Iranian Journal of Fisheries Sciences 13(3)522-529 2014

Apparent digestibility coefficients and nutritional value of Iranian cottonseed meal varieties for rainbow trout (*Oncorhynchus mykiss*)

Dadgar S.[1]; Mohd Salleh Kamarudin M.[2]; Ehteshami F.[1,2*]

Received: January 2013 Accepted: May 2014

Abstract

Three different varieties of cottonseed meal (CSM) were evaluated to measure the apparent digestibility coefficient (ADC) of the nutrients using chromic oxide (Cr_2O_3) as an indigestible marker. Five experimental diets were prepared and mixed with 1% of Cr_2O_3, 2% of mineral and vitamin each of which were premixed. Diet 1 was used as the control diet. Diets 2, 3, 4 and 5 were formulated using 70% of the control diet together with 30% of each ofthe cottonseed meal Pak (CSMP), cottonseed meal Sahel (CSMS), cottonseed meal Akra (CSMA), and soya bean meal (SBM), respectively, in three replications.The ADC of the three CSM varieties was measured to be 53.8-62.7%, 60.2-66.6% and 75.6-82.4% for dry matter, fat and crude protein, respectively. Survival rate for all fishes used in this study was more than 98%. Fishes fed with the CSM diets were not significantly different compared with those fed with the SBM diet in terms of survival rate (p>0.05). Apparent protein digestibility of CSMP and CSMS showed no significant difference with SBM (p>0.05). Therefore, it could be concluded that two kinds of CSM could be used as a replacement for SBM in rainbow trout as a protein source.

Keywords: Iranian Cottonseed meal varieties, Soybean meal, Apparent digestibility coefficients, Rainbow trout.

1-Iran Fisheries Research Organization (IFRO), 14965-149, Tehran, Iran
2-Faculty of Agriculture, University of Putra Malaysia, No. 43400, Serdang, Selangor, Malaysia
*Corresponding author's email: ehteshami@upm.edu.my

Introduction

Oilseed meals are known as the most important and widely used protein sources of plant origin. These are produced from the dried residues, after oil is extracted from oilseeds such as soybeans (SB), cottonseed (CS) and so on (Hertrampf *et al*. 2000; Stickney, 2000). Among the oilseed meals used, soybean meal (SBM) and cottonseed meal (CSM) are of significant value due to their high protein content. The evaluation procedure of any plant protein source as fish feed supplement or ingredient involves investigating their major nutritional constitutions and properties. More specifically, the procedure includes analyzing a) crude protein (CP) content, b) amino acid profile and c) the apparent protein digestibility coefficient for a particular fish species (Hardy 1989; Lim 1989; Devendra 1995). The outcome of such evaluation would determine if a given oilseed meal could play any significant role in a fish diet in semi-intensive and intensive aquaculture systems.

The aim of this study was to determine the proximate composition and apparent protein digestibility of the Iranian CSM varieties (CSMP, CSMS and CSMA) and to compare them with a conventional SBM to investigate the feasibility of partially replacing SBM with local CSMs. Rainbow trout (*Oncorhynchus mykiss*) was used as the experimental fish.

Materials and methods

Analyses of proximate composition were done following the AOAC standard procedures (AOAC, 1995). Moisture was determined by oven-drying of samples at 105°C to a constant weight. Crude protein (CP) content was determined indirectly by analysis of total Kjeldahl nitrogen (CP= N× 6.25). Crude lipid was determined by lipid extraction with diethyl ether for six hours in a soxhlet apparatus. Ash was determined in porcelain crucible placed in a muffle furnace at 550°C for four hours. Fiber content was determined using acid-base digestion method. All analyses were done in triplicates.

Five experimental diets were prepared and mixed with chromic oxide (Cr_2O_3) (1% DM), mineral premix (zinc, iron, manganese, copper, iodine, cobalt, and selenium) (1% DM), and vitamin premix (vitamin A, D3, K, E, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, folic acid, biotin, vitamin B_{12}, vitamin C and choline chloride) (1% DM) as an indigestible marker. Diet 1 was used as the reference diet (Table 1), with chromic oxide as an indigestible marker (Temesgen, 2004). Diets 2, 3, 4 and 5 were formulated using 70% reference diet and 30% of each CSMP, CSMS, CSMA and SBM, respectively, as described by Cho and Slinger 1979. Diets were mechanically mixed with distilled water (30g distilled water/ 100g diet mix), and pelleted using a 4mm die noodle- making machine. The pelleted diets were then air-dried for 48h and stored at room temperature until use. Each diet was tested in three replicates.

Table 1: Composition of the reference diet (%).

Ingredients (%)	
Kilka Fishmeal	18.5
Wheat gluten	13.5
Corn meal	17.5
SBM	31.0
Vitamin premix	2.0
Mineral premix	2.0
Soybean oil	14.5
Chromic oxide	1.0

Fifteen digestibility tanks (100 L) were supplied with flow through spring water (temperature; 11 - 13°C, 1 m^3/s) and each was 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 five 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.

Feces (spaghetti-like green strings) were collected two times a day (0830 and 1630h) just before each feeding, pooled together for each treatment and dried for 3-4 hours. Dried feces were then frozen at -20˚C. The experiment was undertaken for three weeks. Crude protein contents of the diets and feces were determined. Feces were analyzed separately to determine their respective values of dry matter and CP. The ADCs of the experimental diets were calculated based on chromic oxide (Cr_2O_3) as a non-absorbable indicator.

Apparent digestibility coefficients (ADCs) for dry matter, crude protein and fat in the diets were determined with the following equations (Cho and Kaushik, 1990):

ADC of dry matter (%) =100× [1-(dietary Cr_2O_3/fecal Cr_2O_3]

ADC of nutrients (%) =100× [1-(*F*/*D*×*D*cr/*F*cr)]

Where *F* is the percent of nutrient in feces, *D* is the percent of nutrient in diet, *D*cr is the percent of chromic oxide in diet, and *F*cr is the percent of chromic oxide in feces.

The ADCs for dry matter, crude protein and fat were calculated from the respective digestibility coefficients for the reference diet and test diets on the basis of the 30% substitution of test ingredient in the reference diet (Cho *et al*., 1982).

ADC of test ingredient (%) = 100/30× (ADC in test diet - 0.7 ADC in reference diet).

Gossypol was measured as described by Botsoglou (1991). A 2g sample of fish liver was blended for 2 min with 50ml of acetonitrile-water (40:10, v/v) containing 2% of ascorbic acid. After the precipitated protein settled down, the supernatant liquid was filtered through Whatman No.

40 paper, and the first 5 ml of the filtrate extract was pipetted into a 50ml volumetric flask and 0.05ml of hydrochloric acid was added. The flask was then placed in a 65°C water-bath for 100 min. After cooling to room temperature, the flask contents were transferred into a 250ml separating funnel and 50ml of 0.3% aqueous ascorbic acid followed by 0.5ml of hydrochloric acid were added. The suspension formed was extracted with 25ml of chloroform and the separated bottom layer was filtered through anhydrous sodium sulfate on Whatman No. 40 paper into a 100ml flask to be further evaporated under vacuum at 35˚C.

Traces of solvents were removed with a stream of nitrogen and the remaining residue was dissolved in 1 ml of acetonitrile. Then, aliquots (25µl) of sample extracts were injected into the chromatograph and analyzed at a mobile phase flow-rate of 1.5 ml/min, a detection wavelength of 254nm, a chart speed of 15 cm/h and a recorder sensitivity of 0.050 a.u.f.s. Chromatography was performed at 30˚C to isolate the column from fluctuations at ambient temperature. The mobile phase consisted of two solvents, methanol and water, both containing 0.1% of phosphoric acid. The water used in the mobile phase was glass-distilled water that had been further purified by passing it through a C_{18} column. Elution of gossypol was carried out by programming the methanol-water mobile phase composition (v/v) as follows:

2 min isocratic at 82: 18; 2 min linear gradient to 92:8; 5 min isocratic at 92:8; was discarded. A 25ml aliquot of the clear 3min purge at 99: 1, and 10 min equilibration at 82: 18. After each day's work, the column was flushed with water until free from acidity and maintained filled with methanol.

Calibration graphs were prepared daily by running 25µl aliquots from the series of the working solutions and plotting the recorded peak heights versus the amount of gossypol injected. The concentration of gossypol in the samples was calculated by reference to this calibration graph and multiplication by appropriate dilution factor as follows:

Gossypol in samples (ppm) = (QV.2)/ (0.025 W)

Where Q = amount of gossypol found (ng),
V = volume of final sample dilution (ml) and
W = weight of sample (g).

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. LSD test to identify the significance of difference between any pair of treatment means was performed. All differences were Considered as significant at $p<0.05$.

Results

The proximate composition of the reference and experimental diets (%) is presented in Table 2. The crude protein and gross energy contents of the reference diet (Diet 1) were measured at 35.4 % and 1514.5 KJ/100g diet, respectively. The crude protein content of the experimental diets (2, 3, 4 and 5) were determined at 35.1, 34.2, 34.4, 35.9, respectively. The

gross energy content of the experimental diets 2, 3, 4 and 5 were measured at 1518.3, 1517.2, 1517.7, and 1518.4 KJ/100g diet (Table 2).

Table 2: Proximate composition of the reference and experimental diet (%average of three samples).

	Diet *				
	1	**2**	**3**	**4**	**5**
Crude protein (%)	35.4	35.1	34.2	34.4	35.9
Moisture (%)	11.5	10.8	11.2	10.9	10.8
Crude fat (%)	9	9.4	9.9	9.8	9.1
Crude fiber (%)	4.9	6.2	9.7	9.5	5.2
Ash (%)	12.1	14.5	14.9	14.8	13.8
NFE (%)	27.1	24	20.1	20.6	25.2
DM (%)	88.5	89.2	88.8	89.1	89.2
GE(KJ/100g)	1514.5	1518.3	1517.2	1517.7	1518.4

*Diet 1 was the control Diet and Diets 2, 3, 4 and 5 were formulated using 70% control diet and 30% of each CSMP, CSMS, CSMA and SBM, respectively.

The free gossypol contents of CSMP, CSMS, and CSMA were measured at 0.003%, 0.078%, and 0.192%, respectively and the total gossypol contents of CSMP, CSMS, and CSMA were found to be at 0.009%, 0.295%, and 0.475%, respectively.

Chemical analyses resutls for CSMP, CSMS and CSMA were as follows: 93.34, 92.26 and 92.78% dry matter, 36.9, 24.37 and 28.1% crude protein, 10.6, 6.94 and 9.03% crude fat, 4.72, 21.35 and 20.1% fiber, and 945, 903 and 924 KJ/100 g energy, respectively. The proximate composition of CSM varieties, SBM, and the other ingredients analyzed in this study is depicted in Table 3.

Table 3: Proximate composition of CSM varieties, SBM and other ingredients (% as fed basis).

Ingredient(%)	**Kilka fishmeal**	**Corn meal**	**SBM**	**Wheat Gluten**	**CSMP**	**CSMS**	**CSMA**
CP	60.70	8.50	42.10	69.34	36.90	24.37	28.10
Crude Fat	10.35	3.06	3.15	1.58	10.60	6.94	9.03
Fiber	0.96	2.14	5.58	2.85	4.72	21.35	20.10
Ash	15.70	1.40	5.16	3.55	9.14	4.06	4.95
NFE	4.21	72.76	34.01	13.48	31.98	35.54	30.06
Moisture	8.08	12.14	10	9.2	6.66	7.74	7.22
GE (KJ/100 g)	1596	1428	945	1512	943	903	924
DM	91.92	87.86	90	90.8	93.34	92.26	92.78

Apparent digestibility coefficients (ADC) for dry matter, fat and CP in CSMP, CSMS, CSMA, and SBM are presented in Table 4. There were significant differences

between ADC values for dry matter, CP, and crude fat (p<0.05). ADC values of SBM were higher than those of CSM varieties.

The ADC values of nutrients in CSM varieties showed a decreasing tendency with increasing total and free gossypol and there were significant differences between the ADC values of nutrients of CSMP and CSMA (p<0.05), while there were no significant differences between CSMP and CSMS, and CSMS and CSMA (p>0.05).

Table 4:Apparent protein, dry matter, and fat digestibility (%) of CSM varieties and SBM.

Digestibility (%)	SBM	CSMP	CSMS	CSMA
Dry matter	69.2 ± 0.4 [a]	62.7 ± 0.3 [b]	58.5 ± 0.2 [bc]	53.8 ± 0.2 [c]
Crude Protein	87.3 ± 0.4 [a]	82.4 ± 0.3 [a]	78.3 ± 0.2 [ab]	75.6 ±0.2 [b]
Fat	78.5 ± 0.6 [a]	66.6 ± 0.4 [b]	61.4 ± 0.4 [bc]	60.2 ± 0.3 [c]

Means in rows with the same letter are not statistically different (p>0.05).

Means in rows with the same letter are not statistically different (p>0.05).

For all treatments, the survival percentage was more than 99%.

Discussion

Measuring the digestibility coefficients of feed ingredients could provide an insight into thenutrient utilization enabling more accurate ingredient substitutions in diets designed for a 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 productsbetter than that of plant products (Cho *et al.,* 1982; Sullivan and Reigh, 1995). The present study showed that ADC values of nutrients in CSMP, CSMS, and CSMA were comparable to those reported by Cheng and Hardy 2002 and those in other oilseed meals (Morales *et al.,* 1999). However, dry matter, CP, and fat digestibility of CSM varieties were lower than those of SBM, probably due to the high fiber contents (Jones, 1979). High digestibility rate of crude protein of CSM varieties in this experiment confirmed the results obtained by Cheng and Hardy (2002) on the effect of total gossypol concentrations on reducing the digestibility of protein. In addition, results indicated that the comparative nutritional ADCs of CSM varieties were similar to those of the other oilseed meals such as canola.

The present study showed that the ADCs of majority nutrients in the Iranian varieties of CSM were singnificantly different. Because of CSMP`s lower gossypol level, the ADCs of CP, crude fat and dry matter were significantly higher than those of CSMA and CSMS. The results were in agreement with Mbahinzireki *et al*. (2001) who reported that ADCs of CP decreased as dietary gossypol level increased in tilapia (*Oreochromis* sp.) feeds. Cheng and Hardy

(2002) reported that ADCs of CP was 94.8% in canola meal for rainbow trout. Morales *et al.,* (1999) reported that ADCs of dry matter and CP in SBM were 52.8 to 81.4% and 78.7 to 88.9%, respectively, for rainbow trout. This was in line with the findings of the present study which ADCs of dry matter and CP in SBM were 69.2% and 87.3%, respectively. Overall, fishes fed with the CSM diets were not significantly different compared to those fed with the SBM diet in term of survival rate (p>0.05). SBM could be replaced by CSMP and CSMS as a protein source for trout feeding since there is no significance difference between their apparent protein digestibility (p>0.05).

References

AOAC., 1995. Official methods of analysis, Association of Official Analytical Chemists**.** 16th ed. Arlington.

Botsoglou, N. A., **1991.** High-performance liquid chromatographic method for the determination of free gossypol in chicken liver. *Journal of Chromatography*, 587, 333–337.

Cheng, Z. J. and Hardy, R.W., 2002. Apparent Digestibility Coefficient and Nutritional value of Cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 212, 361-372.

Cho, C. Y. and Slinger, S. J., 1979. Apparent Digestibility Measurement in feedstuffs for rainbow trout.pp: 239-248 in Finfish Nutrition and Fish feed Technology, vol.2, J.E.Halver and K. Tiews, eds. Berlin: Heenemann gmbh.

Cho, C. and Kaushik, S. J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Review of Nutrition and Dietetics*, 61, 132–172.

Cho, C. Y., Slinger, S. J. and Bayley, H. S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology*, 73, 25–41.

Devendra, C. ,1995. Composition and nutritive value of browse legumes. In: D'Mello, J.P.F. and Devendra, C., (Eds.), Tropical legumes in animal nutrition. CAB International, London. pp. 49-66.

Hardy, R.W., 1989. Diet preparation. In: J.E.Halver (Ed.), Fish nutrition, 2nd Edition. Academic Press Inc. New York. pp. 476-554.

Hajen, W. E., Beames, R. M., Higgs, D. A. and Dosanjh, B. S., 1993. Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in seawater: 1. Validation of technique. *Aquaculture*, 112, 321-332.

Hertrampf, J. W. and Piedad-Pascual, F., 2000. Handbook on ingredients for aquaculture feeds. Kluwer Academic, Dordrecht. 567P.

Jones, J. D., 1979. Rapeseed protein concentrates preparation and evaluation. *Journal of Oil Chemistry Society*, 56, 716–721.

Lim, C. ,1989. Practical feeding Tilapia. In: Lovell, T., (Ed.), Nutrition and

feeding of fish. Van Mustard Reinhold Press, New York, pp.163-176.

Mbahinzireki, G. B., Dabrowski, K., Lee, K. J., El-Saidy, D. and Wisner, E. R., 2001. Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal- based diets in recirculating system. *Aquaculture Nutrition*, 7, 189-200.

Morales, A. E., Cardenete, G. and Higuera, M. ,1999. Re-evaluation of crude fiber and acid- isolable ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 179, 71-79.

Stickney, R. R. (Ed). ,2000. Encyclopedia of aquaculture. John Wiley, New York. 1063P.

Sullivan, J.A., and Reigh, R.C., 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilis* ♀ × *Morone chrysops* ♂). *Aquaculture*, 138, 313–322.

Temesgen, G.G. M., 2004. Utilization of *Gliricidia sepium* leaf meal as protein source in diets of Mozambique Tilapia (*Oreochromis mossambicus*). PhD Thesis, UPM, Malaysia. 156P.