Comparison of Soybean meal and Cottonseed meal variety Pak (CSMP) on growth and feed using in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Apparent digestibility coefficients (ADC) were calculated by using chromic oxide in the diet as an indigestible marker to evaluate the Iranian Cottonseed meal (CSMP). Then, the nutritional value of cottonseed meal (CSM) as soybean meal (SBM) substitute in quality low cost rainbow trout (*Oncorhynchus mykiss*) ration was investigated. 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 100L 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 103.4g, respectively. Average feed conversion ratio (FCR) of fish fed diets 1 to 6 was measured as of 1.28, 1.31, 1.31, 1.27, 1.29, and 1.25, 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, specific growth ratio (SGR), daily weight gain (DWG) 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 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.

Keywords: Rainbow trout, Cottonseed meal (CSMP), Gossypol, Soybean meal (SBM), Growth

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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 (Anon., 2002). 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 (Cho & Slinger, 1979). 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 (Anon., 2004a) 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 (Robinson & Daniels, 1987; Smith *et al*., 1988; Barros *et al*., 2002).

In recent years, there has been a considerable challenge to use Cottonseed meal (CSM) as a substitute in fish diets (Cheng & Hardy, 2002; Rinchard, 2003; El-Saidy, 2004; Lee *et al.,* 2006), 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 (Withers & Carruth, 1915; Menaul, 1923, Castillon & Alschuk, 1950). Furthermore, the low concentration of lysine in some varieties of cottonseed is another limiting factor for the usage of CSM in monogastric aquatic animals (Rinchard *et al.,* 2002).

Currently, a new variety of Iranian cottonseed (CSMP) with low free and total gossypol has been widely commercialized in Iran with an annual production of more than 800,000 tons (Anon., 2004a). 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 (Anon., 2004b).

Materials and methods

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 (1995). Gossypol was determined by high-performance liquid chromatography (HPLC) according to the standard method of American Oil Chemists Society (AOCS, 1998).

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 (Temesgen, 2004). Diets 2 and 3 were formulated using 70% reference diet and 30% of each CSMP and SBM, respectively (Cho & Slinger, 1979). Diets were mechanically mixed with distilled water (40g/100g diet mix), pressure-pelleted, and stored at –25°C till the experiment.

Nine digestibility tanks (100L) supplied with flow through spring water (temperature; 11-13°C, $1m^3/s$) were each stocked with 20 fish (50±5g 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 a commercial feed (Chineh Co., Tehran, Iran) for 1 week before feeding the experimental diets and fecal collection. Fecal collection lasted for three weeks (Hajen *et al*., 1993). Fish were fed the test diet at the rate of 2% (fresh body weight basis) per day and twice a day (0900 and 1700). All uneaten food was siphoned out an hour after each feeding. See Table 1 for detailed proximate composition of the experimental diets. The ADC value was calculated based on Cho & Slinger (1979) as follows:

ADC = 100 × (1-((Cr_2O_3 g in diet/ Cr_2O_3g in feces) × (fecal nutrient or energy level / dietary nutrient or energy level))).

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

ADC = (ADC of test diet- 0.7 ADC of the reference diet) / 0.3.

Eighteen fiberglass tanks (100L) supplied with flow through spring water (temperature; 11-13°C, $1m^3/s$) were each stocked with 30 fish (initial mean body weight of 50±5g). 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 3600Kcal/Kg energy). The fish were fed the test diets at 2% body weight three times a day for 8 weeks. Following MS 222 anaesthesiasation at 100ppm, 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 (Hardy, 1989). Gossypol was measured using the standard method by American Oil Chemists Society (AOCS, 1998). 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 (1995) methods.

All statistical analyses were performed using SPSS version 6 (SPSS, Inc., Chicago, IL). In order to compare the results of statistical test with that of conventional ANOVA, one-way analysis of variance was performed. Duncan's multiple range (Duncan, 1955) test for means and LSD test to identify the significance of difference between any pair of treatment means were used. All differences were regarded as significant at P<0.05.

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
Chemical composition (%, as is basis, average of three samples)	
Crude protein	35.٤
Crude fat	9
Moisture	11.5

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

Ingredients	Diets [a]					
	1	2	3	4	5	6
Corn meal	17.5	16.5	16.3	15.6	15.1	14.6
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	19.5	20	20	20
Wheat gluten	13.5	12.7	13.6	13.8	14.3	14.8
Soya oil	14.5	14.6	14.6	14.6	14.6	14.6
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
Chemical composition (% as is basis, average of three samples)						
Crude Protein	34.87	34.77	34.65	34.78	35.93	34.75
Energy (kcal/kg)	3612	3617	3628	3606	3625	3610

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.

Results

Experiment # 1; Digestibility study

The free and total gossypol contents of CSMP were found to be 30mg/Kg and 90mg/Kg, respectively. Chemical analyses of CSMP were as follows: 91.92% dry matter, 36.9% crude protein, 10.6% crude fat, 4.72% fiber, and 2250Kcal/Kg energy (as is basis). Apparent digestibility coefficients for dry matter, Crude fat and CP in CSMP and SBM are presented in Table 3. There were no significant differences in ADCs of CP among the different diets. Significant differences existed in ADC values for dry matter and crude fat ($P<0.05$). ADC of dry matter and crude fat was higher in SBM than CSMP.

Experiment # 2; feeding trial

Fish initial weight, weight gain, FCR, SGR, DWG and survival rate were presented in Table 4. No significant differences were found in initial weight, weight gain, SGR, DWG or survival rate among fish fed with different diets. However, there was significant difference in FCR ($P<0.05$). 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 indicated that diets did not present any significant alteration in length and weight. It is also indicated that there were significant differences in FCR among fish fed with different diets (Fig. 1; $P<0.05$). In addition, our results indicated that there were no significant differences in SGR and DWG, respectively, among fish fed with different diets (Figs. 2 & 3; $P>0.05$). 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 127.2mg/Kg (wet weight basis) for fish fed the diets 2-6, respectively. There were no indications of toxicity in all fish samples.

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 ± 0.4^{a}	87.3 ± 0.4^{a}	78.5 ± 0.6^{a}
CSMP	62.7 ± 0.3^{b}	82.4 ± 0.3^{a}	66.6 ± 0.4^{b}

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

Item	Diets					
	1	2	3	4	5	6
Initial weight (g)	47.7[a]	49.1[a]	49.2[a]	48.2[a]	49.2[a]	47.7[a]
Final weight (g)	100.6[a]	102 [a]	102.9[a]	103.3[a]	103.9[a]	103.4[a]
Weight gain (g)	52.9[a]	52.9[a]	53.7[a]	55.1[a]	54.7[a]	55.7[a]
Total length (cm)	20.3[a]	20.3[a]	20.3[a]	20.4[a]	20.4[a]	20.4[a]
FCR	1.28[bc]	1.31[a]	1.31[a]	1.27[bc]	1.29[b]	1.25[c]
SGR (%)	1.26[a]	1.21[a]	1.23[a]	1.26[a]	1.25[a]	1.28[a]
DWG (gd^{-1})	0.90[a]	0.88[a]	0.89[a]	0.91[a]	0.91[a]	0.92[a]
Survival (%)	100	100	100	99	100	100

*** The rows with the same letters are not statistically different (P>0.05).**

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

Item	Diet *					
	1	2	3	4	5	6
Moisture	72.6±0.1	72.2±0.1	72.1±0.3	72.5±0.2	72.9±0.1	73.1±0.1
CP	13.8±0.5	13.7±0.6	13.7±0.6	13.6±0.1	13.7±0.5	13.9±0.7
Fat	10.8±0.9	11.1±0.3	12.3±0.6	11.9±0.1	12.4±0.6	12.6±0.4
Ash	2.2±0.2	2.4±0.1	1.8±0.3	1.6±0.1	1.5±0.1	1.6±0.1

*** No significant differences were found among different dietary treatments.**

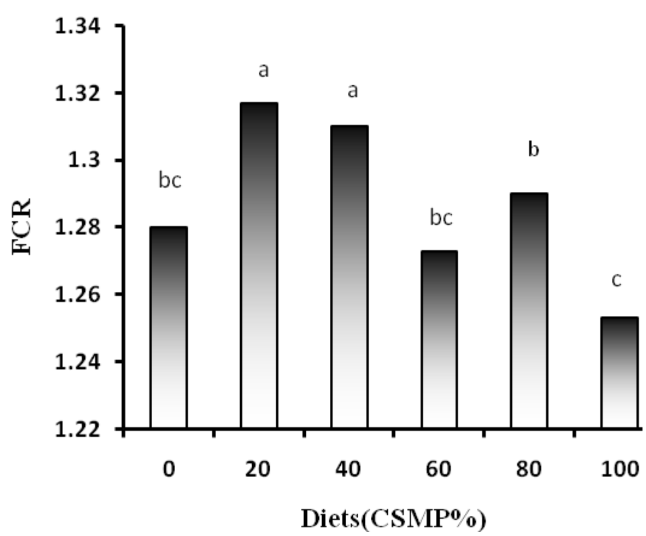

Figure 1: The relationship between FCR and diets

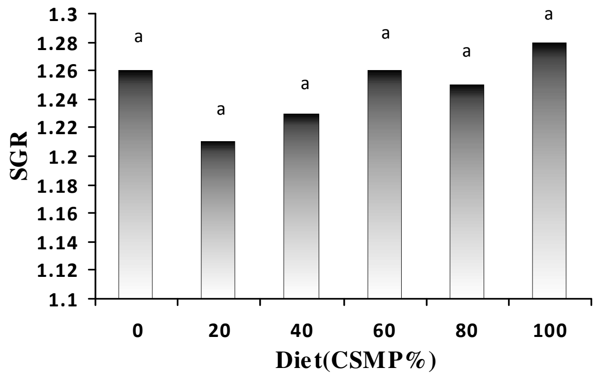

Figure 2: The relation between SGR and diets

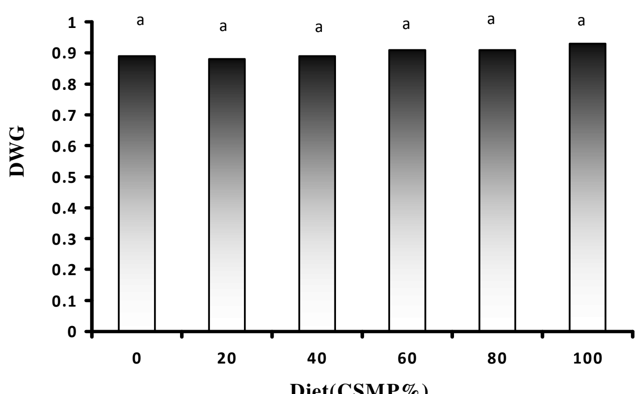

Figure 3: The relation between DWG and diets

Discussion

In general, the primary concerns of using CSM as a protein source in fish feeds are its gossypol content, low levels of lysine and methionine as well as high crude fiber level. Gossypol is the main pigment of CSM that is toxic to some animals (Colin-Negrete *et al.,* 1985), humans (Qian *et al.,* 1984) and fishes (Herman, 1970; Dabrowski *et al.,* 2000; Rinchard *et al.,* 2000). There have been several studies in which CSM was used as a protein source in diets for warm-water fish species (Robinson & Daniels, 1987; Robinson & Brent, 1989; Robinson, 1991) and cold-water fish (Herman, 1970; Fowler, 1980; Blom *et al.,* 2001) despite its reported low availability of lysine, and the presence of gossypol (Barros *et al.,* 2002; Cheng & Hardy, 2002). Robinson and Brent (1989) used solvent extracted CSM at the inclusion levels of 0%, 10% and 20% in channel catfish feeds in a 132-day study. The findings showed that fish fed diets containing 10% and 20% CSM had similar WG, FCR, and survival rate compared to those fed a diet containing 0% CSM (44.7% SBM). Free gossypol levels in fish fillets were below detectable limits. Therefore, they reported that CSM could be used at inclusion level of 15% in catfish feeds.

In an earlier study, Robinson and Daniels (1987) investigated the use of CSM as a partial or complete substitute for SBM in catfish diets. They reported that fish fed with a diet containing glandless CSM to replace all SBM had similar growth, FCR, CF and survival compared to fish fed a SBM-based diet. Similarly, free gossypol levels in fish fillets were below detectable limits for fish fed those two types of CSM-based diets. Robinson (1991) further evaluated the effect of supplementing lysine in CSM when it partially or totally replaced SBM in channel catfish feeds. The results revealed that CSM could replace 50% of SBM without lysine supple-mentation, and replace 100% of SBM when lysine was supplemented, without suppress weight gain, FCR, and survival.

Herman (1970) reported reduced growth in juvenile rainbow trout (cold-water fish) fed with diets containing 300mg free gossypol/Kg diet. Using Pacific salmon, Fowler (1980) used both SBM and CSM as protein sources to partially replace FM. He reported that Chinook salmon fingerlings fed with a diet containing 34% CSM with gland grew as fast as those fed with a diet containing 37% FM. In addition, Coho salmon fed with a diet containing 22% CSM with gland performed as well as fish fed a diet containing 37% FM. These findings were in line with those of Blom *et al.* (2001) that adult rainbow trout fed with diets in which CSM completely replaced FM had normal growth and survival rate over a 6-month period, but the reproductive performance of adult female trout decreased.

In the present study, CSMP was successfully used to replace SMB in rainbow trout diets. The total gossypol level measured in feed at 100% SBM replacement by CSMP 90mg/Kg wet weight basis and the free gossypol content was 30mg/Kg wet weight basis, respectively. The best inclusion level of CSMP in experiment 2 which led to the highest weight gain and the lowest FCR was 100%. This is well explained by the gossypol content of these varieties. The tolerance limit of juvenile rainbow trout to total gossypol was reported to be 165mg/Kg (Cheng & Hardy, 2002), which was supported by other studies carried out by Dabrowski *et al.* (2002) and Lou *et al.* (2006). On the contrary, Herman (1970) claimed that free gossypol did not negatively affect the growth of trout at the concentration lower than 290mg/kg. In the current study, the total gossypol content of the diets CSMP-100% was measured 28mg/kg which was considerably below the reported toxic levels by the other studies (Herman, 1970; Cheng & Hardly, 2002).

In a study conducted by Yildirim *et al.* (2003) on channel catfish (*Ictalurus puctatus*) fed diets containing graded levels of gossypol–acetic acid, the toxic concentration was reported to range from 300 to 1200mg total gossypol/kg diet. Hence, the complete substitution of SBM by CSMP in this study not only did not suppress the growth factors but also was statistically comparable with 100 % SBM-based diet. Therefore, by taking the economic aspects into consideration, complete replacement of SBM in the rainbow trout diet by CSMP could be recommended. Moreover, CSMP is currently being produced in large quantity in Iran and the majority is being used as a ruminants feed ingredient at a comparatively cheaper price (USD 0.15) than SBM (USD 0.35).

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مقایسه کنجاله تخم پنبه واریته پاک (**CSMP**) و کنجاله سویا بر رشد و مصرف خوراک در ماهی قزل‌آلای رنگین کمان (***Oncorhynchus mykiss***)

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چکیده

در این مطالعه ابتدا با استفاده از اکسید کرم (بعنوان نشانگر)، قابلیت هضم ظاهری کنجاله تخم پنبه واریته پاک (CSMP) محاسبه گردید. سپس کنجاله تخم پنبه یاد شده بعنوان جایگزین اقتصادی کنجاله سویا در جیره غذایی ماهی قزل‌آلای رنگین کمان (*Oncorhynchus mykiss*) از نظر ارزش غذایی مورد تحقیق قرارگرفت. در این مطالعه، شش جیره غذایی حاوی سطوح مختلف کنجاله تخم پنبه واریته پاک (CSMP) به عنوان جایگزین کنجاله سویا در سطوح مختلف فرموله گردیده در مجموع به ۵۴۰ ماهی قزل‌آلای رنگین کمان با میانگین وزن ۵±۵۰ گرم خورانده شدند. در شروع آزمایش مذکور، ماهیان به طور تصادفی در تانکهای فایبر گلاس ۱۰۰ لیتری تقسیم شده و ۷ روز هفته، روزی سه بار به مدت ۶۰ روز تغذیه گردیدند. در آزمایش اول قابلیت هضم ظاهری کنجاله تخم پنبه واریته پاک (CSMP) وکنجاله سویا بدین شرح محاسبه شدند: ماده خشک به ترتیب ۶۲.۷ و ۶۹.۲ درصد، پروتیین خام بترتیب ۸۲/۴ و ۸۷/۳ درصد، چربی بترتیب ۶۶/۶ و ۷۸/۵ درصد. پس از ۸ هفته آزمایش بر روی تغذیه، میانگین وزن ماهیان تغذیه شده با جیره‌های ۱ تا ۶ به قرار ۱۰۰.۶، ۱۰۲، ۱۰۲/۹، ۱۰۳/۳، ۱۰۳/۹ و ۱۰۳/۴ گرم محاسبه گردید. همچنین میانگین FCR ماهیان تغذیه شده با جیره‌های ۱ تا ۶ به قرار ۱/۲۸، ۱/۳۱، ۱/۳۱، ۱/۲۷، ۱/۲۹ و ۱/۲۵ بود. درصد بازماندگی برای هر شش جیره آزمایشی ۹۹ درصد بود. قابلیت هضم ظاهری مواد مغذی در CSMP و کنجاله سویا متفاوت بود. وزن حاصله ماهیان، DWG ،SGR و درصد بازماندگی در تیمارها اختلاف معنی‌داری با یکدیگر نداشتند ($P>0.05$) ولی تیمارها درخصوص FCR با یکدیگر اختلاف معنی‌دار داشتند ($P<0.05$). در بحث اقتصادی، جایگزینی کامل CSMP با کنجاله سویا مقرون به صرفه بوده و براساس نتایج حاصله از آزمایش تجزیه گوسیپول، هیچ نشانی از سمیت در کبد ماهیان تغذیه شده با جیره‌های حاوی سطوح مختلف CSMP مشاهده نشد.

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