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Digestibility and Utilization of Canola Meal in Angel Fish (*P. scalare* Lichtenstein 1823) Feeds

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Abstract: A 12 week feeding experiment was conducted in an aquarium (80×40×40 cm) to determine the potential use of canola meal as a partial replacement of fish meal in the isonitrogenous (approximately 44% crude protein) diet for angel fish fries with an initial average weight of about 0.91 g. Diets were formulated to include 0, 8, 16, 24, 32 and 40% (CM0, CM8, CM16, CM24, CM32 and CM40, respectively) of fish meal protein as a substitute by canola meal. Growth performance (weight gain, specific growth rate) decreased significantly, when the replacement level of fish meal protein was increased from 24% and higher, the CM40 diet was the lowest in all groups. When the replacement level of fish meal protein 16% (diet CM16) Feed Conversion Ratio (FCR) was the lowest and Protein Efficiency Ratio (PER) was the highest. There were no significant differences in the moisture, lipid, crude protein and ash content in whole body. Fish were fed with pelleted experimental diets to satiation and the feces were collected by siphoning. The apparent digestibility of dry matter ranged from 80.92-88.49%, protein from 91.16-93.71% in the experimental groups. The high level of canola meal in diets was negatively affected in terms of both dry matter and of protein digestibility. These results support the use of canola meal as important replacement protein source for fish meal of angel fish.

Key words: Angel fish, canola meal, growth, digestibility, fish meal, Turkey

INTRODUCTION

The ornamental fish has gained an enormous popularity worldwide during the last few decades and further interest appears to be continuously growing. Reflecting a high demand, culture facilities for ornamental fish have expanded (Boonyaratpalin and Sermwatankul, 2003). The trading and farming of aquarium fish and plants remain one of the most profitable aspects of aquaculture with a global trade of around US $7.2 billion annually (Chong, 2003).

However, high price of feeds in aquarium industry is a factor which prevents the expansion of the production. The price of ornamental fish feeds are 10-60 times higher that of aquaculture feeds. The prices of the feed targeted for a single ornamental species vary dramatically compared to prices of the food fish feeds, each of which is targeted for a specific species. Another major difference is that feeds for ornamental fish are marketed in much smaller packages, the largest being just over 0.5 kg. In contrast, the smallest commercial package of aquaculture feed we know of is 22 kg. Investigations on various aspects (price, palatability, color enhancement, growth supporting characteristics, maturation and spawning) of commercially available diets were conducted using a variety of species of freshwater ornamental fishes (Tamaru and Ako, 2000).

Angel fish (*P. scalare*) living in South America river is a freshwater cichlid that is one of the most valuable aquarium species. When compared with other fish, their body structures and elegant swimming styles in water are some of the elements which give them attractiveness (Wolfsheimer, 1983). Despite their high economic importance, researches on food requirements in tropical fish feeds are still at insufficient levels today. Angel fish producers prefer mostly live foods (artemia, tubifex, daphnia and mosquito larvae) for growing up fish. Production of live foods and conservation possibilities are quite limited in comparison with the formulated dry feeds. For this reason, formulation of convenient feed rations for ornamental fish carry importance for aquarium sector (Sales and Janssens, 2003). Fish meal, which is the most ideal source of protein in feed rations is inconvenient as being feed ingredients due to the fact that it has been found rarely and it has a high price. Especially for the formulations of the feeds produced in

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abundance species, cheaper ingredients are required as an alternative for fish meal (Chong, 2003). Researches show that oilseeds have a potential that will be able to be a protein source in fish feeds. The most important vegetable protein sources used in fish feeds is soybean meal, sunflower seed meal, cotton seed meal, rape-seed meal and corn gluten (Francis *et al*., 2001).

Canola Meal (CM) is the protein product produced from rapeseed low in erucic acid and glicosinolates. This vegetable protein meal has been used in various fish diets. Although, there are a lot of study on aquaculture about the use of the replacement of plant protein source to fish meal, researches about ornamental fish feeds are quite restricted. Available data show that currently about 30-50% of fish meal can be successfully replaced in fish feeds by plant protein sources (Francis *et al*., 2001), although there may be important differences depending on the species. The feasibility of canola meal as a practical ingredient has already been reported for some fish; Chong *et al*. (2002) and Chong (2003) discus (*Symphysodan aequifasciata*), Webster *et al*. (1997) and Lim *et al*. (1998) channel cat fish (*Ictalurus punctatus*), Kenji *et al*. (1999) red seabream (*Pagrus major*), Galdioli *et al*. (2001) piaucu (*Leporinus macrocephalus*), Thiessen *et al*. (2004) rainbow trout (*O. mykiss*), Mwachireya *et al*. (1999) and Burel *et al*. (2000), trout (*O. mykiss*) and turbot (*Psetta maxima*), Maina *et al*. (2002) tilapia (*O. niloticus*), Tibbetts *et al*. (2004), (*Melanogrammus aeglefinus*), Wu *et al*. (2006) yellowfin seabream (*Sparus latus*), Abbas *et al*. (2008) carp, Zhang *et al*. (2008) Yellow Croaker (*Pseudosciaena crocea*). Researchers reported that high inclusion levels of canola meal reduce weight gain and feed efficiency. Although, alternative protein sources showed considerable potential in replacement of FM, they also associated with negative qualities such as low protein content less than ideal amino acid balance, presence of Antinutritional Factors (ANFs) and high proportion of fiber or ash.

This study was designed to evaluate the effect of canola meal level in formulated on weight gain, feed conversion ratio, digestibility and survival rates.

MATERIALS AND METHODS

Diet preparation: Composition and chemical analyses of the experimental diets are shown in Table 1. Six isoenergetic and isonitrogetic diets were formulated with different levels of canola meal protein as percentage replacement for fish meal protein (0, 8, 16, 24, 32 and 40%). These diets were also formulated to contain 44% protein to the nutrition of requirement of the angel fish fry (Degani, 1993). Diets were also formulated to be isocaloric with digestible energy content of 3500 kcal kg^{-1} diet. Chromium oxide (0.5%) was also added to experimental diets for *in vivo* digestibility analysis. All ingredients were mixed thoroughly in a mixer for 30 min. The diets were made into pellets of 1.0 mm diameter by a laboratory pellet machine after mixing. Feed was then air dried. Samples (diets, fish and feces) were analyzed for dry matter, crude

Table 1: Feed formulation and proximate composition of experimental diets

	Experimental groups					
Ingredients	CM0	CM8	CM16	CM24	CM32	CM40
Fish meal (68.53% protein)	45.53	41.88	38.24	34.60	30.96	27.32
Canola meal (34.67% protein)	0.00	7.20	14.40	21.60	28.80	35.99
Soybean meal (46.44% protein)	2.50	2.50	2.50	2.50	2.50	2.50
Maize starch	19.37	15.32	11.30	7.20	3.12	0.00
Maize germ meal	12.00	12.00	12.00	12.00	12.00	12.00
Blood meal	6.60	6.60	6.60	6.60	6.60	6.60
Wheat meal	0.50	0.50	0.50	0.50	0.50	0.50
Vegetable oils	10.00	10.50	10.96	11.50	12.02	11.59
Vitamin mix.*	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix.**	1.00	1.00	1.00	1.00	1.00	1.00
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50
Dry matter proximate composition (%)						
Dry matter	91.55±0.72	91.89±0.94	91.87±0.75	91.66±0.80	91.99±1.01	91.83±0.81
Moisture	8.45±0.72	8.11±0.94	8.13±0.76	8.34±0.80	8.15±1.01	8.17±0.81
Protein	43.96±0.16	44.27±0.29	43.92±0.59	43.94±0.90	43.83±0.14	43.55±1.23
Lipid	12.41±0.15	13.05±0.03	13.30±0.12	13.47±0.31	13.91±0.21	14.18±0.20
Fiber	0.41±0.05	1.29±0.08	2.30±0.12	3.38±0.22	3.83±0.08	4.69±0.19
Ash	12.45±0.14	10.36±0.10	10.33±0.23	10.24±0.24	10.06±0.19	9.33±0.31
NFE***	22.32±0.62	22.92±0.61	22.02±0.17	20.63±0.20	20.36±0.06	20.08±0.10
Digestible energy (kcal kg^{-1})****	3500	3500	3500	3500	3500	3500

*Eryamix 107 (Vit A: 4,000,000 IU kg^{-1}, Vit D3: 400,000 IU kg^{-1}, Vit E: 40,000 mg kg^{-1}, Vit K: 2,400 mg kg^{-1},Vit B1: 4,000 mg kg^{-1}, Vit B2:6,000 mg kg^{-1}, Niasin: 40,000 mg kg^{-1}, Cal-D-Pantothenate:10.000 mg kg^{-1}, Vit B6: 4,000 mg kg^{-1}, Vit B12: 10 mg kg^{-1}, D-Biotin: 100 mg kg^{-1}, Folic acid: 1200 mg kg^{-1}, Vit C (Stay C): 40 000 mg kg^{-1}, Inositol: 60,000 mg kg^{-1}); **Eryamin-Fish (Mangan: 60,000 mg kg^{-1}, Iron: 60,000 mg kg^{-1}, Zinc: 80,000 mg kg^{-1}, Copper: 5,000 mg kg^{-1}, Cobalt: 200 mg kg^{-1}, Iodinet: 1,000 mg kg^{-1}, Selenium: 150 mg kg^{-1}, Magnesium: 80,000 mg kg^{-1}); ***Nitrogen-free extract = Dry matter (protein + lipid + fibre + ash%); ****Digestible energy value was calculated from published values for the diet ingredients (NRC, 1993)

protein, crude fiber and ash using standard methods (AOAC, 1995). These samples were analyzed for dry matter at 65°C for 24 h in a vacuum oven. Crude protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method and fiber by drying and ashing after the extraction with 0.5 M H_2SO_4 and 0.5 M NaOH. Ash content was determined after incineration at 550°C for 12 h in a muffle furnace. Crude lipid was determined using a chloroform-methanol extraction procedure (Folch *et al*., 1957). Fecal samples were collected twice daily 4 h after feeding for 84 days. Fecal samples collected from the same tank were pooled together in a bowl, pocked in cellophane bags and stored in a freezer. Uneaten diet was siphoned out using a 2 cm pipe 20 min after feeding. The whole body of fish and feces were determined using the ammonium-molybdate method described content of Cr_2O_3 in diet and feces were determined spectrophotometrically according to Furukawa and Tsukahara (1966). Two Apparent Digestibility Coefficients (ADC) were calculated according to Cho *et al*. (1982);

$$\text{ADC (Dry matter (\%))} = 100 - 100\ \frac{\text{Marker in feces (\%)}}{\text{Marker in diet (\%)}}$$

$$\text{ADC (Protein (\%))} = 100 - 100\ \frac{\text{Marker in diet (\%)}}{\text{Marker in feces (\%)}} \times \frac{\text{Protein in feces (\%)}}{\text{Protein in diet (\%)}}$$

Fish and feeding trial: Angel fish fry were obtained from Ortaca Vocational School University of Mugla. Fishes were graded and stocked in glass aquariums (150×50×60 cm), fed a commercial pelleted feed for 2 weeks prior to experimental stocking for acclimatization purposes. At the end of the acclimatizing period, 25 fish (mean weight 0.91±0.01 g) were stocked into each glass aquarium in the size of 80×40×40 cm and were performed in triplicate.

A static water system with continuous aeration and daily water change (20% of volume) to maintain water quality was used. All fish were fed to satiation by hand, three times (Falaye and Jauncey, 1999). The total feeding period was 12 weeks. At the end of the experiment, random sampling of the fishes from every aquarium was carried out for determination of carcass composition (AOAC, 1995).

Calculations and statistical analysis: Growth and feed utilization performances were determined based on these parameters:

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Weight gain (g)} = \text{Mean final weight} - \text{Mean initial weight}$$

$$\text{Specific Growth Rate (SGR \% day}^{-1}) = \frac{(\ln W_t - \ln W_{t-1})}{T} \times 100$$

Where:

W_t = The mean final weight

Wi_{t-1} = The mean initial weight

T = Total experimental feeding days

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain of fish (g)}}{\text{Total protein given (g)}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed fed (g)}}{\text{Total wet weight gain (g)}}$$

Data from each treatment were subjected to one-way Analysis of Variance (ANOVA). The data are presented as mean±SE of three replicate groups; statistical analysis was performed using the SPSS 11.0 for windows. Duncan multiple range test was used to compare the mean values between individual treatments. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Growth, feed utilization and digestibility: At the end of the experiment, the angel fish growth performance and feed utilization are shown in Table 2. High survival was observed in all dietary treatment and there was no significant difference because of dietary canola meal

Table 2: Growth performance and feed utilization of juvenile angel fish after 12 weeks of canola meal replacement study

Diets	Initial weight (g)	Final weight (g)	Weight gain (g)	Amount feed consumed (g)	SGR (% day^{-1})	FCR	PER	Survival (%)
0 CM	0.91±0.01	5.24±0.21^{a}	4.33±0.19^{a}	11.96±0.32^{a}	1.86±0.04^{a}	2.80±0.04^{a}	0.92±0.03^{a}	94.67±1.33
8 CM	0.92±0.01	5.03±0.17^{a}	4.13±0.19^{ab}	10.56±0.16^{b}	1.82±0.05^{ab}	2.66±0.04^{a}	0.95±0.04^{a}	90.67±3.53
16 CM	0.92±0.01	4.57±0.15^{a}	3.66±0.18^{b}	10.17±0.30^{b}	1.71±0.05^{b}	2.83±0.06^{a}	0.96±0.00^{a}	94.67±2.67
24 CM	0.91±0.01	3.87±0.14^{b}	2.97±0.14^{c}	8.68±0.13^{c}	1.50±0.04^{c}	3.03±0.10^{b}	0.80±0.03^{b}	90.67±5.33
32 CM	0.91±0.01	3.74±0.13^{b}	2.83±0.15^{c}	8.60±0.20^{c}	1.46±0.05^{c}	3.12±0.08^{b}	0.78±0.06^{b}	89.33±3.33
40 CM	0.91±0.00	3.16±0.12^{c}	2.24±0.02^{d}	7.90±0.12^{d}	1.26±0.02^{d}	3.54±0.01^{c}	0.75±0.03^{b}	94.67±1.33

Each values is the mean (±SE) from three replicates with means with the same letter being not significantly different (Duncan $p<0.05$)

replacement ($p>0.05$). Weight gain and PER were significantly reduced while FCR increased as the proportion of canola meal replacement in the diet increased ($p<0.05$). A significant difference between diets CM0-CM16 and CM24-CM40 was observed in the growth performance parameters indicating that up to 16% of fishmeal protein could be replaced by canola meal without causing significant reduction in growth and feed utilization. Specific Growth Rate (SGR) values ranged from 1.86-1.26 and either weight gain or growth rates were affected by bulk incorporation.

Data on the Apparent Digestibility Coefficient (ADC) of protein and dry matter in the experimental diets are shown in Table 3. The apparent protein and dry matter digestibility values for different experimental diets ranged between 91.16-93.89 and 80.92-88.49%, respectively. High canola meal levels in the diets were associated with reduced apparent protein and dry matter digestibility. The Apparent Digestibility Coefficient (ADC) of protein and dry matter decreased significantly when the replacement level of fishmeal protein was increased from 24% and higher ($p<0.05$).

The proximate compositions of the various experimental diets were shown in Table 4. There were no significant differences in the dry matter, moisture, protein, lipid and ash content in whole body, although the protein content in whole body decreased with the increase of proportion of canola meal in the dietary diets but the difference was not significant.

In the present study, the weight gain of angel fish fed the diets in which the level of canola meal protein replacing fish meal protein exceeded of 16% (diets CM24, CM32 and CM40) were significantly lower than those in the other dietary groups including the control group.

Table 3: Apparent digestibility coefficient (%) for dry matter and protein of experimental diets used for canola meal replacement study

	Experiment	
Diets	ADC (Dry matter %)	ADC (Protein %)
CM0	88.49±0.11[a]	93.71±0.07[a]
CM8	88.04±0.63[a]	93.80±0.01[a]
CM16	87.16±1.07[ab]	93.89±0.24[a]
CM24	84.87±0.52[b]	92.60±0.22[b]
CM32	81.50±0.54[c]	91.19±0.08[c]
CM40	80.92±1.04[c]	91.16±0.16[c]

Each values is the mean (±SE) from three replicates with means with the same letter being not significantly different ($p<0.05$)

Many studies have shown that in comparison with fish meal protein, canola meal reduced fish growth performance. Davies *et al.* (1990), Higgs *et al.* (1982) and Francis *et al.* (2001) reported significantly lower weight gain and feed intake that high canola meal levels in the diets. The reasons for the decrease of growth performance are the existence of components such as phytic acid, tannin, sinapine in the composition of canola meal. Phytic acid affects negatively benefiting from phosphorus (Borgeson, 2005), protein digestibility by intensifying in the peel part of the tannin grain (Yalcin, 2001) sinapin by giving bitter taste (Satoh *et al.*, 1998). In the present study, this replacement level with canola meal protein is lower than that of found in fresh water fish such as tilapia (*Sarotheredon mossambicus*) Jackson *et al.* (1982), coho salmon (*Oncorhynchus kisutch*) Higgs *et al.* (1983), channel catfish (*Ictalurus punctatus*) (Mays and Brown, 1993), tilapia (*O. niloticus*), discus (*Symphysodon aequifasciata)*. Some omnivorous freshwater fish can utilize canola meal. Extremely high substitution of fishmeal protein with canola meal in the diet of tilapia (*Sarotheredon mossambicus*) was reported, the fish grew successfully with diets in which 50% of fishmeal protein was replaced by canola meal (Jackson *et al.*, 1982). Similar results were found with replacement of up to 36% of fishmeal protein with canola meal in the diet of channel catfish (*Ictalurus punctatus*) (Mays and Brown, 1993). But for many marine fish species, they have poor tolerance for canola meal, Kenji *et al.* (1999) reported that substitution of 10% of fishmeal protein with canola meal protein did not decrease the growth performance of red sea bream (*Pagrus major*).

The FCR in fish fed diets with canola meal protein level in excess of 32% was significantly higher in the other dietary groups including the control group ($p<0.05$). When the canola meal protein replacement level increased from 0-16%, the PER increased significantly, while with the increase in canola meal protein replacement level from 24-40%, the PER decreased significantly ($p<0.05$). Similar results were observed in tilapia (Davies *et al.*, 1990), *Leporinus macrocephalus* (Galdioli *et al.*, 2001), trout (*O. mykiss*) (Thiessen *et al.*, 2004). This may suggest that the fish fed diets canola meal replacement level is under or equal 16% in the diets for angel fish.

Table 4: Carcass proximate analysis (wet weight %) of juvenile angel fish after 12 weeks of canola meal study

	Experimental groups					
Contents	CM0	CM8	CM16	CM24	CM32	CM40
Dry matter	29.58±4.20	28.50±0.62	29.89±3.97	30.67±0.95	26.78±2.30	30.86±1.34
Moisture	70.42±4.20	71.50±0.62	70.11±3.97	69.33±0.95	73.22±2.30	69.14±1.34
Protein	20.91±0.15	20.68±1.40	20.32±1.30	19.99±1.05	19.37±0.61	19.05±0.39
Lipid	5.96±0.83	6.20±0.26	5.20±1.03	5.42±0.66	5.62±1.05	5.63±0.07
Ash	2.10±0.06	2.11±0.15	2.24±0.25	2.56±0.42	2.05±0.06	2.29±0.35

Each values is the mean (±SE) from three replicates with means with the same letter being not significantly different ($p<0.05$)

The Apparent Digestibility Coefficient (ADC) of protein and dry matter in the experimental diets were decreased significantly when the replacement level of fishmeal protein was increased from 24% and higher. The major limiting factor in the digestion of canola meal is the low dry matter digestibility of this feedstuff. This is probably attributable largely to the relatively high crude fiber content (11.1%) Hilton and Slinger (1986). The protein digestibility of the canola meal was considerably lower than that of other protein meals (Cho *et al*., 1982), the reduction in the availability of the EAA in the canola meal would probably not result in any overt EAA deficiencies in fish fed diets adequate in protein. A similar response was observed by Mwachreiya *et al.* (1999) in trout (*O. mykiss*) and Burel *et al.* (2000) in turbot (*Psetta maxima*). High levels of canola meal (32-40%) affect the taste of feed; feed intake has decreased in fish. This case has also been seen at trout (*O. mykiss*) (Thiessen *et al*., 2004) and channel catfish (*Ictalurus punctatus*) (Lim *et al*., 1998).

CONCLUSION

In the present study, the results of proximate composition indicated that the moisture, protein, lipid and ash content were not affected by the level of fish meal protein replaced by canola meal.

At the end of this research, it was found that angel fish (*P. scalare*) could tolerate adding of canola meal protein by 16% in their feeds. Growth performance, feed conversion ratio, dry matter and protein digestibility were negatively affected in high levels supplement (>24%) of canola meal. Canola meal was not an effective aspect for the nutrient contents and survival rate.

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