Journal of Animal and Veterinary Advances 9 (6): 1088-1093, 2010
ISSN: 1680-5593

The Evaluation of Heated Culban, *Vicia peregrina* as a Partial Replacement for Fish Meal in Diets of Nile Tilapia, *Oreochromis niloticus*

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Abstract: This study was conducted to determine the potential nutritional value of heated culban (*Vicia peregrina*) seed at the different levels (0, 10, 20 and 30%) in diet as a plant protein source for Nile tilapia (*Oreochromis niloticus*). The fish fed experimental diets grew from an initial body weight of 38 g to a final body weight of 120 g for a rearing period of 80 days. Growth parameters, feed utilization and meat proximate composition were found similar in all experimental groups. It is concluded that culban seed can be successfully used as an inexpensive plant protein source at least up to a level of 30% in tilapia diets without adverse effects on fish performance.

Key words: Growth, Nile tilapia, *Oreochromis niloticus*, *Vicia peregrina,* experimental diet, body weight

INTRODUCTION

Fish meal, owing to its nutritional quality is an important component of feed for most cultivable fish species (Khan *et al*., 2003). Fish meal production is also rather heavily confined to a small area in some regions of the world resulting in it becoming more expensive and difficult to obtain in many countries practising aquaculture. The high cost of fish meal can be deterrent to optimal diet formulation in aquaculture systems (Hossain *et al*., 2001, 2002). In addition, the use of animal protein for herbivorous or omnivor fish such as tilapia and carp could be unnecessary. Given this, the use of alternative, perennially available plant proteins with low seasonal nutritional quality variability is considered an important objective in aquaculture (Olvera-Novoa *et al*., 2002). Locally available plants are often less expensive sources of energy and protein for commercial feed formulations. Meals made from feed peas are intermediate in terms of both a good energy source and a reasonable amount of protein to the ration.

However, inclusion levels in feed formulations must be tempered by cost, processing consideration, nutrient availability as well as palatability of the meal to target species. As a result, information related to nutrient availability and the biological response of the target species is essential to the successful utilization of a given ingredient (Davis *et al*., 2002). The grain legumes have not been extensively used in fish feeds, they represent a good source of dietary protein and energy (Siddhuraju and Becker, 2002). Recently, researchers have begun to evaluate the acceptability of grain legume such as lupin (Sudaryono *et al*., 1999; Glencross *et al*., 2003), cowpea and rice bean (Eusebia, 1991), *Cassia fistula* (Adebayo *et al*., 2004), feed pea (Borlongan *et al*., 2003), *Sesbenia aculeate* (Hossain *et al*., 2001, 2002) in fish diets. Culban (*Vicia peregrina*), a pea is a legume crop widely available and abundant in region of South Mediterranean of Turkey. Its seeds contain 25% protein with a partially balanced amino acid and fatty acid profile (Table 1). Culban has not been tested yet as a protein source by now on an aquatic animal, except mirror carp (Buyukcapar and Kamalak, 2006).

This study was designed to determine the potential nutritional value of heated culban (*Vicia peregrina*) seed at the different levels in diet as a plant protein source for Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental design: This study was carried out in an indoor system at the Freshwater Fish Experimental Station of the Faculty, the Faculty of Fisheries of Çukurova University, Adana-Turkey. As experimental fish Nile tilapia, *Oreochromis niloticus* reared in this station was used. The fish, average weights of 35-40 g, corresponding to fish ages of about 4 mount, were selected from the general population and then acclimatized to the

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Table 1: Proximate compositions, amino acids and fatty acids profiles and condensed tannin amount (on as is basis) in culban seeds meal

Items	Amount	Fatty acids (%)	Amount
Proximate composition (%)			
Crude protein	25.2	(C14:0)	0.58
Crude fat	1.5	(C15:0)	0.25
Dry matter	92	(C15:1)	0.12
Crude ash	3.9	(C16:0)	10.64
Fibre	8.1	(C16:1)	0.24
Amino acids (mg/100 g)		(C17:0)	0.26
EAA		(C17:1)	0.11
His	432	(C18:0)	4.94
Ile	883	(C18:1n9c)	16.36
Leu	1906	(C18:2n6c)	51.58
Lys	1832	(C20:0)	1.31
Met	240	(C18:3n3)	6.41
Phe	1204	(C20:1n9)	0.13
Thr	1112	(C21:0)	0.29
Val	1124	(C20:2)	0.25
Total EAA	8733	(C22:0)	0.49
Non-EEA		(C22:1n9)	0.48
Aspartic asid	6950	(C23:0)	0.18
Pro	1247	(C24:0)	0.33
Ser	1210	(C24:1n9)	0.13
Tyr	761	Undefined FA (%)	4.49
Ala	1029	Saturated FA (%)	19.36
Gly	1008	Monounsaturated FA (%)	17.62
Glu	4170	Polyunsaturated FA (%)	58.54
Total non-EAA	16375		
Condensed tannin (g kg^{-1})	14.7		

experimental conditions for three weeks before the start of the actual experiment. During this period, the fish were fed a basal diet (or control diet). Sixteen fish were stocked per glass aquarium (300 L) with three replicates for four experimental diets. The fish were fed by hand three times a day at a rate of 3% body weight during the experimental period of 80 days. Body weight of all fish per treatment replicates were measured every 15 days. During the measurements, the fish were anaesthetized using 2-phenoxyethanol at a dose of 0.15 mL L^{-1}.

Compressed air with air stone was used for maintaining soluble oxygen throughout the experiment. Water in each aquarium was changed at a rate of 80% volume per day. Uneaten feed and faces were siphoned out daily.

Aquariums were checked daily and mortalities (if present) were recorded. Temperature, pH and dissolved oxygen level in water of aquaria were measured daily, while ammonia and nitrite were analysed weekly. Throughout the experimental period, the water temperature was between 26 and 27°C, dissolved oxygen level was above 6.2 mg L^{-1}, pH was around 7.8, ammonia (as NH_4) and nitrite were below 0.05 mg L^{-1}. All aquaria were maintained under a constant photoperiod (12 h light: and 12 h dark) created by fluorescent lamps. Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Experimental diets and diet preparation: Four different experimental diets containing 0, 10, 20 and 30% heated culban seed meals, each of which were isonitrogenous and isoenergetic were formulated to evaluate the nutritive value of culban for tilapia. Dietary feed ingredients were ground into a meal with a laboratory grinder and then blended into a homogenous doughy consistency by adding water and pelleted by pressing through a 3 mm die in a grinding machine. The pellets were dried in an oven at 45°C for 48 h and then stored in a refrigerator until required. The ingredients were provided Pinar A.S., Izmir, Turkey. Culban, Vicia peregrina was collected from Kahraman Maras, Turkey. Culban seeds were dried in an oven at 45°C for 48 h and then ground into a meal with a laboratory grinder and were heat-treated in a oven at 121°C for 10 min (Rehman and Salariya, 2005) to eliminate the possible detrimental effects of antinutritional factors within culban. Results of proximate compositions, amino acids and fatty acids analyses in culban seeds meal were given in Table 1. Formulation and proximate compositions of experimental diets were shown in Table 2.

Chemical analyses:

Proximate composition: Crude protein, crude fat, crude fibre, crude ash and moisture analyses were performed on dried culban seed, experimental diets and fish fillets. Fillets from three fish in each treatment were pooled and then minced using a meat mincer and stored at -20°C until required. The analyses were conducted in triplicate.

Table 2: Formulation and proximate composition of experimental diets

	Experimental diets			
Ingredients (g kg^{-1})	Control	10% culban	20% culban	30% culban
Fish meal	200	186	170	154
Soybean	406	390	362	342
Culban seed	0	100	200	300
Maize flour	302	226	161	84
Sun flower oil	70	78	85	95
DCP (di-calcium phosphate)	6	7	8	9
Limestone	5	5	5	5
Salt (NaCl)	3	3	3	3
Methionin	1	1	1	1
Lysine	1	1	1	1
Bentonite	1	1	1	1
Vitamin and mineral premix*	5	5	5	5
TOPLAM	1000	1000	1000	1000
Proximate composition (g kg^{-1})				
Dry matter	889	883	890	899
Crude protein	352	353	352	349
Crude fat	95.0	95.5	102.4	109.2
Crude ash	67.8	66.3	65.0	64.0
Crude fibre	33.6	35.9	39.0	40.2
Gross energy (kJ g DW^{-1})	18.0	18.0	18.0	18.0
Condensed tannin	1.25	2.56	3.99	5.40

*Per 5 kg vitamin and mineral premix: 20,000,000 IU vitamin A, 200,000 IU vitamin D3, 200,000 mg vitamin E, 12000 mg vitamin K_3, 20,000 mg vitamin B_1, 30,000 mg vitamin B_2, 200,000 mg niacin, 50,000 mg Ca-panthothenate, 20,000 mg vitamin B_6,50 mg vitamin B_{12}500 mg D-biotin, 1.200 mg folic acid, 200,000 mg and 300,000 mg inositol.1,200,000 mg cholin chloride, 40,000 mg manganese, 30,000 mg Zinc, 800 mg Copper, 1000 mg iodine, 150 mg Selenium, 40,000 mg magnesium

Tannin: Total condensed tannin was determined by the butanol-HCL method, as described by Makkar *et al.* (1995). Mimosa tannin was used as an external standard.

Amino acid: Amino acids analyses were performed on culban seed meal. Samples were hydrolysed in 6 N HCl at 110°C for 24 h in an evacuated sealed ampoule. Excess acid from the hydrolysate was removed by flash evaporation under reduced pressure. The analysis was carried out using an Eppendorf Biotronic LC 3000 Amino Acid Analyzer (Eppendorf-Biotronic, Hamburg, Germany), according to standard program. The analyses were carry out in duplicate.

Fatty acids: Fatty acids analyses were performed on culban seed meal. The fatty acids in the total lipids were saponifed into the free form by saponification with 0.5 N methanolic NaOH, fallowed by esterification with 14% BF_3 (w/v) in methanol. All samples were analysed using a Thermoquest Trace gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column (30 m ×0.25 mm i.d., 0.20 μm film thickness of polyethylene glycol) (Supelco Inc., Bellefonte, PA, USA) and a Flame-Ionization Detector (FID). Helium (30 mL min^{-1}) was used as the carrier gas. The samples were injected at 120°C. After 2 min the temperature was raised at 5°C min^{-1} to 220°C, where it was kept for 8 extra min. The temperatures of the injector and the detector were set at 240 and 250°C, respectively. The fatty acids methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Company; the fatty acid methyl mixture No. 189-19). The analyses were carry out in duplicate.

Statistical analysis: All data were expressed as mean±standard error. Data taken from each treatment diet on each sampling period were analysed by Analysis of Variance (one-way ANOVA) and the significant differences (if present) in ANOVA were ranked with Tukey's multiple comparison test at the 5% level of significance in SPSS 11 for windows.

RESULTS AND DISCUSSION

Growth performance, feed conversion rate and survival rate: All fish grew normally and no specific signs of disease were observed throughout the experiment period. Fish weight increased over three fold at the end of the feeding trial of 80 days (Table 3). All diets were accepted equally well by the fish.

Total weight gain among experimental groups varied from 78.84-81.86 g, specific growth rate from 1.40-1.43 and feed conversion rate from 1.94-1.97 (Table 4). However, in terms of both growth parameters and feed utilization, no significant differences were found among the experimental groups ($p>0.05$).

Proximate composition: As percentage, protein content in the fish fillets from experimental groups ranged from 18.73-19.31, lipid from 1.74-1.83, moisture from 76.57-77.59 and ash from 1.32-1.41. However, these differences among the experimental groups were not statically significant ($p<0.05$) (Table 5).

Growth parameters and feed conversion ratio between fish fed diets with or without culban were found similar. The present results indicate that culban can be successfully used as an alternative plant protein source up to a level of 30% in tilapia diets without adverse affects on growth or feed utilization.

Culban has not been used in tilapia or other fish diets, except study conducted by Buyukcapar and Kamalak (2006) who stated that heated culban could be supplemented up to a level of 30% in mirror carp diets. The level of plant protein sources included in fish diets is restricted to the presence of antinutritional components and high cellulose amount.

The critical levels of dietary peas which retard growth have been determined to range 9.7-17.6 in various fish species (Hossain *et al*., 2001, 2002; Borlongan *et al*., 2003; Adebayo *et al*., 2004; Ramachandran *et al*., 2005). Higher inclusion level (30%) of culban in the study was used. The reason of this can be caused by several factories: Processing technology of a dietary feed material is very important as it can be strong effects on some anti-nutritional components and nutrient digestibility of fish diets. Buyukcapar and Kamalak (2006) who found similar result with ours but in different fish species, declared that using of culban up to a level of 30% in mirror carp diet was due to heating process of culban. Elevated levels of dietary cellulose have been reported to affect adversely nutrient digestibility (Kirchgessener *et al*., 1986) and growth (Dioundick and Stom, 1990) in fish. However, since culban contains relatively low level (8%) of cellulose, cellulose amount among experimental diets showed a little change (from 3.36-4.02). Moreover, as tilapia has a relatively long digestive tract which may allow for more efficient digestion of plant ingredient, this fish could tolerate easily this increase in cellulose level in their diets.

Even though peas are considered to contain relatively low levels of anti-nutritional components, rearing parameters of the fish could be impaired by such factors (Francis *et al*., 2001).

It would be essential to present sufficient information about anti nutritive factors in culban seed to allow adequate interpretation of the results and comparison to the other research. This is particularly important in the current experiment. However, no information is available on anti-nutritive factors except for condensed tannin (1.47%, Table 1) in the culban seed used in this experiment.

As shown in Table 1, culban has in addition to low cellulose, a balanced amino acid and fatty acid profile. It contains many essential amino acids and polyunsaturated fatty acids (58%). Nevertheless, inclusion of culban at high levels may cause an imbalance of essential amino acid profile in the diet. To compensate for this, we supplemented the experimental diets with lysine and methionine, two of potentially limiting amino acids.

In the present study, results of proximate composition analysis indicated that protein, lipid, moisture and ash in fillets of Nile tilapia were not affected by inclusion of culban seeds in their diets. Similarly, many researchers reported that addition of plant protein sources in fish diets did not affect generally proximate composition of fish fillets (Moyano *et al*., 1992; Pongmaneerat *et al*., 1993; Regost *et al*., 1999; Zhou *et al*., 2005).

Table 3: Weight of the fish fed experimental diets at the rearing periods

Rearing periods	Experimental diets			
	Control	10% culban	20% culban	30% culban
0 Gün	38.17±0.36	38.12±0.40	38.29±0.41	38.21±0.40
20 Gün	53.70±0.69	53.17±0.76	53.86±0.76	54.22±0.64
40 Gün	72.53±1.06	72.05±1.21	73.64±1.17	74.14±0.90
60 Gün	95.68±1.71	95.11±1.59	97.16±1.79	98.24±1.19
80 Gün	117.23±2.43	116.95±2.04	118.09±2.42	120.07±1.79

Table 4: Rearing parameters of the fish fed the experimental diets, for a rearing period of 80 days

Rearing parameters	Diets			
	Control	10% culban	20% culban	30% culban
Initial weight (g)	38.17±0.36	38.12±0.40	38.29 ±0.41	38.21 ±0.40
Final weight (g)	117.23±2.43	116.95±2.04	118.09±2.42	120.07±1.79
Total weight gain (g)	79.06±0.83	78.84±0.96	79.80±1.55	81.86±0.74
Daily weight gain (g.day^{-1})	0.98±0.01	0.99±0.01	1.00±0.02	1.02±0.01
Weight gain (% day^{-1})	207.11±1.99	206.79±1.52	208.37±3.65	214.27±2.33
Specific growth rate (% day^{-1})	1.40±0.01	1.40±0.01	1.41±0.01	1.43±0.01
Feed conversion ratio	1.97±0.01	1.97.01±0.01	1.97±0.02	1.94±0.01
Survival rate (%)	100	100	100	100

Table 5: Proximate compositions of the fish fed experimental diets

Proximate composition (%)	Diets Control	10% culban	20% culban	30% culban
Crude protein	19.31±0.13	18.95±0.42	19.04±0.64	18.73±0.79
Crude fat	1.80±0.09	1.74±0.07	1.83±0.03	1.78±0.04
Moisture	76.90±0.80	77.59±0.25	76.57±0.42	77.36±0.17
Crude ash	1.32±0.06	1.36±0.04	1.41±0.02	1.39±0.06

CONCLUSION

It is said that heated culban seed can be successfully used as an inexpensive plant protein source at least up to a level of 30% in Nile tilapia diets without adverse effects on growth performance, feed utilization and meat proximate composition. Based on the positive results of these studies fish feed industry can be encouraged to evaluate the use of pea meals in trial formulations and long term growth trials. However, because culban inclusion over 30% level in diet was not tested in this study, additional experiments are required to test over that level.

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