Growth Performance and Body Composition in Mirror Carp (Cyprinus carpio) Fed Culban Seed (Vicia peregrina) with Different Heat Treatments ^[1]

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Summary

This study was conducted to determine the potential nutritional value of differently heat-treated (121°C 10, 20, 30 min) culban (*Vicia peregrina*) seed at different levels (0, 20, 30, 40%) in diet as a plant protein source for mirror carp (*Cyprinus carpio*). Fish fingerlings were randomly allocated at a stocking rate of 12 fish per aquaria with three replicate aquariums under each dietary treatment. The fish were hand fed twice a day at 8.30 am and 16.30 pm at 3% of their body weight during the experimental period of 60 days. The fish fed experimental diets grew from an initial body weight of 7 g to final body weight of 20 g for rearing during the course of the experiment. Growth parameters, feed utilization and whole body proximate composition were found similar in all experimental groups. It is concluded that heat-treated culban seed can be successfully used as an inexpensive plant protein source a level of 40% (121°C 10-30 min) in mirror carp diets without adverse effect on fish performance.

Keywords: Mirror carp, Cyprinus carpio, Heated culban, Vicia peregrina, Growth

Farklı Sürelerde Isıtılmış Culban (*Vicia peregrina*) Tohumuyla Beslenen Aynalı Sazanlarda (*Cyprinus carpio*) Büyüme Performansı ve Vücut Kompozisyonu

Özet

Bu çalışma, bitkisel protein kaynağı olarak farklı seviyelerde (%20, 30, 40) aynalı sazan diyetlerine, farklı sürelerde ısıtılarak (121°C'de 10, 20, 30 dk) katılmış culbanın (*Vicia peregrina*) besin değerinin belirlenmesi amacıyla düzenlenmiştir. Balıklar rastgele her akvaryuma 12 adet ve 3 tekerrürlü olarak stoklanmıştır. Balıklar günde iki kez sabahları 8.30, akşamları ise 16.30'da vücut ağırlığının %3'ü oranında 60 gün süreyle elle yemlenmiştir. 60 günlük deneme süresince balıklar 7 g'dan 20 g ağırlığa ulamışlardır. Bütün deneme gruplarında, büyüme parametreleri, yem tüketimi ve tüm balık vücut kompozisyonları bakımından benzer bulunmuştur. Sonuç olarak ucuz bir bitkisel protein kaynağı olarak ısıtılmış culbanın (121°C 10-30 dk) yemlerde %40 oranında katılması balık performansına herhangi bir olumsuz etki göstermediği belirlenmiştir.

Anahtar sözcükler: Aynalı sazan, Cyprinu carpio, Culban, Vicia peregrina, Büyüme

INTRODUCTION

Research investigating cheaper alternative protein and energy feedstuffs for the development of low-cost pelleted feeds suitable for use by the small-scale farmers has become priorty in developing countries. Locally available plants are often less expensive sources of energy and protein for commercial feed formulations. Meals made from peas are intermediate in terms of both a good energy source and a reasonable amount of protein to the ration ¹. However, inclusion levels in feed formulations needs to be tempered by cost, processing consideration, nutrient

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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 ². Alternative plant ³⁻⁵ and animal proteins ^{6,7} have been studied by fish nutritionists and feed industries. Grain legumes have no been extensively used in fish feeds, although they represent a good source of dietary protein and energy ⁸. The acceptability of grain legumes such as lupin ^{9,10}, *Cassia fistula* ¹¹, *Vicia peregrinea* ¹², feed pea ¹³, *Sesbenia aculeate* ¹⁴, *Vicia narbonensis* ¹⁵, *Pisum elatius* ¹⁶, *Lathyrus sativus* ¹⁷.

The use of grain legumes in aquaculture diets is potentially restricted by inadequacies in protein composition, relatively high levels of carbohydrates, the presence of anti-nutritional factors, and/or reduced palatability ^{18,19}. Trypsin inhibitors are generally considered as the most important anti-nutritional factor in plant proteins. Triypsin inhibitors and some other anti-nutritional factors are heat lable and are inactive by heat treatments²⁰. In general, the extent to which trypsin inhibitor is destroyed by heating, particle size and moisture conditions ²¹. At 100°C, however, nearly all of the trypsin inhibitor activity is destroyed after 10 min²². 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). Little is known about the dietary effects of duration of heat treatment of culban seed meal in mirror carp. Buyukcapar and Kamalak¹² who stated that raw and heated (only 121°C 10 min) culban could be supplemented up to levels of 10 and 30% respectively. Optimization of heat treatments is necessary because over processing reduces protein availability ²³. This study was conducted to determine the effects of duration of heat treatment of culban seed at different levels in diet as a plant protein source for mirror carp (Cyprinus carpio).

MATERIAL and METHODS

Mirror carp (*Cyprinus carpio*) fingerlings were obtained from the brood stock maintained at the State Hydraulic Works in Adana, Turkey. The fish (about 5 g) were transferred to the Department of Fisheries, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam. Mirror carp reared and acclimatized for 10 days in a 250-L fiberglass tank at 28.2±0.2°C. During this period they were fed with a diet containing approximately 350 g/kg protein, 80 g/kg lipid, 7 g/kg ash and energy content of 13.54 MJ/kg dry matters until they reached a body weight of approximately 7 g.

Fish fingerlings were randomly allocated at a stocking rate of 12 fish per aquaria with three replicate aquariums under each dietary treatment. The fish were hand fed twice a day at 8.30 am and 16.30 pm at 3% of their body weight

during the experimental period of 60 days. They were individually weighed at the beginning of the experiment (day 0) and at day 20 40, and 60. At the beginning of the experiment 10 fish from the same stock were sampled to determine the whole body composition. At the end of the experiment, 5 fish per aquarium were sampled to determine the whole body composition in groups. Heated culban was included in the diets at different levels (0, 200, 300, 400 for diets Control, Ht₂ Ht₃ and Ht₄, respectively). The growth parameters of fish fed the experimental diets were compared to fish fed a fish meal and soyabean meal based control diet. The control diet contained 40% fish meal and 15% of soybean meal as main protein sources.

In a recirculation system containing a set of aquaria, each with a capacity of approximately 80-L and water depth 50 cm, water quality was monitored throughout the experiment. Temperature, pH and dissolved oxygen were recorded daily. Mean temperature, pH and dissolved oxygen were $27.1\pm0.02^{\circ}$ C, 7.5 ± 0.08 and 6.9 ± 0.05 mg L⁻¹. respectively. Water quality was controlled by biologic filter and electronic heater. Water was re-circulated through the filter at a rate of 5 L/min. Additional aeration was provided by an air pump.

Culban seed was sun dried and ground into powder to pass a 1 mm sieve. Ground culban was heat-treated in autoclave at 121°C for 10, 20, 30 min to eliminate the possible detrimental effect of anti-nutritional factors ²⁴. The chemical composition of Fish meal, soybean meal, and culban are given in *Table 1*.

Growth performances were evaluated by body weight gain (BWG), feed conversion ratio (FCR), feed intake (g) per fish (FI), specific growth rate (SGR %) and protein efficiency ratio (PER).

Body weight gain (BWG) (g) = [final body weight (W_2) (g) - initial weight (W_1) (g)]

Feed conversion ratio (FCR) = [dry feed consumed (g)/live body weight (g)]

Specific growth rate (SGR %) = $[(InW_2 - InW_1)/days] \times 100$

Feed intake (g) per fish = [Total feed consumption (g) per aquaria]/(number of fish per aquaria)

Protein efficiency ratio (PER) = [body weight gain (g)/protein fed (g)]

In the feeding experiment fish meal was replaced with heat-treated culban on a dry matter basis to maintain crude protein and energy levels. The composition of the experimental diets is given in *Table 2*.

Ingredients were mixed, formed into a moist pellet about 2 mm in diameter and then dried in a forced-air oven at 45°C. Ten isonitrogenous and isoenergetic diets were formulated to evaluate nutrition value of heated culban for carp fingerling (*Table 2*). Culban, experimental Table 1. Proximate compositions, amino acids and fatty acids profiles and condensed tannin amount (on as dry basis) in culban seed meal

Items	Amount	Fatty acids (%)	Amount	
Proximate composition (g/kg)				
Crude protein	253.1	(C14:0)	0.58	
Crude fat	15.3	(C15:0)	0.25	
Dry matter	930.2	(C15:1)	0.12	
Crude ash	38.9	(C16:0)	10.64	
Fiber	80.0	(C16:1)	0.24	
Essantial amino acids (g/kg)		(C17:0)	0.26	
Methionine	10.8	(C17:1)	0.11	
Valine	10.6	(C18:0)	4.94	
Isoleucine	9.5	(C18:1n9c)	16.36	
leucine	16.8	(C18:2n6c)	51.58	
Tyrosine	6.4	(C20:0)	1.31	
Phenylalanine	10.1	(C18:3n3)	6.41	
Histidine	14.9	C20:1n9)	0.13	
Lysine	16.3	(C21:0)	0.29	
Threonine	8.8	(C20:2)	0.25	
Non-essantial amino acids (g/kg)		(C22:0)	0.49	
Alanine	10.0	(C22:1n9)	0.48	
Aspartic Acid	36.4	(C23:0)	0.18	
Glutamic acid	40.2	(C24:0)	0.33	
Glycine	8.4	(C24:1n9)	0.13	
Proline	9.7	Undefined FA (%)	4.49	
Serine	11.7	Saturated FA (%)	19.36	
Hydroxiproline	0.0	Monounsaturated FA (%)	17.62	
		Polyunsaturated (%)	58.54	
Antinutritional factor				
	Raw	Heated (10 min)	Heated (20 min)	Heated (30 min)
Condensed Tannin (g/kg)	15.3	7.8	7.9	7.6

diets and fish samples were analyzed for their proximate composition according to the methods of AOAC²⁵. Table 2 shows the formulation, proximate composition of the 10 diets. Condensed tannin was determined by butanol-HCl method as described by Makkar et al.²⁶. Amino acids composition of culban was determined by GC-ID at Scientific and Technology Research Council of Turkey (MAM) using Phenomox EZ Feast GC-FID Hydrolyzed Amino acid analyses Kit. Fatty acids analysis was 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, followed by esterification with 14%BF₃ (w/v) in methanol. All samples were analysed using using a Thermo quest Trace gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column (30 m x 0.25 mm i.d., 0.20 µm film thickness of polyethylene glycol) (Supeco Inc., Bellefonte, PA, USA) and a Flame-Ionization Detector (FID). Helium (30 mL/min) 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 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 carried out in duplicate.

A completely randomized design was adopted with three replicates per diets. One-way analysis of variance (ANOVA) was carried out to determine the effect of diets on growth and whole body composition parameters using General Linear Model (GLM) of Statistical for windows (1993). Significance between individual means was identified using the Duncan's multiple comparison tests. Mean differences were considered significant at P<0.05.

One-way analysis of variance (ANOVA) was applied to determine whether the effects of the diets on growth and whole body composition parameters using SPSS 11 for

	Dose of Culban 200 g/kg (20%) Duration			Dose of Culban 300 g/kg (30%) Duration			Dose of Culban				
Ingredients (g/kg)							400 g/kg (40%)				
							Duration				
	Control	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	
Fish Meal	400	360	360	360	340	340	340	320	320	320	
Soybean meal	150	150	150	150	150	150	150	150	150	150	
Culban	0	200	200	200	300	300	300	400	400	400	
Maize flour	367	202	202	202	122	122	122	42	42	42	
Sunflower oil	72	77	77	77	77	77	77	77	77	77	
DCP ¹	1	1	1	1	1	1	1	1	1	1	
Vit-min ²	5	5	5	5	5	5	5	5	5	5	
Salt	1	1	1	1	1	1	1	1	1	1	
Methionine	2	2	2	2	2	2	2	2	2	2	
Lysine	2	2	2	2	2	2	2	2	2	2	
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	
Composition (g/kg)											
Crude protein	380.01	389.2	389.2	389.2	394.5	394.5	394.5	399.5	399.5	399.5	
Crude Fat	131.67	129.38	129.38	129.38	125.79	125.79	125.79	122.3	122.3	122.3	
Crude ash	65.22	66.33	64.3	65.4	66.8	65.9	66.45	64.5	63.4	65.6	
Crude fibre	40.36	42.96	42.96	42.96	44.46	44.46	44.46	45.9	45.9	45.9	
Dry Matter	892.5	889.2	888.5	888.4	890.4	889.3	892.1	890.9	891.2	889.5	
Gross energy (MJ/kg) ³	19.9	20.0	20.0	20.0	19.9	19.9	19.9	19.9	19.9	19.9	

¹ Di calcium Phosphate

² Per 5 kg vitamin-mineral premix: 20.000.000 IU vitamin A, 2.00.000 IU vitamin D_y 200.000 mg vitamin E, 12.000 mg vitamin K_y 20.000 mg vitamin B, 30.000 mg vitamin B $_y$ 200.000 mg niacin, 50.000 mg Ca-panthothenate, 20.000 mg vitamin B $_{65}$ 50 mg vitamin B $_{12}$ 500 mg D-biotin, 1.200 mg folic acid, 200.000 mg vitamin C and 300.000 mg inositol.1.200.000 mg cholin chloride, 40.000 mg manganese, 30.000 mg Zinc, 800 mg Copper, 1.000 mg iodine, 150 mg Selenium, 40.000 mg magnesium.

³ Gross energy was estimated using the following coefficients: 23.6 KJ/g for crude protein; 39.5 KJ/g for crude fat and 17.2 KJ/g for carbohydrates (NRC, 1993)

windows Significance differences between individual means were evaluated using the Duncan's multiple comparison tests when a significant (P<0.05) effect was detected.

RESULTS

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 60 days (*Table 3*). All diets were accepted equally well by the fish.

Final weight among experimental groups varied from 21.9-18.77 g, specific growth rate from 1.65-1.88%, feed conversion rate from 1.36-1.54 and protein efficiency ratio from 1.90-1.71 (*Table 3*). However, in terms of both growth parameters and feed utilization, no significant differences were found among the experimental groups (P>0.05).

The effect of diets on the whole body composition of mirror carp (*Cyprinus carpio*) fingerlings at the end of the feeding trials are given in *Table 4*. The final protein, fat, ash

and DM contents of fish were higher than the initial levels. No significant differences among the experimental groups (*Table 4*).

DISCUSSION

The critical levels of dietary peas which retard growth have been determined to range 9.7-30 in various fish species ^{1,11,12,15-17,27-29}. Higher inclusion level (40%) of culban in the study was used. The reason of this can be caused by several factors; processing technology of dietary feed material is very important as it may have strong effects on some antinutritional components and nutrient digestibility of fish diets. Yanar et al.¹ who found similar result with ours but in different fish species, declared that the use of culban up to a level of 30% in tilapia diet was due to heating processes (121°C 10 min) of culban. Elevated levels of dietary cellulose have been reported to affect adversely nutrient digestibility ³⁰ and growth ³¹ in fish. However, since culban contains relatively low level (8%) of cellulose, cellulose amount among experimental

Dose of Culban

Parameters		Dose of Culban 200 g/kg (20%) Duration			Dose of Culban 300g/kg (30%) Duration			Dose of Culban 400 g/kg (40%) Duration													
												Control	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
											Initial weight (g)*	7.18±0.38	7.12±0.41	7.15±0.39	7.06±0.38	7.17±0.47	7.19±0.44	7.16±0.40	7.19±0.44	7.15±0.46	7.19±0.42
Final weight (g)*	20.63°±2.62	20.36°±2.33	20.14°±3.23	21.9ª±2.42	21.10±2.85	20.15±3.03	20.94±2.53	20.84±2.82	18.77±2.88	19.68±2.7											
Body weight gain (g)*	13.45±0.87	13.24±0.92	12.99±0.41	14.85±0.72	13.92±0.94	12.95±0.79	13.78±0.15	13.64±0.49	12.12±0.50	12.49±0.8											
Feed intake (g)*	18.88±3.95	18.99±3.98	18.97±3.97	19.11±3.99	19.13±4.00	18.72±3.91	18.99±3.97	18.77±3.93	19.2±4.01	18.82±3.9											
Feed conversion ratio*	1.42±0.12	1.44±0.09	1.46±0.06	1.36±0.06	1.38±0.10	1.45±0.08	1.37±0.05	1.37±0.01	1.54±0.07	1.52±0.10											
Specific growth rate (%)*	1.75±0.11	1.74±0.05	1.72±0.07	1.88±0.13	1.79±0.12	1.71±0.06	1.78±0.05	1.77±0.09	1.65±0.09	1.67±0.12											
Protein efficiency ratio*	1.76±0.14	1.79±0.06	1.77±0.09	1.90±0.12	1.86±0.15	1.77±0.04	1.85±0.13	1.80±0.07	1.71±0.12	1.73±0.13											
Survival rate (%)	100	100	100	100	94	96	100	98	94	100											

Table 4. The whole body composition analyses (%) (on wet weight basis) of mirror carp fed test diets (n = 15) Tablo 4. Deneme diyetleri ile beslenen aynalı sazanın tüm vücut kompozisyonu (%) (yaş maddede) (n = 15) **Dose of Culban** Dose of Culban

Parameters			200 g/kg (20%)			300g/kg (30%)			400 g/kg (40%)			
			Duration			Duration			Duration			
	Initial	Control	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	
Protein*	9.37±0.32	13.79±0.28	13.83±0.37	14.15±0.18	14.38±0.39	14.65±0.35	14.20±0.42	13.86±0.30	14.16±0.56	14.58±0.19	13.87±0.24	
Fat*	3.57±0.19	6.78±0.11	7.010±0.45	6.99±0.15	6.89±0.27	6.52±0.36	6.69±0.42	6.83±0.36	6.62±0.23	6.64±0.17	6.5±0.33	
Ash*	2.07±0.18	2.38±0.14	2.28±0.10	2.42±0.24	2.30±0.12	2.9±0.03	2.22±0.07	2.59±0.02	2.23±0.10	2.69±0.11	2.72±0.24	
DM*	19.02±0.90	27.32±0.88	27.41±0.49	27.12±0.88	27.29±0.69	26.55±0.50	26.88±0.90	26.46±0.56	28.20±0.51	26.47±.065	28.34±0.72	
* All betwee	* All between-group differences were not significant (P>0.05)											

diets showed a little change (from 4-4.5%). Moreover, as mirror carp has a relatively long digestive tract which may allow for more efficient digestion of plant ingredients, this fish could easily tolerate this increase in cellulose level in the diets ¹.

It would be essential to present sufficient information about anti-nutritive factors in culban seed to allow adequate interpretation of the result and comparison to the other research. This is particularly important in the current experiment. However, no information is available on antinutritive factors except for condensed tannin in the culban seed used in this experiment. The growth performance of mirror carp fingerlings fed diets containing up to 40% of heat-treated culban was comparable with control diets (Table 3) possible due to reduction in condensed tannin in culban by heat treatment (Table 1). Makkar et al.³² indicated that the presence of tannin and trypsin growth inhibition was related to their lower nutritive value. Although peas are considered to contain relatively low levels of anti-nutritive factors, performance of the fish could nevertheless be impaired by them ³³. Extrusion decreases the efficiency of the majority of these ³⁴.

In the present study, results of proximate composition analysis indicated that protein, lipid, moisture and ash in whole body of mirror carp were not affected by the dietary inclusion of culban seeds. Similarly, many researchers reported that addition of plant protein sources in fish diets did not affect generally proximate composition of fish body ^{1, 35-37}.

Culban has been used in mirror carp diets by Buyukcapar and Kamalak¹² who stated that heated (only 121°C 10 min) culban could be supplemented up to level of 30% in mirror carp diets. However, the present results indicate that heated culban can be successfully used as an alternative plant protein source a level of 40% in mirror carp diets without adverse affects on growth or feed utilization. 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 40% levels in diets was not tested in this study, additional experiments are required to test over the tested level.

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