Comparative evaluation of soybean meal and carob seed germ meal as dietary ingredients for rainbow trout fingerlings

Maria N. Alexis

National Centre for Marine Research, GR 16604, Elliniko, Athens, Greece.

Received December 8, 1989; accepted May 15, 1990.

Alexis M. N., *Aquat. Living Resour.*, 1990, 3, 235-241.

Abstract

Carob seed germ meal (CSGM), and soybean meal (SBM), were tested as ingredients of diets for rainbow trout (*Oncorhynchus mykiss*) fingerlings at three levels of inclusion, contributing 20 to 50% of the total protein of the diet. Fish growth decreased as the level of the plant by-product in the diet increased. Diets containing CSGM resulted in lower growth than comparable SBM diets, especially at inclusion levels contributing 37 and 53% of the total protein. High dietary CSGM levels decreased body protein and increased liver protein indicating possible amino acid deficiencies and/or imbalances at high inclusion levels. Mortality was also high at the high dietary CSGM level. The results are discussed with reference to the antinutritional factors contained in the raw materials tested and their possible effects on protein availability.

Keywords : Nutrition, rainbow trout, *Oncorhynchus mykiss*, carob seed, soybean.

Évaluation comparatives des farines de soja et de germes de caroube comme aliments pour de jeunes truites arc-en-ciel.

Résumé

Des farines de germe de caroube (FGC) et de soja (FS) ont été testées comme aliments sur des jeunes truites arc-en-ciel (*Oncorhynchus mykiss*). Trois taux d'incorporation de farines correspondant à des concentrations de 20 à 50 % des protéines totales de l'aliments sont étudiés. Le taux de croissance des poissons diminue lorsque la proportion en sous-produits végétaux augmente. Les aliments contenant de la FGC entraînent des taux de croissance faibles par rapport aux aliments contenant FS et spécialement lorsque le taux d'incorporation de FGC est compris entre 37 et 54 % de protéines totales. Un taux élevé en FGC abaisse le taux de protéine du corps et lève le taux de proteiné du foie, indiquant alors des déficits et/ou déséquilibres possibles en acides aminés. La mortalité est aussi élevée à concentration maximale de FGC. Les résultats sont discutés ayant comme référence la composition en facteurs antinutritionnelles dans les matières premières testées et leur effet sur la disponibilité protéinique.

Mots-clés : Nutrition, truites arc-en-ciel, *Oncorhynchus mykiss*, germe de caroube, soja.

INTRODUCTION

The use of plant materials of high protein content in fish diets is desirable due to their low prices and continuous market availability. The most widely tested plant materials are leguminous seeds, and especially soybeans, either in extracted or full fat form, due to their high protein content and good amino acid profile. Several other oil seed meals have been tested (Tacon and Jackson, 1985). Recently lupin products have been used for replacing either fish meal or soybeans (de la Higuera *et al.*, 1988, Hughes, 1988, Gomes and Kaushik, 1989) with variable success.

The main limitations in the use of plant materials are certain amino acid deficiencies (Harris, 1980) and the presence of chemical compounds collectively known as antinutritional factors (Liener, 1980, Tacon and Jakson, 1985), which lower the nutritional quality and sometimes cause mortalities. Specification of the factors reducing fish performance and determination of the maximum dietary levels of plant materials compatible with good fish growth are the first steps for further possible improvement of their quality and increment of their levels of dietary inclusion.

Carob seed germ meal (CSGM) is a by-product of the carob gum producing industries. It has a high protein content and a biological value similar to that of soybean meal (SBM) when fed to rats (Drouliskos and Malefaki, 1980), while being considerably less expensive (about half the price). The potential therefore of using CSGM instead of SBM in trout diets is of an economic interest. Inclusion of CSGM in practical rainbow diets (Alexis *et al.*, 1985) has resulted in conversion efficiencies inferior to those obtained by feeding diets containing similar levels of SBM at first feeding, whereas differences in conversion efficiencies were less pronounced at the end of the experiment.

A more detailed comparison of GSGM with SBM was undertaken in the present study. Three different levels of inclusion of these two by-products were fed to rainbow trout fingerlings. Fish growth, feed utilisation, body and liver composition and some hematological characteristics are reported and discussed.

MATERIALS AND METHODS

Twelve groups of 200 rainbow trout, having an average weight of 1 g per fish were used for the experiment. The fish were maintained in $2 \times 0.45 \times 0.40$ m fiberglass tanks, having a water flow of 0.5 l/sec. The water temperature was 11-13°C, pH 7.4-7.8, conductivity 235-265 $\mu S.cm^{-1}$ and hardness 2.2-2.6 mol/l $CaCO_3$.

The composition of the raw materials used for formulating the diets is given in *table* 1. The amino acid composition of the fish meal was provided by the suppliers, while the values obtained by Malefaki (1981) were used for the other two by-products. The protein digestibilities of the raw materials used were presented in a previous publication (Alexis *et al.*, 1989).

Six diets were formulated and assigned to duplicate tanks of fish (*table* 2). Diets 1-3 contained CSGM, 4-6 SBM at increasing levels, so that the amount of

Table 1. — Composition of the raw materials used for formulating the diets (SBM: soybean meal, CSGM: carob seed germ meal).

	Proximate analysis (%)		
	Herring meal	SBM	CSGM
Protein	69.8	47.1	38
Fat	11	1.8	3.5
Ash	10.8	6.4	5
Moisture	8.5	7.8	8.4
	Amino acid content (% protein)		
Met + Cys	3.39	1.56	2.79
Thr	4.01	4.35	3.16
Trp	1.07		
Val	5.28	4.63	4.06
Ile	4.58	3.40	3.13
Leu	7.17	7.23	5.78
Tyr + Phe	6.49	8.17	5.39
His	1.92	2.35	2.29
Lys	7.03	6.40	5.16
Arg	5.10	6.26	11.55

protein contributed by each by-product increased from about 20 tp 50% of the total protein.

Fish were fed by hand, at a daily rate of 3% of their body weight, three times a day seven days a week. A sample of 50 fish from each tank was weighed every fortnight. Fish were starved for 24 hours before each weighing. The experiment lasted for 83 days. The whole population of each tank was weighed at the end of the experiment.

A sample of 140 fish of the initial population as well as a sample of 20 fish from each tank, removed at the end of the experiment, were used for body composition analysis. A number of the fish removed at the end of the experiment were also used for determining liver characteristics as well as hematocrit values. Body composition analysis was performed by using standard procedures, as previously described (Alexis *et al.*, 1985). Blood for hematocrit determination was taken after severing the caudal artery of the fish.

The method of Olomucki and Bornstein (1960) was used for determining antitrypsin activity of SBM. The cresol binding value determined was 4.4 mg/g meal. The total tannin content of CSGM was found to be 2.5% (Filioglou and Alexis, 1989). Statistical examination of results was performed by one-way analysis of variance. Tuckey's test was used for multiple comparisons. Results are reported at 5% probability level.

RESULTS

The weight gain of the fish throughout the growing period is presented in *figure* 1. Fish fed diets containing CSGM exhibited weight gains inferior to fish fed comparable SBM diets. Differences in weight gain

Table 2. – Formulation (% composition) and proximate analysis (% weight composition) of experimental diets.

	Diets					
	1	2	3	4	5	6
			Composition (%)			
Herring meal	50.7	36.9	26.5	50.3	36.4	28.3
SBM		–		19.0	36.5	44.6
CSGM	21.8	42.4	57.6	–		–
Raw corn starch	11.4	4.7	–	14.1	9.8	8.5
a-cellulose	2.1	1.4	1	1.4	–	
Bone meal	0.5	0.8	1.2	1.0	1.9	2.0
Guar gum	1.9	1.9	1.9	1.9	1.9	2.0
Linseed oil	9.6	10.0	10.0	10.2	11.4	12.4
Met	1.2	1.1	1.0	1.3	1.3	1.4
			Proximate analysis %			
Protein	44.9	43.0	41.4	45.4	43.9	42.2
Digestible protein	33.6	29.4	26.2	37.3	36.7	35.5
Fat	15.9	15.5	15.0	16.1	16.1	16.3
Ash	7.1	6.9	6.9	7.7	8.2	7.9
Moisture	6.1	6.7	7.1	5.7	5.9	5.6
% of total protein	18.5	37.5	52.9	19.7	39.2	49.8

Each diet also contained (%), choline chloride 0.5, vitamin premix 0.08, mineral premix 0.2 and BHT 0.02.

Vitamin premix (expressed as mg/kg of finished feed except where otherwise indicated): thiamine 10, riboflavin 20, pyridoxine 10, calcium pantothenate 40, nicotinic acid 150, biotin 1, folic acid 5, vitamin B_{12} 0.02, inositol 40, vitamin A 2500 I.U., vitamin D_3 500 I.U., vitamin E 60 I.U., vitamin K_3 5, ascorbic acid 400.

Mineral premix: A commercial mixture of minerals (Suivit) was used, fortified with $AlCl_3 \cdot 6H_2O$, KI and $CoCl_2 \cdot 6H_2O$ to contain in mg/2 g mixture: Fe 200, Al 0.3, Zn 300, Cu 200, Mn 63, I 5.3, Co 7.3.

were significant only at levels of CSGM contributing more than 20% of the dietary protein (*fig.* 2*a*). For both sets of diets, weight gains tended to decrease with increasing levels of plant by-products. However, the difference in weight gain between the two lower inclusion levels of SBM did not differ (*fig.* 1). Feed efficiency and protein retention decreased as the level of plant by-products in the diet increased (*fig.* 2*b-c*). The values of feed efficiency and percent protein retention obtained from the diet containing the highest CSGM level were significantly lower than those of all the other dietary treatments. When the % protein retained was calculated in terms of digestible protein instead of crude dietary protein content, values were not significantly different between CSGM and SBM containing diets (*fig.* 2*c*).

The proximate analysis of the fish of all experimental groups is presented in *table* 3. All fish had similar protein content with the exception of fish fed diet 3, which had a significantly lower protein content. The lipid content of the fish increased with the SBM content of the diet. The lipid content of the fish receiving the CSGM diets did not show any specific trend.

Fish fed the highest level of CSGM had a higher lipid content despite the fact that these fish had the lowest weight gain. The ash content did not differ significantly among the groups fed the SBM containing diets, while an increase in the ash content of fish fed the CSGM diets was apparent. Moisture content was similar with the exception of fish fed diet 2 and 3, which exhibited a significantly higher moisture content than the other groups. Mortality was much higher in the groups fed the highest CSGM level.

Hematocrit values of fish receiving either CSGM or SBM at similar dietary levels did not differ significantly (*table* 4). Hematocrit values decreased as the level of plant by-products in the diet increased.

Hepatosomatic indexes were similar within groups (*table* 4). Hepatosomatic indexes of fish fed the SBM containing diets were consistently higher than of those fed the CSGM containing diets. Moisture levels and glycogen content of the livers did not differ significantly.

Liver protein levels were similar within groups. Fish fed the CSGM containing diets had on the average higher liver protein content than those fed SBM. Significant differences were observed only at the highest SBM inclusion level. Fat content of the livers of fish receiving the SBM diets were on the average higher and the ash content lower than those of fish receiving the CSGM diets. The ash content of the livers decreased with increasing levels of plant by-products in the diet.

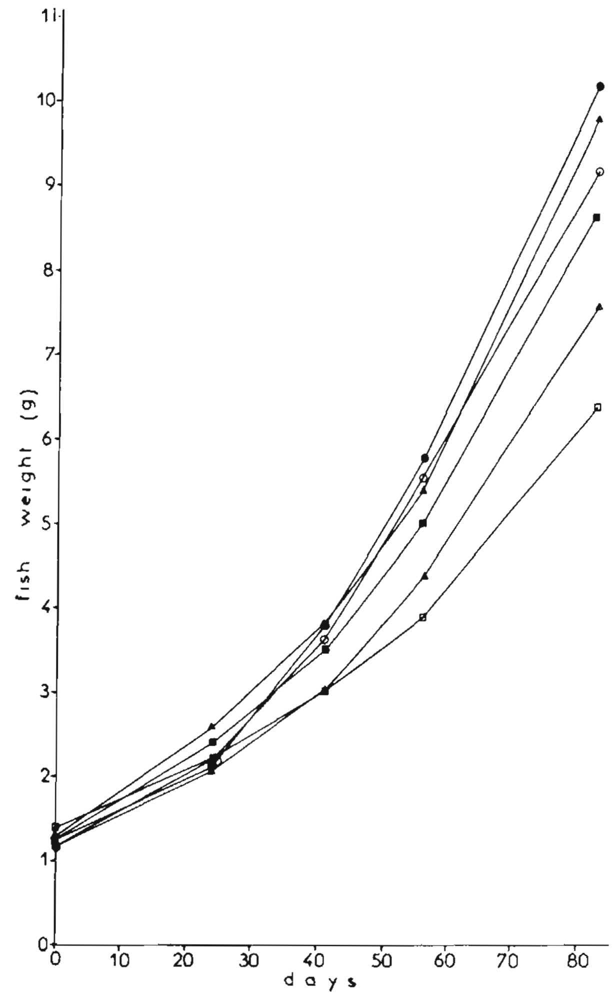

Figure 1. — Fish weight throughout the growing period. Diet 1 (○), diet 2 (△), diet 3 (□), diet 4 (●), diet 5 (▲), diet 6 (■).

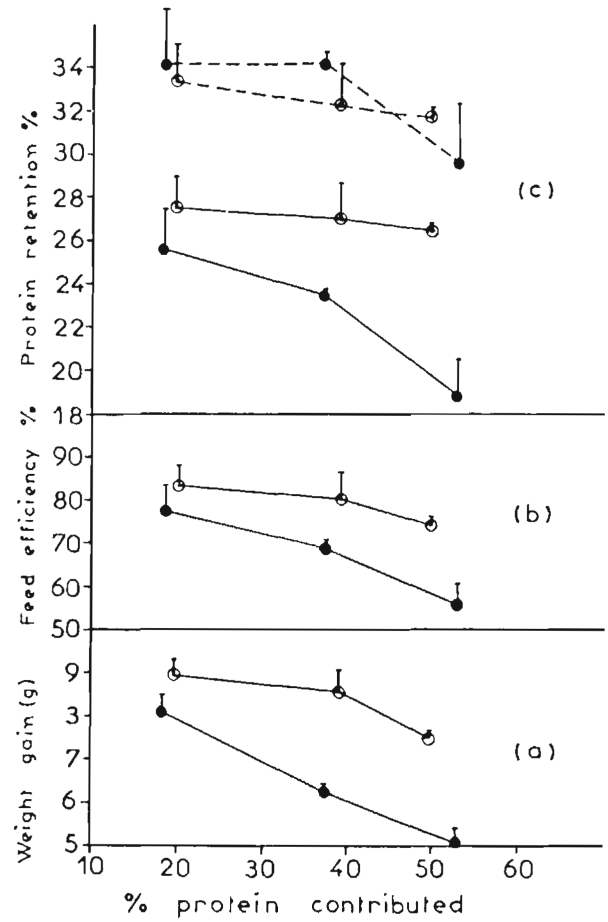

Figure 2. — Weight gain, feed efficiency, protein retention (———) and digestible protein retention (– – –) in relation to the protein contributed by each by-product, SBM (○) and CSGM (●).

DISCUSSION

Use of SBM as protein source

The diets containing SBM had a digestible protein and fat content best suited for rainbow trout of this size (Watanabe *et al.*, 1979). However, differences in weight gain were observed among the fish fed the three dietary SBM levels.

The degree of heating of SBM has been shown to be the main factor affecting its nutritional value (Sandholm *et al.*, 1976, Ketola, 1982, Viola *et al.*, 1983). Proper heating improves nutritional value by destroying antitrypsin activity. The protein digestibility of the soybean meal used in the present study was high, about 87% (Alexis *et al.*, 1989), indicating sufficiently heated soybeans with a minimal amount of antitrypsin activity (Sandholm *et al.*, 1976). Overheating reduces the nutritional value of soyabean mainly by reducing the available lysine content (Viola *et al.*, 1983). The available lysine content of the product used could therefore be low enough to create deficiency at the highest inclusion level, thus contributing to the lower growth rate observed.

The increase in the lipid content of the fish fed with SBM may have resulted from the higher digestibility of carbohydrates contained in SBM than in raw corn starch (Cho and Slinger, 1979) and therefore diets containing higher SBM levels would have had higher available energy.

Use of CSGM as protein source

The reduction in weight gain of the fish fed increasing CSGM levels was more pronounced than that observed with SBM. The main antinutritional factors reported for carob pods are tannins (Nachtomi and Alumot, 1963). CSGM has been shown to contain tannins (Filioglou and Alexis, 1989).

Table 3. — Body composition and mortalities of rainbow trout fed experimental diets for 83 days (% wet weight)*.

Diet	Crude protein	Crude fat	Ash	Moisture	Mortalities**
1	14.9 (0.1)	10.20 (0.05)	1.98 (0.08)	72.4 (0.1)	2
2	14.7 (0.1)	9.53 (0.11)	2.38 (0.07)	72.7 (0.1)	3
3	14.2 (0.1)	10.12 (0.07)	2.62 (0.04)	73.0 (0.1)	27
4	14.9 (0.2)	9.51 (0.11)	2.33 (0.07)	72.3 (0.2)	2
5	14.8 (0.2)	9.65 (0.07)	2.35 (0.08)	72.3 (0.1)	9
6	15.0 (0.1)	9.92 (0.07)	2.33 (0.03)	72.2 (0.1)	1

* Values are the averages of the two replicates.
Values in parentheses are the standard deviations of the measurements.
**Average number of fish/tank.

Table 4. — Hepatosomatic index, liver composition and hematocrit values of experimental groups.

	Diet					
	1	2	3	4	5	6
Hepatosomatic index	1.84 (0.42)	1.63 (0.35)	1.70 (0.39)	2.01 (0.62)	2.13 (0.54)	1.97 (0.42)
Hematocrit	29.0 (4.7)	25.1 (4.4)	22.3 (4.7)	25.0 (3.6)	22.2 (5.2)	20.8 (3.3)
Moisture	76.4 (0.6)	75.9 (2.3)	76.1 (0.9)	76.5 (1.0)	76.3 (2.3)	76.0 (0.6)
Proteins	16.9 (1.1)	16.9 (1.0)	17.5 (1.3)	15.9 (1.2)	16.0 (1.1)	15.5 (1.0)
Fat	3.8 (0.4)	3.7 (0.5)	4.3 (0.5)	4.9 (0.5)	5.2 (0.3)	4.3 (0.4)
Glycogen	3.4 (1.7)	3.1 (1.4)	2.8 (1.3)	3.7 (1.6)	2.7 (1.4)	3.6 (1.4)
Ash	1.5 (0.1)	1.3 (0.2)	1.2 (0.2)	1.4 (0.1)	1.3 (0.2)	1.0 (0.2)

Hepatosomatic index and hematocrit values are the average of 20 measurements. Moisture and ash contents of 4 measurements and protein, fat and glycogen contents of 10 measurements.
The values in parentheses are the standard deviations of the measurements.

Tannins create palatability problems due to their astringent taste (Joslyn and Goldstein, 1964). It is generally accepted that palatability problems exist, with rainbow trout, specifically for fish weighing less than 10 g (Crampton, 1985). A constant feeding level was used in the present experiment, so that possible palatability problems were not tested.

The main antinutritional characteristics of tannins are related to their ability to reduce feed digestibility. From *in vivo* and *in vitro* experiments, it has been suggested that protein digestibility is reduced by tannins by either a direct binding to certain parts of the protein molecule or through a non-competitive inhibition of the digestive enzymes (Tamir and Alumot, 1969, 1970). Extracts of CSGM were also found to decrease trypsin activity *in vitro* (Filioglou and Alexis, 1987) and protein digestibility measurements performed with rainbow trout resulted in values lower than those of SBM, ranging from about 50-70% (Alexis *et al.*, 1989, Filioglou and Alexis, 1989). The variability was possibly due to different amounts of the seed coat present in different batches of the meal.

It was calculated that the digestible protein content of the diets used in the present experiment decreased from about 37% to 26% as the level of CGSM in the diet increased (*table* 2). Fish fed diet 1 containing 37% digestible protein indicated a performance similar to that of the diets containing SBM, while reduction of growth parameters was particularly pronounced for the highest CSGM level. The decrease in digestible protein might therefore be the main reason for the poor performance of the fish receiving the diets containing high levels of CSGM. However, the reduction in fish growth and the changes in feed conversion are much greater than those observed by Watanabe *et al.* (1979), among diets covering the same range of protein contents (26-36%) at similar fat levels (16%). The percentage of digestible protein retained at the highest CSGM inclusion level is also lower than the values obtained at the two lower inclusion levels (*fig.* 2 *c*), despite the fact that protein retention increases with decreasing protein content of the diet (Steffens, 1981). These observations indicate that there might be other factors in addition to protein digestibility which reduce the nutritional value of CSGM.

From the amino acid composition of CSGM (*table* 1) it appears that amino acid requirements for trout were met or exceeded at all CSGM levels. Possible methionine deficiency (Drouliskos and Malefaki, 1980) was prevented by supplementing the diets with synthetic methionine.

Tannins have been shown to bind to hydrophobic parts of the protein molecule (Mitaru *et al.*, 1984), hydrophobic amino acids like histidine, isoleucine and valine indicating comparatively larger increases in digestibilities (21-24%) with reconstitution (high moisture storage, which deactivates tannins) of sorgum, than other indispensable amino acids (14-18%). A decrease in the availability of hydrophobic amino acids due to their interaction with tannins could therefore create deficiencies and imbalances, which might be an additional factor in the reduction of fish performance with increasing dietary CSGM content.

The high protein content of the livers in contrast with the low body protein of the fish receiving the highest CSGM levels might be an indication of an amino acid imbalance, since experiments with rats have shown that amino acid imbalance stimualtes protein synthesis in the liver and reduces protein retention by the muscle (Sanahuja and Rio, 1967, Yoshida *et al.*, 1966).

Fish receiving diet 3, the highest CSGM content, had the lowest body protein and a high fat and moisture content. Low body protein in fish receiving high CSGM containing diets has been observed previously (Alexis *et al.*, 1985, 1986). Experiments with chicks have shown that condensed tannins purified from fava beans (Marquardt *et al.*, 1977) increased fat retention and decreased protein retention, when added to diets. A similar effect was observed in the current study at the highest CSGM inclusion with an increase in the fat and a decrease in the protein content of the fish. Liver weights of chicks receiving high levels of growth inhibitors were also reduced (Ward *et al.*, 1977) in accordance with our present observations.

The high mortality of fish receiving the diet containing the highest CSGM level indicates that there may have been a toxic effect of the CSGM at least for fish of this size.

CONCLUSION

CSGM appears to be a protein source of lower nutritional value than SBM. Levels contributing about 20% of dietary protein did not significantly affect fish performance, higher inclusion levels resulted in lower weight gains. The main reason for the reduced fish performance appears to be the low protein digestibility of the CSGM. It appears that at a high level on inclusion (50% of dietary protein) fish metabolism is affected and a toxic effect exists resulting in high fish mortalities.

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