static or declining clupeid fish populations that are harvested for FM, any negative market disturbance, supply disruption, or availability problem, can lead to dramatic increases in the commodity price (Tacon et al., 2000). Further, the capture of wild fish used to feed cultured fish is unsustainable at current levels according to most experts (Naylor et al., 2000). Current developments in aqua-feeds production are seeking the substitution of FM by alternatives such as terrestrial plant material, rendered terrestrial animal products, krill, seafood by-products or materials of protest origin. The National Organics Standards Board (NOSB) has proposed limiting the use of FM in organically certified aquaculture products with a 12-year phase-out schedule (Board, 2008). These developments are being driven by both economic and ethical concerns.

As the tilapia industry expands, there is a need to formulate nutritious, economical diets that do not rely on FM as a major protein source. One approach to reducing FM in Nile tilapia diet is to replace it with alternative, less expensive animal or plant protein ingredients. This would alleviate the dependence on marine-derived protein, allow for continued expansion of global aquaculture, utilize renewable ingredients, and help decrease production costs. The use of environmentally friendly approach is desirable in modern aquaculture and cheese processing byproduct meal (CPBM) fulfills this objective; however it is readily available and renewable ingredient. This by-product achieves a protein content of 34% to 89% (USDEC, 2004); that nominees it to partially or totally replace FM in fish diets. Therefore, this study was conducted as a preliminary study to evaluate the use of CPBM in fish diets instead of FM and its impact on growth, survival, feed efficiency, and body composition of Nile tilapia, *Oreochromis niloticus* (L.).

MATERIALS AND METHODS

Diet preparation

Cheese processing byproduct meal was obtained from local cheese manufacture produces Domiatta cheese from caw milk. It was centrifuged at 10,000 *g* for 30 min and oven dried at 55 ^{o}C for 24 hours. AOAC method (AOAC, 1990) was used to determine its proximate chemical composition. Moisture, crude protein, total lipid, and total ash contents of CPBM (on dry matter basis) were 77.1, 42.2, 14.3, and 28.4%, respectively.

Five diets were formulated to be isonitrogenous (30.0% crude protein) and isolipidic (7.5% total fat) with CPBM replacing herring FM at different levels. All diets contained a constant level of plant protein from soybean meal, corn meal and wheat bran to complete the protein requirement. These diets were formulated to contain the same protein and lipid contents (Table 1). The control diet (D1) was prepared with herring FM as the only protein source. In the remaining four diets (D2 – D5) 25, 50, 75, or 100% of herring FM protein substituted by CPBM protein. The dietary ingredients were thoroughly mixed and moistened by the addition of 100 ml warm water per kg diet and then made into pellets by a mincing machine. The pellets were cut into shape manually, dried in an oven at 55 ^{o}C till constant weight was obtained and stored in a freezer at -2 ^{o}C until use.

TABLE 1. Ingredients and chemical composition of the experimental diets (on dry matter basis).

Ingredients	Cheese processing byproduct (%)				
	0.0 (Control)	25	50	75	100
	D1	D2	D3	D4	D5
Herring fish meal [1]	10.1	7.6	5.1	2.5	0.0
Cheese processing byproduct	0.0	4.4	8.7	13.1	17.4
Soybean meal [2]	43.1	43.1	43.1	43.1	43.1
Corn meal	17.4	17.4	17.4	17.4	17.4
Wheat bran	14.5	14.5	14.5	14.5	14.5
Cod liver oil	2.1	2.1	2.1	2.1	2.1
Corn oil	1.8	1.8	1.8	1.8	1.8
Vitamins premix [3]	1.0	1.0	1.0	1.0	1.0
Minerals premix [4]	2.0	2.0	2.0	2.0	2.0
Starch	8.0	6.1	4.3	2.5	0.7
Total	100	100	100	100	100
Chemical analyses (%)					
Moisture	7.5	7.4	7.6	7.8	7.7
Crude protein	30.4	30.2	30.3	30.5	30.6
Ether extract	7.4	7.3	7.5	7.6	7.4
Ash	7.1	7.4	7.8	8.2	8.6
Crude fiber	5.0	4.9	4.7	4.8	5.1
Nitrogen-free extract [5]	50.1	50.2	49.7	48.9	48.3
GE (kcal/100g) [5]	447.1	445.4	445.9	444.6	440.9
P/E ratio	68.0	67.8	68.0	68.6	69.4

[1] Danish fish meal72% protein, 14.2% crude fat, and 11.0% ash obtained from TripleNine Fish Protein, DK-6700 Esbjerg, Denmark.

[2] Egyptian soybean flour 44% protein, 1.1% crude fat, and 7.9% ash obtained from National Oil Co., Giza, Egypt.

[3] Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[2] Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.

[3] Nitrogen-free extract = 100 – (crude protein + total lipid + crude fiber + total ash).

[4] Gross energy (GE) was calculated from (NRC, 1993) as 5.65, 9.45, and 4.1 kcal/g for protein, lipid, and carbohydrates, respectively.

Fish culture and feeding regime - Nile tilapia, *O. niloticus* (L.) were obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, abo-Hammad, Sharqia, Egypt. Before starting the experiment, fish were acclimated and hand-fed to apparent satiation twice a day for 2 weeks. For the experiment, 15 100-L aquaria were used and oxygenated to saturation by air pumps and 20 fish (3.5 ± 0.1 g) were stocked in each aquarium. The tested diets were administered to five fish groups with three replicates per each. Fish were hand-fed for satiation twice daily (at 9:30 and 14:00 hours), 6 days a week for 12 weeks. Settled fish wastes along with three-quarter of aquarium's water were siphoned daily. Siphoned water was replaced by clean

and aerated water from a storage tank. Every 2 weeks fish were group-weighed. Fish were starved for a day before weighing. During the experiment, the water quality was checked periodically. The water temperature ranged from 24.2 to 26.4 ^{o}C, pH from 7.4 to 7.6, dissolved oxygen was 4.9 – 5.3 mg/L, and unionized ammonia was <0.2 mg/L.

Fish growth and feed utilization - At the end of the experiment, fish per each aquarium were harvested, counted, and weighed. Fish growth and feed utilization variables were calculated as follows:
Weight gain (g) = final weight – initial weight;
Weight gain % = 100 x weight gain / initial weight;
Specific growth rate (SGR; %/day) = 100 (Ln final weight – Ln initial weight) / days;
Feed conversion ratio (FCR) = feed intake (g) / weight gain (g);
Protein efficiency ratio (PER) = weight gain (g) / protein intake (g);
Apparent protein utilization (APU; %) = 100 [protein gain in fish (g) / protein intake in feed (g)];
Energy utilization (EU; %) = 100 x (energy gain / energy intake).

Chemical analysis of diets and fish - The proximate chemical analyses of the tested diets and fish samples were done for moisture, crude protein, total lipid, and total ash according to the standard methods of AOAC (1990). Moisture content was estimated by drying the samples to constant weight at 95 ^{o}C in drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 ^{o}C for 6 hours.

Economic evaluation - The cost of feed to raise unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal, 12.0; CPBM, 3.0; soybean meal, 2.5; corn meal, 1.50; wheat bran, 1.40; starch, 3.0; fish oil, 9.0; corn oil, 7.0; vitamin premix, 7.0; mineral mixture, 3.0. An additional 50.0 LE/ton manufacturing cost.

Statistical analysis - The obtained data in this study are presented as means ± SD of three replicates. One-way analysis of variance was used to test the effects of the diets. Duncan's Multiple range test was used for mean comparisons. Differences were regarded as significant when $P < 0.05$. Second-order polynomial regression analysis of the relationship between the fish growth and the replacement levels of protein of CPBM was used to estimate the optimal replacement level of protein of FM by CPBM in the diets for Nile tilapia. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

RESULTS

Fish displayed an active feeding behavior, particularly during the morning meal. Final body weight, weight gain, weight gain %, and SGR were insignificantly ($P<0.01$) influenced by the dietary CPBM except that fed 100% CPBM diet, which exhibited the lowest growth performance (Table 2). No significant differences were observed in survival among the treatments since its range was 96.7 - 100 % ($P > 0.05$; Table 2).

TABLE 2. Growth performance and feed utilization for Nile tilapia fed diets containing different levels of cheese processing byproduct meal (CPBM) for 12 weeks.

	CPBM levels (%)				
	0.0 (Control)	25	50	75	100
	D1	D2	D3	D4	D5
Initial weight (g)	3.6±0.06	3.6±0.07	3.5±0.09	3.5±0.09	3.5±0.06
Final weight (g)	30.2±0.38 ab	30.8±0.29 ab	30.8±0.42 ab	31.8±0.61 a	29.5±0.61 b
Weight gain (g)	26.6±0.32 ab	27.2±0.30 ab	27.3±0.38 ab	28.3±0.52 a	26.0±0.55 b
Weight gain %	738.9±3.0 c	755.6±7.7 bc	780.0±6.5 b	808.6±6.6 a	742.9±3.5 c
SGR (%/day)	2.53±0.004 b	2.56±0.005 b	2.59±0.009 ab	2.63±0.009 a	2.54±0.005 b
Survival rate (%)	98.3±1.7	100.0±0.0	98.3±1.7	96.7±3.3	100.0±0.0

Means having the same letter in the same row is not significantly different at $P < 0.05$.

Feed intake increased in all groups during the experiment, but it decreased significantly only at 100% CPBM (D5; $P < 0.05$; Table 3). Indeed, feed intake increased in all aquaria during the course of the experiment, as fish grew, but it was low in group fed D5. Feed conversion ratio showed similar values for fish fed D1 – D5; it varied between 1.35 in D1 and 1.39 in D2 (Table 3). Similarly, PER, APU, and EU value showed insignificant differences ($P > 0.05$) among the different treatments (D1 – D5) and their ranges were 2.57 – 2.64, 44.2 – 45.8%, and 25.1 – 26.3%, respectively.

TABLE 3. Growth performance and feed utilization for Nile tilapia fed diets containing different levels of cheese processing byproduct meal (CPBM) for 12 weeks.

	CPBM levels (%)				
	0.0 (Control)	25	50	75	100
	D1	D2	D3	D4	D5
Feed intake (g feed/fish)	36.0±0.46 ab	37.8±0.51 a	37.6±0.51 a	38.4±0.76 a	35.3±1.01 b
Feed conversion ratio	1.35±0.037	1.39±0.044	1.38±0.073	1.36±0.025	1.36±0.035
Protein efficiency ratio	2.63±0.020	2.57±0.064	2.60±0.028	2.64±0.047	2.60±0.073
Protein utilization (%)	44.8±1.70	44.2±0.93	45.8±0.84	45.8±1.62	45.4±0.92
Energy utilization (%)	25.5±0.69	25.1±0.56	25.8 ±0.68	26.1±0.90	26.3±0.52

Means having the same letter in the same row is not significantly different at $P < 0.05$.

The chemical composition of the whole fish body is given in Table 3. All fish displayed a change in the whole body composition (compared with that at the start of the experiment), which consisted mainly in a decrease of moisture percentage and a corresponding increase in total lipid content. No significant changes in moisture, crude protein, total lipid, and total ash contents in fish body were found due to the inclusion of CPBM in fish diets and their ranges were 74.4 – 75.1%, 65.8 – 66.5%, 18.3 – 18.6%, and 13.8 – 14.3%, respectively.

TABLE 3. Proximate chemical analyses (%; on dry weight basis) of Nile tilapia whole-body fed diets containing different levels of cheese processing byproduct meal (CPBM) for 12 weeks.

	CPBM levels (%)				
	0.0 (Control)	25	50	75	100
	D1	D2	D3	D4	D5
Moisture	75.1±0.32	74.7±0.28	74.4±0.49	74.5±0.44	74.5±0.52
Crude protein	66.1±0.71	65.8±0.50	66.5±0.67	65.8±076	66.2±1.22
Total lipid	18.5±0.68	18.3±0.17	18.4 ±0.63	18.6±0.35	18.3±0.75
Total ash	14.2±0.68	14.3±0.34	13.8±0.93	13.9±0.90	14.1±051

Means having the same letter in the same row is not significantly different at $P < 0.05$.

It is noticed that the incorporation of CPBM (D2 – D5) herein reduced the price of one kg diet as compared to the control group (Table 4). Average cost to produce on kg gain in weight for D1 – D5 were 4.59, 4.45, 4.14, 3.81, and 3.40 LE, respectively. However, CPBM inclusion reduced the cost to produce one kg gain by 3.1, 9.8, 17.0, and 25.9% for D2 – D5, respectively (Table 4).

Table 4: Economic efficiency for production of one kg gain of Nile tilapia fed diets containing different levels of cheese processing byproduct meal (CPBM) for 12 weeks.

	CPBM levels (g/kg diet)				
	0.0 (Control)	25	50	75	100
	D1	D2	D3	D4	D5
Feed cost (L.E./kg)	3.4	3.2	3.0	2.8	2.5
FCR (kg feed/kg gain)	1.35	1.39	1.38	1.36	1.36
Feed cost per kg gain (L.E.)	4.59	4.45	4.14	3.81	3.40
Cost reduction per kg gain (L.E.)*	0.0	0.14	0.45	0.78	1.19
Cost reduction per kg gain (%)**	0.0	3.1	9.8	17.0	25.9

* Cost reduction per kg gain (L.E.) = feed cost per kg gain of control (L.E.) - feed cost per kg gain of CPBM treatment (L.E.);

** Cost reduction per kg gain (%) = 100 [cost reduction per kg gain (L.E.) in D2-D5 / feed cost per kg gain of control (L.E.)].

DISCUSSION

The present study indicated that the partial substitute of FM protein by CPBM protein has no significant adverse effect on the growth response and feed utilization for Nile tilapia; meanwhile higher amounts of CPBM protein (D5) retarded fish growth and feed utilization significantly. These results suggest that it is possible to replace up to 75% of FM protein with CPBM protein without significant adverse effect on fish growth response.

This is the first time to our knowledge that CPBM has bean demonstrated to be effective in replacing FM in fish diets although other authors have demonstrated FM replacement potential for a variety of plant and animal meals. Based on diet intake, the palatability of the tested diets

(D1 – D4) appeared to be better than D5 (100% CPBM; Table 3). Palatability may be in part responsible for the significant differences in weight gain and FCR among the tested diets. The highest level of substitution, which was not significantly different from the control in growth performance, was 75% CPBM (Table 2).

Many authors reported that between 30% and 75% of dietary FM could be replaced by animal by-products. Abdelghany (2003) evaluated the use of gambusia, *Gambusia affinis*, fish meal (GFM) in practical diets for red tilapia, *O. niloticus* x *O. mossambicus.* He formulated six isonitrogenous diets (35%) in which GFM replaced 0.0, 10, 25, 50, 75, or 100% of the protein supplied by herring FM. He demonstrated that GFM is a suitable protein source in practical diets for Nile tilapia and could replace HFM up to 50%; however, fish growth and feed and protein utilization were retarded for diets containing 100% GFM. Furthermore, Ahmad (2008) used the same diets as Abdelghany (2003) for Nile tilapia and he found that the optimum GFM level was obtained at 75%.

The complete replacement of CPBM with FM (100% GFM) reduced the fish growth. The growth reduction in fish fed the diet containing 100% CPBM may be attributed to reduced palatability or attractiveness of the diet causing a reduced diet intake. Also, the low fish growth at 100% CPBM diet may be attributed to the low availability of certain EAA or to EAA imbalance (the data are not included here) resulting in growth retardation.

The obtained results herein are in concomitant with previous studies used animal byproducts sources to partially or totally replace FM for red tilapia, *O. niloticus* x *O. mossambicus* (Abdelghany 2003; Ahmad 2008), sunshine bass, *Morone chrysops* x *Morone saxatilis* (Muzinic et al. 2006), gibel carp, *Carassius auratus gibelio* (Yang et al. 2006), and black Sea turbot, *Psetta maeotica* (Yigit et al. 2006). On the other hand, Rodriguez-Serna et al. (1996) found that commercial defatted animal by-product meal (a combination of BM, MBM, feather meal and FM) supplemented with soybean oil completely replaced FM in the diets fed to Nile tilapia for 7 weeks, with no adverse effects on fish performance. El-Sayed (1998) totally replaced FM by shrimp meal (SM), blood meal (BM), meat and bone meal (MBM), BM+MBM mix and poultry by-product meal (PBM) in six isonitrogenous (30% crude protein), isocaloric (400 kcal GE 100/g) diets for Nile tilapia. He found that the growth of fish fed SM, PBM and MBM was not significantly different from those fed the FM-based diet, while a reduction in fish performance was noticed when BM or BM+MBM replaced FM in the control diet.

No significant changes in the proximate whole-body composition were observed because of the changes in CPBM levels in fish diets. These results suggested that fish efficiently ingested, digested, and assimilated CPBM protein. These results are in agreement with Abdelghany (2003) and Ahmad (2008) who reported that partial or complete replacement of FM with GFM did not affect body composition (protein, fat, and dry matter) of red tilapia and Nile tilapia, respectively. Takagi et al. (2002) did not find significant changes in whole-body composition of yearling red sea bream because of inclusion of low-fat poultry by-product (with 6.7% fat) in fish diets. Yang et al. (2006) found that no significant changes were observed in whole-body moisture and fat content resulted from the different replacement of FM with PBM.

Most of the works reviewed have evaluated FM replacements in tilapia feeds from biological or nutritional viewpoints. Little attention has been paid to economic analyses of these protein sources. Only a few studies have been conducted into this subject and these have indicated that those unconventional protein sources were more economical than FM because of their local availability at low prices. Cost–benefit analysis of the test diets herein indicated that CPBM was economically superior to FM. Similar results were reported by other workers. The economic evaluation of animal by-product meals replaced FM for Nile tilapia indicated that these sources were economically superior to FM, even at total replacement levels (Rodriguez-Serna et al., 1996; El-Sayed, 1998).

Small-scale fish farmers in developing countries are constrained by both the availability and the cost of pelleted fish diets produced commercially. Hence, there is a real need to encourage fish farmers to formulate their own pelleted diets using CPBM produced near their farms as far as possible. As a conclusion of this study, it is suggested that without amino acid

supplementations, CPBM could safely replace FM up to 75% in practical diets for Nile tilapia. These results may allow for formulation of less expensive diets for Nile tilapia and may reduce the diet costs for producers.

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