

Partial or Total Replacement of Soybean Meal with Iranian Cottonseed Meal in Diets for Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Apparent digestibility coefficients (ADC) were calculated in experiment 1 by using chromic oxide in the diet as an indigestible marker to evaluate the Iranian Cottonseed meal (CSMP). Experiment 2 was carried out to investigate the nutritional value of cottonseed meal (CSM) as soybean meal (SBM) substitute in quality low cost rainbow trout (*Oncorhynchus mykiss*) ration. In this study, six formulated feeds consisting different levels of CSMP (0, 20, 40, 60, 80, 100%) substitution of SBM were fed to a total of 540 rainbow trout with initial mean body weight of 50 ± 5 g. Fish were randomly stocked into eighteen 100 L. fiberglass tanks with 30 fish per tank and 3 tanks per diet and fed to apparent satiation 3 times a day and 7 days per week for 60 days. The ADC of CSMP and SBM were measured as of dry matter, 62.7 and 69.2%; crude protein, 82.4 and 87.3%; fat, 66.6 and 78.5%, respectively. After an 8 week feeding trial, the average weight gain of fish fed with diets 1 to 6 was: 100.6, 102, 102.9, 103.3, 103.9, and 103.4 g, respectively. Average feed conversion ratio (FCR) of fish fed diets 1 to 6 was measured as of 1.280, 1.317, 1.310, 1.273, 1.290, and 1.253, respectively. For all six treatments, the survival percentage was more than 99%. ADC value for most nutrients of CSMP was different from those of SBM. Weight gain and survival rate were not significantly different ($P > 0.05$) for fish fed with CSMP diets compared to the control diet but the differences of FCR, specific growth ratio (SGR), and daily weight gain (DWG) were significant among different diets ($P < 0.05$). In the feasibility study, complete replacement of SBM by CSMP revealed to be economic and based on the gossypol analysis, total gossypol levels was not observed for toxicity on liver of fish fed by CSMP, indicating the possibility of total replacement of SBM by CSMP in rainbow trout fed formulations.

Key words: Rainbow trout, Iranian, cottonseed meal Pak (CSMP), gossypol, soybean meal (SBM).

INTRODUCTION

In recent years, aquaculture industry has developed rapidly in many countries. The aquaculture production improves 15% annually and is predicted to continue to grow fast in future^[1]. The sustainability of aquaculture development is absolutely dependent on the availability of quality and cheap feedstuffs. Feed costs generally account for more than half of operating costs of aquaculture operations^[8]. Fishmeal (FM) and soybean meal (SBM) are the main raw components in manufactured fish feeds as a protein resource. However, as excellent a source of protein as FM is, it is expensive to use as a protein source in manufactured fish feed. The production of FM is a multi-step process including catching the fish, processing it, and then testing the meal for nutrient value. Moreover, only limited species of fish are available as a source for FM

and the populations of these species is relatively constant. With demand for FM increasing and government constraints protecting against over-fishing, availability of FM is decreasing and its price is increasing. This rising expense is a driving force behind the constant search for alternative protein sources and due to its high protein content, SBM has the potential to be a full or partial replacement for FM in manufactured fish feed.

SBM has its own drawbacks such as its increasing price in Iran (Agriculture Statistic yearbook of Iran, 2005) and several antinutritional factors such as phytic acid contain in Soybean. phytic acid can reduce zinc availability and reduce protein digestibility in salmonid diets.

Extensive research has been conducted on the usefulness of SBM alternative feedstuffs to decrease the production costs^[22,21,3].

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In recent years, there has been a considerable challenge to use cottonseed meal (CSM) as a substitute in fish diets [6,20,10,16], and there has been a general agreement that CSM is an acceptable candidate to partially replace FM and SBM in fish ration. However, gossypol, the principal pigment of cottonseed, has been identified as a toxic substance in CSM that limits the use of this feedstuff in animal diets [28,18,5]. Furthermore, the low concentration of lysine in some varieties of cottonseed is another limiting factor for the usage of CSM in monogastric aquatic animals [20].

Currently, a new variety of cottonseed (Pak) with low free and total gossypol has been widely commercialized in Iran with an annual production of more than 800,000 tons in 2004 (Agriculture Statistic yearbook of Iran, 2005). This variety of cottonseed is currently just used for livestock feeding. This study was conducted to investigate the possibility of partial and complete replacement of this variety in rainbow trout diet with SBM. Rainbow trout is an important commercial aquaculture species in Iran with an annual production of more than 150,000 tones (Iranian Fisheries Annual report, 2004).

MATERIAL AND METHODS

2.1. General Description and Preliminary Experiment:

This study was carried out in two stages in a commercial trout farm (Sarshar farm) located at Tonekaboon, Iran. CSMP used in this study was a by-product of the mechanically extracted cottonseed oil. The chemical analysis was done based on AOAC [1]. Gossypol was determined by high-performance liquid chromatography (HPLC) according to the standard method of American Oil Chemists Society [2].

2.2. Experiment # 1; Digestibility Study: This experiment was conducted to evaluate the apparent digestibility coefficients (ADC) of cottonseed meal. Diet 1 was used as the reference diet (Table 1), which served as an internal control with chromic oxide (1%) as an indigestible marker [27]. Diets 2 and 3 were formulated using 70% reference diet and 30% of each CSMP and SBM, respectively as described by Cho & Slinger [8]. Diets were mechanically mixed with distilled water (40 g/ 100 g diet mix), pressure-pelleted, and stored at - 25 °C till the experiment.

Nine digestibility tanks (100 L.) supplied with flow through spring water (temperature; 11 - 13 °C, 1 m³/s) were each stocked with 20 fish (50 ± 5 g body weight initially) in early March. The tanks were kept indoor allowing ambient light to enter through a glass roof. Fish were assigned randomly to these three diets and

consumed their respective diets. Sampling of faecal matter was done by settling sedimentation [12], after the one-week feeding period and continued for three weeks. Feces were frozen and freeze dried before analyses. Diets were fed (2% body weight basis) three times a day [8]. See Table 1 for detailed proximate composition of the experimental diets. The ADC value was calculated following Cho & Slinger, [8], as follows:

$$\text{ADC} = 100 \times (1 - ((\text{Cr}_2\text{O}_3 \text{ g in diet} / \text{Cr}_2\text{O}_3 \text{ g in feces}) \times (\text{fecal nutrient or energy level} / \text{dietary nutrient or energy level})))$$

The ADC of the test ingredients was then calculated as follows:

$$\text{ADC} = (\text{ADC of test diet} - 0.7 \text{ ADC of the reference diet}) / 0.3$$

Table 1: Composition of reference diet (%)

Ingredients	%
Kilka Fish meal	18.5
Wheat gluten	13.5
Corn meal	17.5
SBM	31
Vitamin premix	2
Mineral premix	2
soybean oil	14.5
Chromic oxide	1
<i>Chemical composition (% , as is basis, average of three samples)</i>	
Crude protein	35.39
Crude fat	9.02
Moisture	11.5

2.3. Experiment # 2; Feeding Trial: Eighteen fiberglass tanks (100 L.) supplied with flow through spring water (temperature; 11 - 13 °C, 1 m³/s) were each stocked with 30 fish (initial mean body weight of 50 ± 5 g). The tanks were kept outdoors and each experimental diet was fed to three tanks.

Six pelleted isocaloric test diets were formulated to obtain CSMP-SBM substitution of 0, 20, 40, 60, 80 and 100% (Table 2). Experimental diets were ISO-caloric and formulated based on the rainbow trout requirements (35% crude protein (CP) and 3600 Kcal/Kg energy). The fish were fed the test diets at 2% body weight three times a day for 8 weeks. Following MS 222 anaesthesiation at 100 ppm, sampling was done for body weight and total length every two weeks. Fish were starved for 24 hours before each sampling.

At the end of the experiment, three fish from each treatment were sacrificed and pooled for body composition analyses. Fish weight gain, FCR, SGR, DWG and survival were estimated [13]. Gossypol was measured using the standard method by American Oil Chemists Society [2]. At the end of the second experiment, three fish of each diet were sacrificed for gossypol analyses. The chemical compositions of the fish were measured following AOAC [1], methods.

Table 2: Diets and chemical composition for Experiment 2 (% as is basis)

Ingredients	Diets ^a					
	1	2	3	4	5	6
Corn meal	17.5	15.3	13.45	11.44	9.67	7.82
SBM	31	24.8	18.6	12.4	6.2	0.0
CSMP	0.0	6.2	12.4	18.6	24.8	31
Fishmeal(Kilka)	18.5	20.1	20.05	20.3	20.4	20.45
Wheat gluten	13.5	13.9	15.7	17.4	19	20.75
Soya oil	14.5	14.7	14.8	14.86	14.93	14.98
Supplements ^b	5	5	5	5	5	5
Crude protein	35	35	35	35	35	35
Energy (kcal/kg)	3600	3600	3600	3600	3600	3600
<i>Chemical composition (% as is basis, average of three samples)</i>						
Crude Protein	34.75±0.57	35.22±0.45	34.77±0.29	35.65±0.53	34.83±0.61	34.93±0.47
Energy (kcal/kg)	3615	3617	3628	3640	3635	3625

^a Diets 1, 2, 3, 4, 5, 6 contained 0%, 20%, 40%, 60%, 80% and 100% CSMP, respectively.

^b Supplements provided as the following: Trace mineral mix (zinc, iron, manganese, copper, iodine, cobalt, and selenium), Vitamin mix (vitamin A, D3, K, E, riboflavin, pyridoxine, panthothenic acid, nicotinic acid, folic acid, biotin, vitamin B12, vitamin C, choline chloride, L-ascorbyl acid-2-polyphosphate, celufil).

2.4. Calculations and statistical methods: Fish weight gain (final weight – initial weight) and FCR (feed consumed/ weight gain) were calculated. All statistical analyses were performed using SPSS (SPSS, Inc., Chicago, IL). All percentage data were arc-sin transformed prior analysis. In order to compare the results of statistical test with that of conventional ANOVA, one-way analysis of variance was used. Duncan's multiple range ^[9]. test for means and LSD test were used to identify the significance of difference between any pair of treatment means.

RESULTS AND DISCUSSION

3.1. Experiment # 1; Digestibility Study: The free and total gossypol contents of CSMP were found to be 0.003% and 0.009%, respectively. Chemical analyses of CSMP were as follows: 91.92 % dry matter, 36.9 % crude protein, 10.6% crude fat, 4.72 % fiber, and 2250 Kcal/Kg energy (as is basis). Apparent digestibility coefficients for dry matter, fat and CP in CSMP and SBM are presented in Table 3. There were no significant differences in ADCs of CP and crude fat among the different diets. Significant differences existed in ADC values for dry matter (P<0.05). ADC of dry matter was higher in SBM than CSMP.

Table 3: Apparent digestibility coefficients (%) for dry matter, crude fat and crude protein of SBM and CSMP for rainbow trout (% , n=3 tanks)

Ingredients	Dry matter	Crude Protein	Crude fat
SBM	69.2± 0.407 ^a	87.3 ± 0.408 ^a	78.5 ± 0.608 ^a
CSMP	58.5 ± 0.236 ^{bc}	78.3 ± 0.242 ^{ab}	61.4 ± 0.443 ^{bc}

3.2. Experiment # 2; Feeding Trial: Fish initial weight, weight gain, FCR, SGR, DWG and survival rate are presented in Table 4. No significant differences were found in initial weight, weight gain or survival rate among fish fed with different diets. However, there were significant differences in FCR (P<0.05). However, fish fed with diets 1, 4 and 6 showed no significant difference in FCR. Results indicated that SBM could be replaced 100% by CSMP in rainbow trout diets without significantly reducing weight gain, SGR, DWG, survival rate and increasing FCR. There were no significant differences in moisture, Crude protein, fat, or ash in whole fish body (Table 5). Results in figures 1, and 2 indicate the relationship between weight, length and diets, respectively. As shown, diets did not present any significant alteration in length and weight.

Figures 3, 4 and 5, indicate that there were significant differences in FCR, SGR, and DWG, respectively, among fish fed with different diets (p<0.05). Results indicated that the best SGR, DWG, and FCR were obtained from fish fed the 100% dietary CSMP (diet 6). At the end of the second experiment, total gossypol concentrations were measured in the liver samples (3 samples per dietary treatment). The results of the liver analysis have shown that the total gossypol levels were 78.5, 85.4, 112.2, 119.3 and 127.2 mg/Kg (wet weight basis) for fish fed the diets 2-6, respectively. There were no indications of toxicity in all fish samples.

Table 4: Average initial weight, final weight, weight gain, total length, FCR, SGR, DWG and survival rate for rainbow trout fed different diets for 2 months(n=3 tanks)

Item	Diets					
	1	2	3	4	5	6
Initial weight (g)	47.1 ^a	48.6 ^a	49.1 ^a	48.4 ^a	49.2 ^a	47.6 ^a
Final weight (g)	101.4 ^a	103.1 ^a	104.2 ^a	100.5 ^a	101.5 ^a	99.9 ^a
Weight gain (g)	54.3 ^a	54.5 ^a	55.1 ^a	52.1 ^a	52.3 ^a	52.3 ^a
Total length (cm)	20.3 ^a	20.5 ^a	20.5 ^a	20.3 ^a	20.2 ^a	20.1 ^a
FCR (gg ⁻¹)	1.446 ^{ab}	1.503 ^c	1.500 ^c	1.520 ^{ab}	1.556 ^{bc}	1.530 ^a
SGR (%Bwd ⁻¹)	1.260 ^{ab}	1.250 ^c	1.250 ^{bc}	1.250 ^{ab}	1.200 ^{ab}	1.230 ^a
DWG (GD ⁻¹)	0.90 ^{bc}	0.90 ^c	0.92 ^{bc}	0.88 ^{ab}	0.87 ^{ab}	0.86 ^a
Survival (%)	100	99	100	100	100	99

Table 5: Chemical analysis of whole fish body(% , as is basis)

Item	Diets *					
	1	2	3	4	5	6
Moisture	72.9±0.38	72.2±0.95	72.1±0.44	72.7±0.12	72.5±0.17	73.2±0.16
CP	13.8±0.73	13.7±0.77	13.7±0.32	13.6±0.54	13.7±0.83	12.9±0.19
Fat	10.3±0.92	10.8±0.31	11.2±0.56	11.9±0.49	12.4±0.71	12.8±0.25
Ash	2.2±0.05	2.1±0.14	1.9±0.08	1.7±0.05	1.6±0.15	1.8±0.28

* No significant differences were found among different dietary treatments.

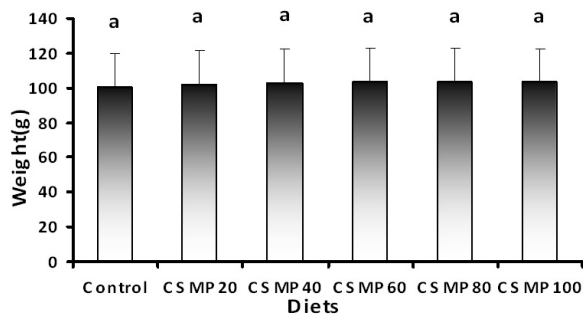


Fig. 1: The relationship between fish final weight and diets.

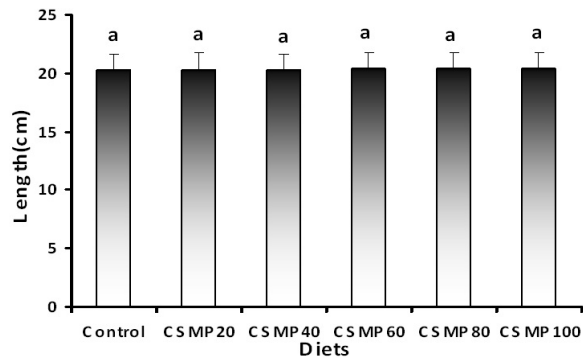


Fig. 2: The relationship between fish total length and diets.

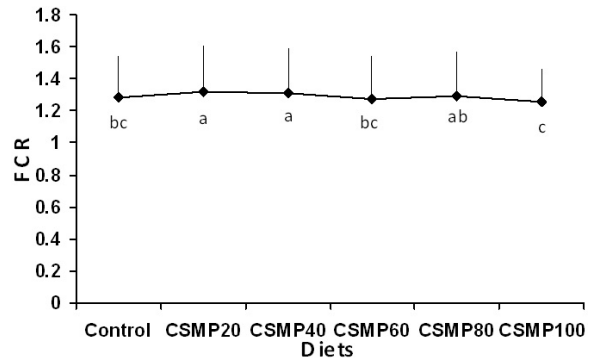


Fig. 3: The relationship between FCR and diets.

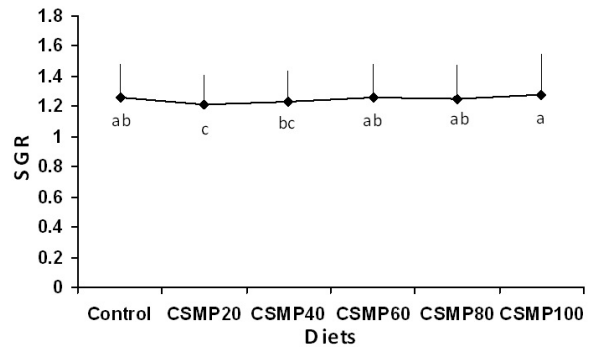


Fig. 4: The relationship between SGR and diets.

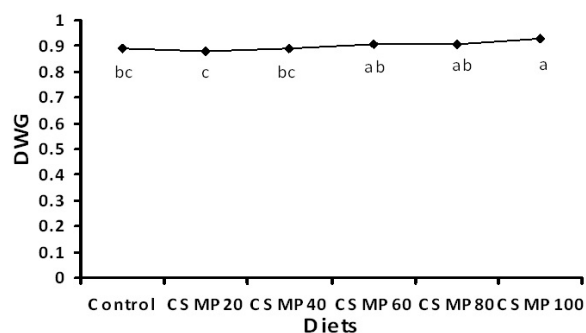


Fig. 5: The relation between DWG and diets.

Discussion: The digestibility coefficients of feed ingredients provide insight concerning nutrient utilization and should enable more accurate ingredient substitutions in diet designed for target fish species. The nutrient digestibility varies depending on the composition of ingredients used. It has been reported that carnivorous fish tend to utilize the dry matter in animal products better than that in plant products [7,24]. The present study showed that ADC values of nutrients in CSMP were comparable to those in other oilseed meals as well as SBM [19]. However, dry matter digestibility of CSMP was lower than that of SBM, probably due to the high fiber contents [15].

High digestibility rate of crude protein of CSMP in experiment 1, confirmed the results obtained by Chang & Hardy [6], on the effect of total gossypol concentrations on reducing the digestibility of protein. In addition, results indicated that the comparative nutritional ADC of CSMP was similar to those of the other oilseed meals.

Hepher (1988) reported that CSM has a lower nutritional value than SBM, which is not supported by the current study. In addition, CSMP has some other important advantages such as lower FCR. This better performance might be attributed to its higher fiber quality. There is a considerable difference between the results obtained by the feeding experiment of the present study (esp. dietary treatments 5 and 6) and those by Cheng & Hardy [6] and Barros *et al.*, [3]. As they have reported that the acceptable level of CSM inclusion in the ration is not higher than the maximum of 10% and more than 55% substitution of SBM by CSM could result in reduced WG. Dorsa *et al.*, indicated that more than 17.4% of CSM in the ration inhibited the growth.

In contrast, it has been shown in this study that CSMP did not negatively affect WG even when 100% replaced SBM, suggesting that CSMP can be included in the rainbow trout diet up to 310 g per Kg. The main reason for these differences is the type and variety of the cottonseed meal used. In line with the results obtained by this study, it has been documented that CSM could be a suitable alternative for partial

replacement of FM and SBM and it can totally replace them if used with supplementary lysine [23,4,22]. However, CSMP, showed an acceptable performance without lysine supplementation.

Gossypol is the main pigment of CSM that is toxic to some fishes. In addition, the results of the investigations of Herman [14], showed that free gossypol did not negatively affect the growth of trout at the concentration lower than 300 mg/kg.

The highest concentration of total gossypol was found in diet 6 (127.2 mg/Kg) with 100% CSMP inclusion. However, Cheng & Hardy [6], reported comparatively higher concentration of 165 mg/Kg at 80% CSM inclusion. The tolerance limit of juvenile rainbow trout to total gossypol was also reported to be 165 mg/Kg [6], which is in good agreement with the results obtained by Lou *et al.*, [17], and the current study. Hence, the free gossypol content in CSMP would not be a barrier for its utilization in fish feed. Interestingly, growth rates observed with fish fed a 100% SBM -based diet and those fed a 100% CSMP-based diet were similar. Moreover, these results confirmed that the best FCR belonged to the 100% CSMP-based diet.

Based on the economic feasibility studies, 100% CSMP-based diet was the cheapest (0.6 UD\$) and was 0.02 US\$ cheaper than the control diet (100% SBM-based diet). CSMP and SBM cost 0.21 and 0.37 USD per kg in Iran, respectively. Thus, trout production can be more profitable using diets containing CSMP. In addition, we report that based on the results obtained, there were not any significant variations in final weight, dry matter and chemical composition of the harvested fish fed SBM or CSMP, which was comparable with that of Soofiani *et al.*, [25]. Furthermore, gossypol of the applied cottonseed meal was not a problem and did not have any inhibitory effects on growth of fish. Our feeding experiment was conducted for a short period. Long-term studies are needed to closely monitor gossypol level in fillets and ensure that gossypol levels remain in safe ranges for human consumption.

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