

Canola Substitution in Nile Tilapia *Oreochromis niloticus* Diets

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Abstract

Global development in the manufacture of aquatic food make the researchers to find out some suitable substitution for fish and soybean meal to reduce the price especially in omnivorous and herbivorous fish like tilapia. Canola as an oil seed plant protein source can be a good candidate. To examine this, five iso-caloric experimental diets (gross energy, 4.61 Kcal/g) were formulated to contain graded levels of 0 (control), 25%, 50%, 75% and 100% canola meal replaced with soybean and fish meal. The results showed that there was a significant negative effect on growth performances by canola enhancement for feed and protein intake reduction because of bitter taste of some anti-nutrients. But, the indices of feed and protein performances such as FCR, PER, PCE did not exhibit any significant difference until 50% replacement. So, it could be predicted that if the problem of palatability discard, canola replacement can be happen until 50% for growing Nile tilapia.

Keywords: Canola; Substitution; Nile tilapia; Diets

Introduction

Global development in the manufacture of aquatic food has effected on fish oil and meal usage but little on their production. This industry has potential for using 70% to 100% of all fish oil and meal products of the world. Availability of high quality fish oil and meal is a major problem; either, according to the limitation of stocking, fish meal production has fixed and because of human consumption from primary sources of fish meal manufacturing, its availability is more and more limited. And finally, the demands caused higher price [1]. Therefore, it's necessary to focus on other protein sources in animal food.

Some plant protein sources especially oil seed have produced a lot globally and have valuable potential for fish meal substitution [1]. Oil seed by-products are major protein sources in aquaculture for omnivorous or herbivorous warm-water fish such as tilapia and catfish [2]. However, it's necessary to pay attention to digestibility, palatability and food efficiency of ingredients as a replacement in animal diets [3].

Too many tilapia are producing in the developing countries, and because of its low price, it is able to consume by vulnerable people. But, aquatic food factories especially for tilapia diets must find out some alternative instead of fish meal due to its price and products; and it is more important for herbivorous fish to reduce food cost and keep its quality. Different kind of protein sources have been studied as a replacement of fish meal such as: fisheries by-products, shrimp meal, livestock by-products (ex. feather meal, blood meal and bone meal), soybean meal, cotton seed meal, peanut meal, sunflower meal, canola meal, sesame meal, coconut meal, palm meal, aquatic plants such as Azolla etc. [4]. So according to the economic issues and availability canola meal was selected as a plant protein source to replace with fish and soybean meal owing to their price.

Tannin, fiber, sinapine, phytic acid and glucosinolate are the famous anti-nutrients of Rape seed. Sinapine has phenolic composition and reduces palatability of diets due to the bitter taste. Phytic acid combines strongly with protein at low pH and cations like zinc, calcium and especially phosphorus at intestine. Glucosinolate as a thioether disturbs thyroid function and limits canola usage as a protein source and effects palatability by making bitter taste [3,5].

Canola as a new variate of Rape seed (*Brassica* sp.) have less uric acid and glucosinolate (<2% and <30 µmol/g, respectively) genetically

[3]. Amount oil seed cultivation, canola places after soybean in the world [1,5]. Canola meal is a good protein source and rich in methionine, cysteine, choline, biotin, folic acid, niacin, riboflavin, thiamine and essential minerals like selenium and sodium [3,5-7]. So, this experiment was plan to evaluate amount of canola replacement in Nile tilapia diets.

Material and Methods

This experiment performed in National Research Center of Saline Water Aquatics, Bafgh, Yazd, Iran. A feeding trial was conducted using a completely randomized design. Proximate analysis of canola (Table 1) and total protein sources, diets (Table 2) and carcass (Table 2) done according to AOAC [8]. Amino acid composition of protein sources analyzed at Atomic Energy Organization of Iran by HPLC. So, according to the proximate and amino acids analysis, five iso-caloric experimental diets (gross energy, 4.61 Kcal/g) were formulated to contain graded levels of 0 (control), 25%, 50%, 75% and 100% canola meal replaced with soybean and fish meal (Table 2). Canola meal, fish meal, soybean meal and wheat flour were used as protein sources. All dry ingredients were thoroughly mixed with distilled water. The mixed dough was extruded through the meat chopper machine into 2-3 mm diameter size and dried in a forced-air drier at 50°C to 60°C for 18 h. The pellets were crushed into desirable particle sizes and stored at -20°C until used.

Male fingerling Nile tilapia (initial weight=26.66 g ± 8.53 g), was produced in National Research Center of Saline Water Aquatics by

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17 α -methyl testosterone [9], were acclimated in indoor tanks for 2 weeks. In this period, the fish were fed the control diet and the canola diets to acclimate to the experimental facilities, conditions, and diets. The fish was randomly distributed into 15 aerated 300 l polyethylene tanks (15 fish/tank) in a flow-through system at flow-rate of 3 L/min. Every experimental diet was fed to three replicate groups of fish at the rate of near satiation twice daily at 8:00 and 14:00 H for 6 weeks. Growth was measured fortnightly after anesthetizing by 150 ppm to 200 ppm Colve meal *Eugenia caryophyllata*. The temperature was adjusted at 29.1°C \pm 0.46°C. The diurnal cycle was 12-h light/12-h dark. Salinity was approximately 8 g/l during the experimental period. Dissolve oxygen, pH, nitrite and ammonia were 6.03 mg/l \pm 0.41 mg/l, 7.41 mg/l \pm 0.08 mg/l, <0.02 mg/l and 0.0038 mg/l \pm 0.00098 mg/l, respectively. The extra food siphoned 20 min after feeding to estimate total food intake better. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), total food intake (TFi), protein intake

(Pi), protein efficiency ratio (PER), protein conversion efficiency (PCE) were calculated. All data were subjected to on-way ANOVA in SPSS version 16.0. Significant differences among the group means were compared using Duncan's multiple comparison. Data are presented as mean \pm standard division.

Results

The levels of canola substitution did not have any significant effect on survival ($P>0.05$). But growth indices were effected and best weight gain (WG) and specific growth rate (SGR) showed at the control (185.6 \pm 29.5 and 2.49 \pm 0.24, respectively) and differed significantly by others ($p<0.05$). There was not any significate different between 25% and 50% replacement; while, they were negative at 100% (Table 3).

Feed indices were affected by canola levels ($p<0.05$). At 100% replacement, food conversion efficiency (FCR) was not displayable owing to losing weight. It was significantly higher at 75% replacement compare to control, 25% and 50% canola ($p<0.05$; Table 3). On the other hand, feed intake (FI) significantly better at the control ($p<0.05$); but, it was not significant between 25% and 50% or 75% and 100% replacement ($p>0.05$; Table 3). The differences within all treatments in protein intake (PI) were exactly assemble to FI.

Protein indices differed significantly by canola levels ($p<0.05$). There were not any significant differences in Protein efficiency ratio (PER) and protein conversion efficiency (PCE) within the control, 25% and 50% replacements; adversely by 75%. They not displayable for 100% replacement due to losing weight (Table 3).

Chemical analysis	Canola Meal
Crud Protein (%)	36
Ether Extract (%)	3.4
NFE (%)	36.1
Gross Energy (Kcal/g)	3.84
Crud Fiber (%)	16.3
Ash (%)	7.5
Moisture (%)	7.5

Table 1: Canola analysis.

	Control	Canola 25%	Canola 50%	Canola 75%	Canola 100%
Ingredients composition (% of fed basis)					
Canola meal	0	20.1	40.21	60.31	78.77
Fish meal	23.13	17.29	11.87	6.16	0
Soybean meal	23.15	17	11	5.5	0
Wheat flour	20	16.71	10.54	4.15	0
Corn starch	25.7	15.5	10.5	5.5	0.5
Multi Vitamins 1	1	1	1	1	1
Vitamin C2	0.08	0.08	0.08	0.08	0.08
Choline	0	0.94	0.94	0.94	0.94
Minerals 3	1	1	1	1	1
Amino acids complex 4	0.01	0.01	0.01	0.01	0.01
Methionine	0.25	0.25	0.25	0.25	0.25
Threonine	0.25	0.25	0.25	0.25	0.25
Lysine	0	0.7	0.7	0.7	0.7
Soybean oil	5.34	9.07	11.55	14.06	16.4
total	100	100	100	100	100
Chemical composition (% of DM basis)					
Crud Protein	29.13	29.37	29.43	29.71	29.32
Ether Extract	9.29	11.38	13.57	15.77	18.66
NFE	50.2	45.1	40.7	35.4	30.5
Gross Energy (Kcal/g)	4.58	4.58	4.61	4.62	4.67
Crud Fiber	1.95	5.14	7.7	10.84	13.6
Ash	9.42	8.98	8.55	8.28	7.89
Moisture	7.49	6.2	6.28	5.95	5.64
CP/GE (mg/Kcal)	63.59	64.08	63.77	64.32	62.79

1. Composition: Vitamin A 3600000 IU/kg; D3 800000 IU/kg; E 14400 mg/kg; K3 800 mg/kg; B1 710 mg/kg; B2 2640 mg/kg; B3 11880 mg/kg; Calcium Pantothenate 3920 mg/kg; B6 1176 mg/kg; B9 400 mg/kg; B12 6 mg/kg; Biotin 40 mg/kg; Choline chloride 100000 mg/kg

2. Purity: 50%

3. Composition: Zn 33880 mg/kg; Mn 39680 mg/kg; Cu 4000 mg/kg; Fe 20000 mg/kg; Se 80 mg/kg; I 397 mg/kg; Choline chloride 100000 mg/kg

4. Composition: Vitamin A 30000000 IU/l; D3 1000000 IU/l; E 15000 mg/l; K3 1000 mg/l; B1 4000 mg/l; B2 3000 mg/l; B3 10000 mg/l; B5 5000 mg/l; B6 1000 mg/l; B12 10 mg/l; H 20 mg/l; L-Aspartic acid 3600 mg/l; L-Glycine 2400 mg/l; L-Lysine 3100 mg/l; L-Histidine 800 mg/l; L-Arginine 2100 mg/l; L-Isoleucine 1900 mg/l; L-Leucine 3200 mg/l; L-Tyrosine 1200 mg/l; L-Phenylalanine 1900 mg/l; L-Alanine 2600 mg/l; L-Cystine 600 mg/l; L-Valine 2800 mg/l; DL-Methionine 700 mg/l; L-Threonine 2100 mg/l; L-Serine 2700 mg/l; L-Glutamic acid 6200 mg/l; L-Proline 2100 mg/l

Table 2: Ingredients and proximate composition of experimental diets.

	Control	Canola 25%	Canola 50%	Canola 75%	Canola 100%
Indices					
Initial weight ¹	26.66 ± 8.53	26.66 ± 8.53	26.66 ± 8.53	26.66 ± 8.53	26.66 ± 8.53
Final weight ¹	76.13 ± 7.91	41.95 ± 2.33	41.45 ± 2.47	31.40 ± 0.00	26.63 ± 1.45
WG ³	185.6 ± 29.5 ^a	57.4 ± 8.7 ^b	55.4 ± 9.3 ^b	17.8 ± 0.1 ^c	-
SGR ⁴	2.49 ± 0.24 ^a	1.07 ± 0.13 ^b	1.05 ± 0.14 ^b	0.39 ± 0.00 ^c	-
FI ¹	859.9 ± 26.4 ^a	271.1 ± 65.5 ^b	267.1 ± 31.3 ^b	126.9 ± 1.1 ^c	114.1 ± 8.4 ^c
FCR ⁵	1.45 ± 0.20 ^a	1.46 ± 0.13 ^a	1.51 ± 0.08 ^a	2.22 ± 0.01 ^b	-
PI ¹	231.7 ± 7.1 ^a	74.7 ± 8.0 ^b	73.7 ± 8.6 ^b	35.5 ± 0.3 ^c	31.6 ± 2.3 ^c
PER ⁶	2.60 ± 0.34 ^a	2.48 ± 0.23 ^a	2.40 ± 0.13 ^a	1.61 ± 0.00 ^b	-
PCE ⁷	39.20 ± 5.04 ^a	33.79 ± 4.02 ^a	32.52 ± 2.11 ^a	15.77 ± 0.92 ^b	-
Proximate composition of fish diet					
Crud Protein ²	52.80 ± 0.10 ^a	61.00 ± 1.97 ^b	57.00 ± 0.78 ^{ab}	60.47 ± 4.19 ^b	58.33 ± 2.25 ^b
Ether Extract ²	31.58 ± 1.35 ^a	22.62 ± 1.07 ^c	26.65 ± 0.11 ^b	19.95 ± 1.47 ^c	19.83 ± 3.67 ^c

*Mean with different letters have significant difference (p<0.05).

1. Gram.
2. Based on dry weight.
3. Weight gain (%) = 100*(final mean body weight – initial mean body weight)/initial mean body weight.
4. Specific growth rate (%) = {(loge final body weight – loge initial body weight)/days} * 100.
5. Feed conversion ratio = dry feed fed / wet weight gain.
6. Protein efficiency ratio = wet weight gain / total protein given.
7. Protein conversion efficiency (%) = 100 * (protein gain / protein intake).

Table 3: Growth, feed and protein performances and proximate composition of fish fed the experimental diets.

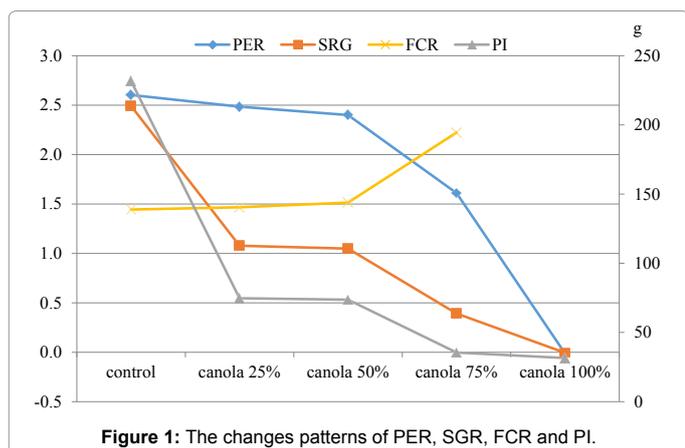


Figure 1: The changes patterns of PER, SGR, FCR and PI.

The control diet has significant lower carcass crude protein (p<0.05), and it was not differing within 25%, 50%, 75% and 100% replacement, significantly (p>0.05). There were not any significant differences in crude fat within 25%, 75% and 100% replacement (p>0.05), and it was higher in control and 50% canola, respectively (Table 3).

Discussion

Canola levels did not affect survival significantly (p>0.05) that is confirmed by Yigit and Olmez [6]. Growth indices showed inverse relationship to the canola levels. There was big difference between the control and others. This inverse relationship reported by Yigit and Olmez on black tilapia fry, too. SGR decreased by canola increasing on Grass carp and best one showed at the control diet [10]. Webster, et al. [11] displayed significant better growth of the control diet on Channel catfish. They found significant difference at WG within 12, 36 and 48% canola replacements; in the way that it decreased by canola enlargement. However, soybean was replaced by canola on Nile tilapia and there was not reported any significant difference at SGR by canola increasing [12].

FI deceased by canola increasing and the best one was at the control. Enami et al. reported lose in FI by plant protein increasing at

diet because of feed volume. This reduction exhibited on fry Nile tilapia beside not significant difference within canola replacement until 40% [6]. In this experiment, against FI, FCR did not significant differences within the control and 25% and 50% canola. Soybean replacement by canola was not effectiveness on FCR on growing Nile tilapia [12]. Other authors did not report significant differences on FCR at diets with canola replacement until 16% and 36% on Rainbow trout and Channel catfish, respectively [11,13].

FCR rose by canola enhancement may be for fiber increasing, according to the crude fiber (more than 8%) of the diets with the high level of canola. High amount of fiber in diets causes rapid cross of feed from gastrointestinal tract and reduction of digestibility time. Yigit and Olmez displayed FCR increasing by canola enhancement happened due to fiber of diets. Totally, these result show that diets palatability is the problem because fish had suitable growth in compare with FI and they get acceptable FCR.

In protein indices (PER and PCE), they did not show significant difference until 50% replacement. Fry Nile tilapia and Grass carp neither until 40% and 32% respectively at PER [6,10]. Except of the reduction in the growth indices because of low palatability that result in low FI and PI; non-significant effect of canola substitution on FCR, PER and PCE at the control, 25% and 50% confirm that canola may be capable to replace until 50% (Figure 1). On the other hand, it seems that canola didn't show considerable negative effects on diet digestibility until 50% replacement. Enami et al. reported that modified and concentrated canola meal were capable to replace fish meal on fish diets and fry Nile tilapia can tolerate it until 10%.

Replacement of 41.8% Rape seed meal on fingerling tilapia *Sarotherodon mossambicus* made a good growth [14]. In addition, canola replacement until 75% did not have any negative effects on GIFT Nile tilapia. Though, Enami et al. recommended canola meal should be used on aquatic diets below 30% because of glucosinolate limitation (under 2650 µmol/Kg diet).

In this experiment, it seems that inexistence of a convince growth was for low FI and in result PI; Indeed, bitter taste of glucosinolate and sinapine caused the diets unpalatable. Another author displayed that

bitter taste of glucosinolate's metabolites (such as Isothiocyanate and Oxazolidinethione) and sinapine make mustard like smell and cause unpalatability in diets [15].

Fiber of the control, 25% and 50% canola was under 8%; so, in the other diets, more than 8% fiber could be the reason of unsuitable growth. More than 50% canola replacement, owing to higher fiber than 8%, did not recommended [3]. And Yigit and Olmez suggested that high fiber in diets of tilapia reduced growth and protein performances.

Environmental condition of canola farms, especially land and farm management, all effect on the number of anti-nutrients of canola. Although, all species of Rape seed have cultured in Iran are canola (too zero species) and their uric acid and glucosinolate reduced genetically; but, agricultural conditions and oil extracting processes effect on anti-nutrients that confirmed by Enami et al.

According to the different results of this experiments with some others; it seems that agricultural condition and oil extracting process effected negatively and anti-nutrients of the canola like glucosinolate and sinapine was more than what was expected. Yigit and Olmez reported that these kinds of anti-nutrients make unpalatability in diet due to their bitter taste.

Carcass protein and lipid of the diets with canola significantly differ with the control that is against the Yigit and Olmez report on fry Nile tilapia.

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