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Keywords
Feeds, Fish-meal free, Nutrition, Soy flour, Soybean meal, Trout

Disciplines
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Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*

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Abstract

Eight experimental diets were formulated for rainbow trout using agricultural byproducts as major ingredients. Each experimental diet contained varying amounts of corn grain, corn gluten meal, corn gluten feed and one of the following: 200 g kg^{-1} peanut meal, 200 or 400 g kg^{-1} soybean meal (SBM), 390 g kg^{-1} low-allergen soy flour, 310 g kg^{-1} soy protein concentrate, 300 g kg^{-1} low-allergen soy protein concentrate or 200 g kg^{-1} SBM + 110 g kg^{-1} blood meal. One diet contained 200 g kg^{-1} SBM and canola oil as the main lipid source. The remaining diets contained 95 g kg^{-1} menhaden oil. Fish fed a commercial trout diet exhibited significantly greater weight gain (322%), and a lower feed conversion ratio (0.89) but significantly lower protein efficiency ratio (2.18) than fish fed the experimental diets. Within the experimental diets, fish fed the 400 g kg^{-1} soy flour diet and the 400 g kg^{-1} soybean meal diet had significantly higher weight gains (276% and 268%) and protein efficiency ratios (2.58 and 2.52), and lower feed conversion ratios (1.02 and 1.03) than fish fed other experimental diets. Fillet flavour varied between treatments. Most notable was the lower fishy flavour and higher chicken flavour of fish fed the diet that contained canola oil rather than menhaden oil. Microscopic evaluation of the liver and five sections of the gastrointestinal tract failed to demonstrate any differences between treatment groups. The ingredient costs of several experimental diets were lower than the estimated cost of a standard commercial trout diet. However, the superior feed conversion ratios of fish fed the control diet resulted in lower feed costs per unit of fish produced.

KEY WORDS: feeds, fish-meal free, nutrition, soy flour, soybean meal, trout

Introduction

Fish feeds are the largest single operating cost in aquacultural production. Consequently, there is a continuing effort to reduce feed cost by using lower-priced ingredients. Fish meal is a major ingredient in many feeds and is commonly targeted for replacement because of its high cost and finite World supply (Hardy & Masumoto 1990; Rumsey 1993). Several low-cost agricultural byproducts are produced in the Midwestern United States and replacing fish meal with these products in diets for rainbow trout (*Oncorhynchus mykiss*) would reduce the cost of feed. However, reducing feed costs by replacing fish meal in diets does not necessarily reduce operating costs in aquaculture.

Many plant byproducts contain lower protein levels and lower levels of essential amino acids than fish meals. In addition, plant protein feedstuffs contain antinutritional factors. For example, soybean meal contains as many as five trypsin inhibitors, non-digestible carbohydrates, lectins, saponins, phytates and possibly allergenic storage proteins (Salunkhe *et al.* 1992), all of which have been implicated in

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hindering digestion in rainbow trout (Spinelli *et al.* 1983; Dabrowski *et al.* 1989; Olli & Krogdahl 1994; Rumsey *et al.* 1994; Bureau *et al.* 1996). In the early 1970s, there was a world-wide shortage of fish meal caused by a significant reduction in harvest of Peruvian anchovy (Spinelli *et al.* 1979). This stimulated efforts to find suitable alternative protein sources (Cho *et al.* 1974; Fowler & Banks 1976; Gropp *et al.* 1976; Spinelli *et al.* 1979; Tiews *et al.* 1979). In general, those studies found that fish fed plant byproducts grew at a slower rate than fish fed diets containing high levels of fish meal. However, in recent years, there have been renewed efforts to increase the amount of plant byproducts in diets for trout. This results from several factors, including improved processing and quality of plant byproducts, better understanding of the nutritional requirements of trout and development of feed-grade amino acids. Recent studies have shown considerable success in the total replacement of fish meal in diets for rainbow trout with plant byproducts, using soy flours and protein concentrates (Rumsey *et al.* 1993; Kaushik *et al.* 1995), and soybean meal and corn gluten meal mixtures (Ketola & Harland 1993; Gomes *et al.* 1995).

The objective of this study was to develop diets for rainbow trout that were free of fish meal and evaluate growth, health and flavour characteristics of fish fed those diets relative to a commercial trout diet.

Materials and Methods

Fish and experimental system

London strain rainbow trout were acquired from the Curtis Creek State Fish Rearing Facility, Indiana Department of Natural Resources, Howe, IN, USA. Fish were transported to the Purdue University Aquaculture Research Facility and held at 12°C in a 2000 L circular tank for 5 weeks prior to transfer to the experimental system.

Three weeks prior to the study, the temperature in the holding tank was increased 1°C day^{-1} to 15°C. One week prior to transfer to the experimental system, a sample of fish were removed for health evaluation. The evaluation included gross examination, cytological evaluation of skin scrapes and gill clippings, and microscopic evaluation of sections of liver, kidney, spleen, skin, muscle, gill and gastrointestinal tract: all were normal. Bacterial culture of liver, spleen, and kidney failed to identify any significant pathogens. Fish used in this study were not fed diets containing soy products prior to acquisition or during quarantine.

The experimental system consisted of 28 aquaria connected to a common filter. The system included biological filtration, screen particle filters, 190 L sand filter, refrigeration units, and supplemental aeration. Water was added continuously to the system with a turnover time of 18–21 h. Photoperiod was maintained at 16 h light:8 h dark. Each aquarium was 120 L with a flow rate of 5–6 L min^{-1} at 15 ± 1°C.

Groups of 15 fish were randomly placed into each aquarium for a 2-week acclimation period. At the beginning of the second week, dietary treatments were randomly assigned to triplicate aquaria. At the onset of the study, the total number of fish was reduced to 12 fish per tank to establish a combined weight of 420–430 g (≈ 35 g per fish). Fish were fed to apparent satiation twice per day (morning and evening) for 8 weeks and were weighed at 4-week intervals. At the initial and middle weighings, fish were sedated with 20 mg L^{-1} tricaine methanesulfonate (MS 222, Argent Chemical Laboratories, Redmond, WA, USA). At the end of the study, all fish were killed by a sharp blow to the cranium.

Diets

Diets were formulated with Mixit-II^{+} (Agricultural Software Consultants, Kingsville, TX, USA) to meet the nutritional requirements of rainbow trout, and to contain 350 g kg^{-1} digestible protein, 120 g kg^{-1} fat, and 15 MJ kg^{-1} digestible energy (Cho & Cowey 1991).

The proximate compositions of ingredients were provided by suppliers and used to formulate the feeds. Available phosphorus values for corn gluten meal (315 g kg^{-1}), peanut meal (417 g kg^{-1}) and soybean meal (168 g kg^{-1}) were from Riche & Brown (1996). For all other ingredients the phosphorous availability was considered negligible. Digestible protein and energy content of ingredients were obtained from values reported by Cho & Cowey (1991) and NRC (1993). All experimental diets were deficient in either lysine or methionine and were supplemented with one or both of those essential amino acids to meet the requirements (Cho & Cowey 1991). Ingredients were mixed, then extruded at Illinois State University with an Insta-Pro model 600 Jr. Extruder (Insta-Pro Extruders International, Des Moines, IA, USA), at 135–160°C. Diets were transported to Purdue University and stored at –20°C during the feeding trial. Gelatinization of the dietary starch upon extrusion was estimated to be 90–95% (Nahil Said, Insta-Pro Extruders International, Des Moines, IA, USA, personal communication). Therefore, the digestible energy of ingredients that contained starch was increased 4.7 MJ kg^{-1} of starch (Cho & Cowey 1991).

The composition of the experimental diets is shown in Table 1. The primary ingredients were soybean meal (500 g kg^{-1} crude protein (CP); hexane extracted, toasted, dehulled, Cargill, Inc., Lafayette, IN, USA), corn gluten meal (600 g kg^{-1} CP) and corn gluten feed (240 g kg^{-1} CP; A.E. Stayley and Co., Lafayette, IN, USA), blood meal (920 g kg^{-1} CP; American Protein Corp., Ames, IA, USA), peanut meal (480 g kg^{-1} CP; ADM, Augusta, GA, USA), menhaden oil (Zapata Proteins, Inc., Mandeville, LA, USA), canola oil (Hunt-Wesson, Inc., Fullerton, CA, USA), soy flour (ADM, Decatur, IL, USA) and two soy protein concentrates (Central Soya, Fort Wayne, IN, USA). The soy flour was extrusion processed to reduce antigenicity and contained 80 g kg^{-1} lecithin. The soy protein concentrates were Profine F® and Profine VF®. Profine F® is a standard soy protein concentrate and Profine VF® is further processed and marketed as a low-allergen product. The mineral premix (US Fish and Wildlife Federal Mineral Premix #3) was added at 1.5 times the recommended level to allow for potential decreases in mineral absorption resulting from the phytic acid content of the feed ingredients. The level of menhaden oil in each diet was held constant to avoid differences in palatability (except for the diet containing canola oil). The remainder of the lipid was provided by soybean oil. The diet that contained canola oil was the only diet that did not meet the *n*-3 fatty acid requirement recommended by Cho & Cowey (1991).

Ingredient costs of feedstuffs were based on 5-year averages of market prices in Chicago, IL, USA, between

Table 1 Ingredient composition, proximate composition, and pellet density of experimental diets fed to rainbow trout

	Dietary treatment								
Ingredient (g kg^{-1})	Control	PM	SB20	SPC1	SPC2	SF	SB40	BM	SB20c
Corn gluten meal	–	360	360	213	206	227	227	209	364
Yellow corn	–	152	156	289	275	193	184	285	156
Corn gluten feed	–	125	125	38	52	31	32	44	123
Peanut meal	–	204	–	–	–	–	–	–	–
Soybean meal	–	–	200	–	–	–	430	200	200
Soy protein concentrates[1]	–								
Profine VF®	–		–	301	–	–	–	–	–
Profine F®	–		–	–	307	–	–	–	–
Soy flour[2], Nutrisoy®	–		–	–	–	387	–	–	–
Blood meal	–		–	–	–	–	–	106	–
Soybean oil	–	7	7	8	10	12	7	6	–
Menhaden oil	–	95		95	95	95	95	95	95
Canola oil	–		–	–	–	–	–	–	102
L-Lysine[2]	–	10.2	6.3	1.8	1.2	1.7	1.3	–	6.4
DL-Methionine[3]	–	1.7	1.1	2.7	2.7	2.2	2.4	3.1	1.1
Dicalcium phosphate	–	32.7	35.0	38.7	38.8	38.4	35.6	39.7	35.2
Vitamin premix[4]	–	3	3	3	3	3	3	3	3
Mineral premix[5]	–	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin C (stay-C, 15%)[6]	–	1	1	1	1	1	1	1	1
Choline chloride	–	7	7	7	7	7	7	7	7
Proximate composition (g kg^{-1} dry matter)									
Crude protein	514	399	391	378	394	380	385	380	394
Crude fat	181	118	115	105	99	113	110	106	117
Crude fibre	12	37	21	16	16	15	19	17	22
Ash	94	55	59	62	64	63	63	59	59
Moisture	77	75	79	87	80	90	88	86	82
Pellet density (kg L^{-1})	5.8	2.9	3.5	2.6	2.0	3.6	3.8	3.6	3.5

[1]Central Soya, Fort Wayne, IN, USA.
[2]ADM Bioproducts, Decatur, IL, USA.
[3]DL-2-amino-4-(methylthio)butyric acid; Rhône-Poulenc Inc., Cranbury, NJ, USA.
[4]US Fish and Wildlife Federal Vitamin Premix #30, provided the following (per kg diet): biotin 0.528 mg, folacin 13.2 mg, niacin 330 mg, pantothenic acid 158.4 mg, pyridoxine 46.2 mg, riboflavin 79.2 mg, thiamine 52.8 mg, vitamin A 9900 IU, vitamin B_{12} 0.033 mg, vitamin D_3 660 IU, vitamin E 528 IU, vitamin K 16.5 mg.
[5]US Fish and Wildlife Federal Mineral Premix #3, provided the following (per kg diet): calcium 0.037 g, copper 1540 mg, iodine 9994.6 mg, iron 600 mg, magnesium 22.17 mg, manganese 22900 mg, phosphorous 0.219 mg, potassium 1.292 mg, selenium 0.42 mg, sodium 0.5385 mg, sulphur 75.35 mg, zinc 75000 mg.
[6]L-ascorbyl-2-polyphosphate; Hoffman-La Roche Inc., Nutley, NJ, USA.

August 1991 and August 1996 (Feedstuffs, Minnetonka, MN, USA). The ingredient cost of a standard commercial trout diet was also calculated. That diet contained 300 g kg^{-1} fish meal, 250 g kg^{-1} corn grain, 200 g kg^{-1} soybean meal, 50 g kg^{-1} corn gluten feed, 100 g kg^{-1} corn gluten meal, 80 g kg^{-1} menhaden oil, a trace mineral and vitamin premix and met the same nutrient restrictions as the experimental diets. The control diet used in the study was a commercial salmon diet, which contained no soybean meal (Nelson & Sons, Inc., Murray, UT, USA).

Proximate analysis of feeds was performed by the USDA National Centre for Agricultural Utilization Research, Peoria, IL, USA. Moisture, nitrogen, crude fat, ash, and crude fibre were determined by AACC methods (1983). Moisture was determined by drying at 135°C for 2 h, nitrogen by micro-Kjeldahl, crude fat by petroleum ether extraction, ash by heating samples at 600°C for 2 h, and crude fibre by digestion with 26 mM H_2SO_4 and 31 mM NaOH and measuring loss on ignition of the dried residue.

Growth and tissue analysis

Percentage growth ((final weight – initial weight)/initial weight), feed conversion ratio (FCR, dry feed intake/wet weight gain), and protein efficiency ratio (PER, wet weight gain/protein intake) were calculated for each replicate at the end of the study. Immediately following the final weigh-out, two midsection muscle samples were removed bilaterally from three fish per tank for proximate composition analysis. The data were statistically analysed on a wet-weight basis. Nitrogen, ash and moisture were determined by AOAC methods (1990). Moisture content was determined by drying samples overnight at 100°C. Dried samples were ground and crude protein was determined by micro-Kjeldahl analysis. Ash was determined by heating samples at 600°C overnight. Livers were removed from six fish per tank for determination of hepatosomatic index (HSI, 100 × liver weight/body weight). Three livers per tank were weighed and dried at 100°C overnight for determination of moisture content. Crude fat was measured in fillet and liver samples by extraction with chloroform and methanol (Folch *et al.* 1957). Percentage dressout (dressout weight/whole wet weight × 100) was determined by removing viscera, and gills from three fish per tank.

Three fish from each treatment were necropsied and examined for gross abnormalities. The gastrointestinal tract (flushed with buffered 100 g L^{-1} formalin) and sections of spleen, kidney, liver, and gill were immersed in buffered 100 g L^{-1} formalin. Seven sections of gastrointestinal tract (proximal stomach, pylorus, pyloric caeca (with accompanying pancreatic tissue), proximal, mid and distal intestine, and rectum) along with one section of spleen, kidney and liver were embedded in paraffin, sectioned at 7 μm and stained with haematoxylin and eosin using standard histological procedures (Sheehan & Hrapchak 1980). Sections of gastrointestinal tract were evaluated, by comparing with experimental controls, for the presence of inflammation and alterations in the architecture of the mucosa and eosinophilic granular layer. The spleen and kidney were examined for differences in haematological activity. The amount of lipidosis present in hepatic sections was graded on a scale of 0–10 (0 = no lipidosis and 10 = completely affected) and compared.

Sensory analysis

Fillets from the remaining fish were submitted for sensory analysis by a 10-member panel trained and experienced in evaluating fish. Fish fillets were transported on ice to the USDA National Centre for Agricultural Utilization Research, Peoria, IL and stored at – 10°C for 3 weeks. Nine flavour descriptors were used to evaluate each sample, including four positive flavours (sweet, nutty, buttery and chicken) and five negative flavours (fishy, cardboard, cereal, grassy and stale). These descriptors were modifications of those described by Johnson *et al.* (1987). Flavour intensity was based on a 0–10 scale with 0 representing no flavour and 10 representing a strong flavour. Fish were removed from frozen storage 2.5 h before analysis. One hour before analysis, 150 g of fish from each treatment were weighed and placed in a 25.4 cm × 40.6 cm oven bag (Reynolds Metals Company, Richmond, VA, USA). Slits, 2.5 cm long, were cut in the top of each bag; these were then closed with a plastic tie and placed into a 22.9 × 33.0 × 5.1 cm pan. Fish were cooked at 175°C for 15 min. As each panellist arrived, 10 g of fish was placed into a 50 mL glass beaker, covered with aluminium foil, placed on a heated aluminium block to maintain the temperature of the sample and presented to individual panellists. Testing was conducted in a temperature-controlled panel room with red lights to mask colour differences. Panellists rinsed their mouths before tasting and between samples with carbon-filtered tap water at 38°C.

Statistical analysis

Data from the feeding trial were analysed with SAS (1979) as a completely randomized design with each aquarium as the experimental unit. Nested analysis was used when individual fish were analysed (e.g. HSI). If ANOVA indicated significant

differences, a Student–Newman–Keuls test was performed to separate mean values. Sensory data were analysed by two-way ANOVA and least significant difference (LSD). Accepted level of significance was $P < 0.05$.

Results

Fish fed the commercial diet exhibited the highest weight gain and lowest FCR (Table 2). Within the experimental diets, fish fed the soy flour diet (SF) and the 400 g kg^{-1} soybean meal diet (SB40) had significantly higher weight gains and significantly lower FCRs than fish fed other diets. Weight gains of fish fed the remaining diets were not significantly different from each other, but the FCR of fish fed diets containing low-antigen soy protein concentrate (SPC1) and 200 g kg^{-1} soybean meal (SB20) were significantly lower than fish fed the diets containing canola oil (SB20c), peanut meal (PM) and the standard soy protein concentrate (SPC2). Feed consumption was significantly higher in fish fed the control, SF and SB40 diets. Consumptions of the remaining diets were not significantly different. Fish fed the SF and SB40 diets exhibited the highest overall PER. The PER of fish fed the control diet was among the lower values. Dressout percentage did not differ significantly between treatments (81–83%).

Table 2 Mean percentage weight gain, total feed consumption, feed conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) of rainbow trout fed diets free of fish meal[1]

Dietary treatment	Weight gain (%)	Total consumption (kg)	FCR[2]	PER[3]	HSI[4]
Control	322.4a	1.22a	0.89a	2.18cde	1.19a
SF	276.3b	1.20a	1.02b	2.58a	1.35ab
SB40	267.8b	1.17a	1.03b	2.52a	1.50bc
SB20	215.0c	1.03b	1.13c	2.28bc	1.68c
SPC1	212.3c	1.00b	1.11c	2.39b	1.58bc
BM	186.5c	0.94b	1.18cb	2.23cd	1.54bc
SPC2	184.8c	0.95b	1.22d	2.08de	1.71c
PM	184.3c	0.94b	1.21d	2.07de	1.70c
SB40c	164.9c	0.88b	1.25d	2.03e	1.60bc
Pooled SEM[5]	0.8004	0.0030	0.0013	0.0025	0.0073

[1]Means of three replicate groups of fish, initial weight of fish = 35 g. Values with the same letters in the same column were not significantly different ($P < 0.05$), as determined by ANOVA and Student–Newman–Keuls test.
[2]Feed conversion ratio = dry feed intake/wet weight gain.
[3]Protein efficiency ratio = wet weight gain/protein intake.
[4]Hepatosomatic index = 100 × (liver weight/body weight).
[5]Pooled standard error of the mean.

Table 3 Mean proximate composition of muscle (crude protein, fat, ash, moisture) from rainbow trout fed various experimental diets. All values are expressed as g kg^{-1} as is basis[1]

Diet	Crude protein	Fat	Ash	Moisture
BM	197a	24a	15	769
Control	192a	28ab	14	763
SF	185ab	36abc	14	773
SPC1	181ab	33abc	15	771
SPC2	181ab	35abc	14	774
SB40	179ab	43c	14	765
SB20c	179ab	34abc	14	767
SB20	178ab	37abc	14	761
PM	169b	42bc	14	771
Pooled SEM[2]	0.0261	0.0190	0.0037	0.0210

[1]Means of nine fish from replicate treatment groups. Values with the same letters in the same column were not significantly different ($P < 0.05$), as determined by ANOVA and Student–Newman–Keuls test.
[2]Pooled standad error of the mean.

Muscle protein concentration was highest in fish fed the 200 g kg^{-1} soybean meal/blood meal diet (BM) and the commercial diet, and lowest in fish fed the peanut meal diet (PM, Table 3). The lowest muscle lipid concentrations were in fish fed diet BM and the commercial diet and highest in fish fed diets SB40 and PM. Muscle moisture content and muscle ash content were not significantly different between treatment groups.

HSIs were significantly different between treatments (Table 2), although there was considerable variation within each treatment. Fish fed the commercial diet had the lowest HSI values, followed by those fed diet SF. Microscopic evaluation of livers also revealed considerable individual variation with no apparent differences between treatments. There were no significant differences in moisture or lipid concentrations of liver samples and average treatment values ranged from 751 to 756 g kg^{-1} and from 36 to 51 g kg^{-1}, respectively.

No gross lesions were observed and no microscopic differences were found between treatment groups in sections of spleen, kidney, pancreas, skin, or gill tissue. The amount of hepatic lipidosis present varied greatly between individual fish but no treatment group effect was identified. No inflammatory infiltrates were found in any of the gastrointestinal sections.

The taste test revealed significant differences in the flavours of fillets (Table 4). No differences were noted in the flavour descriptors nutty, buttery, or grassy. Fish fed most dietary treatments exhibited flavours similar to fish fed the commercial diet; however, fish fed the canola oil diet (SB20c) had the lowest fishy flavour score. Fish fed that diet also had a greater chicken flavour intensity than fish fed the commercial

Table 4 Flavour intensity scores of rainbow trout fillets fed the control and experimental diets (0–10, none to strong)[1]

	Positive flavours					Negative flavours				
Diet	Sweet	Nutty	Buttery	Chicken	Fishy	Cardboard	Cereal	Grassy	Stale	Other
Control	2.23ab	1.83a	0.72a	1.44a	3.10b	1.18ab	0.82ab	0.24a	0.87ab	0.58a
PM	2.56b	2.12a	0.97a	1.38a	3.14b	0.70a	0.51a	0.31a	0.52a	0.45a
SBM20	1.77a	1.56a	0.76a	1.04a	3.76c	0.71a	0.83ab	0.14a	1.34b	0.82a
SPC1	2.37ab	1.78a	0.80a	1.44ab	3.23bc	1.08ab	0.98ab	0.41a	0.72a	0.99a
SPC2	1.80a	1.75a	0.69a	1.19a	3.76c	0.95ab	0.46ab	0.32a	1.15ab	1.15a
SF	2.43ab	2.09a	0.99a	1.45ab	2.99b	0.64a	0.89ab	0.42a	1.33b	0.92a
SBM40	1.84a	1.84a	0.69a	1.08a	3.68c	0.93ab	1.01ab	0.48a	1.41b	1.10a
BM	2.28ab	2.14a	0.65a	1.45ab	3.43bc	1.07ab	0.82ab	0.18a	0.80ab	0.95a
SBM20c	1.72a	1.79a	0.76a	2.03b	2.13a	1.47b	1.39b	0.34a	0.63a	0.79a

[1]Values with the same letters in the same column were not significantly different ($P < 0.05$), as determined by ANOVA and Student–Newman–Keuls test.

Table 5 Cost analysis of experimental feed ingredients fed to rainbow trout[1]

Dietary treatment	Ingredient cost ($/ton ingredients)	Ingredient cost/gain ($ ingredient cost/ton gain)
Control	340.93	303.43
SB40	305.45	314.61
SB20	315.88	346.55
SF	385.57	393.28
SB40C	315.81	394.76
PM	334.24	404.43
BM	365.94	431.81
SPC1	550.19	610.71
SPC2	535.64	648.13

[1]Based on ingredient costs for a practical diet containing 300 g kg^{-1} menhaden fish meal, 200 g kg^{-1} soybean meal, 100g kg^{-1} corn gluten meal and a trace mineral and vitamin premix.

diet, PM, SB20, SB40 or SPC2. Diets SBM20, SPC2, and SB40 exhibited a statistically higher fishy flavour compared with fish fed the commercial diet. It was also noted that fish fed the experimental diets exhibited distinct yellow pigmentation of the skin and flesh. The degree of coloration was not quantified.

Cost analysis (Table 5) revealed that, while several experimental diets were less costly than a standard trout diet, the cost per unit gain of growth was least for the control group. Of the experimental treatments, diet SB40 exhibited the lowest cost per unit gain, which was \$11 ton^{-1} more than the commercial treatment.

Discussion

The relatively high weight gain and low feed conversion of fish fed SB40 and SF diets are consistent with findings in similar studies. Smith *et al.* (1988) evaluated the growth of 10 different strains of rainbow trout fed a diet that contained only 70 g kg^{-1} fish meal and observed little or no significant difference in growth rate and feed efficiency compared with those fed a 300 g kg^{-1} fish meal diet. Gomes *et al.* (1995) reported significantly lower weight gain and FCR in fish fed a diet that completely lacked fish meal and contained primarily full-fat soybean meal and corn gluten meal. While those studies and the present study continue to find a growth reduction in rainbow trout when fed fish-meal free diets, they do represent advancements in the replacement of fish meal with low-cost agricultural byproducts. For example, if fish meal prices rise, least-cost feed formulations, using those formulae identified in this and previous studies may become important. Further, these formulae serve as the basis for further development of fish-meal free diets for trout.

Fish fed the SB40 diet exhibited higher weight gains and lower FCRs than those fed the SB20 diet and those fed the soy protein concentrate diets (SPC1 and SPC2). The reason for this remains unclear but this is similar to the data of Ketola *et al.* (1993) who reported that weight gain of rainbow trout fed a similar formulation was almost 90% of fish fed a commercial diet. Further, the feed conversion ratio was 1.02 in coho salmon fed diets containing 410 g kg^{-1} soybean meal and 300 g kg^{-1} corn gluten meal (Ketola *et al.* 1993). Soybean meal contains higher levels of antinutritional factors than soy protein concentrates (SPCs) (Cheeke & Shull 1985), which are produced by extracting soybean meal with hot alcohol or aqueous acid. Additional processing steps further reduce the effects of highly stable allergenic soy proteins. Adverse reactions by salmonids to dietary soy products can be ameliorated by replacing soybean meal with soy protein concentrates (Rumsey *et al.* 1993, 1994; Olli & Krogdahl 1994; Brown *et al.* 1997). Reactions to selected soy products in previous studies included diarrhoea, growth reduction, intestinal lesions and other changes to the architecture of the gastrointestinal tract. No such gastrointestinal alterations were observed in the present study. However, the primary soy product eliciting these adverse responses have been

full-fat products (van den Ingh *et al*. 1991), not processed soybean meal or further processed concentrates or flours.

In the present study, the lower fishy flavour and lower HSI in fish fed the SF diet compared with those fed SB40 may be attributed to the different soy products used in these diets. The soy flour was heat processed a second time and contained 80 g kg^{-1} lecithin (phosphatidylcholine). The higher protein efficiency ratio of fish fed the SF and SB40 compared with the control is probably a factor of the high protein content of the control. Increasing the protein level in rainbow trout diets improves growth rates but reduces protein efficiency (Steffens 1981; Pongmaneerat & Watanabe 1993).

Sensory analysis revealed that the feed ingredients affect flavours in the fillets. Varying results have been reported regarding the impact of dietary ingredients on fish flavour. Smith *et al*. (1988) found no significant difference in flavours of rainbow trout when fed a diet that contained full-fat soybean meal and soybean oil. Boggio *et al*. (1985) also found no significant effect on flavour of rainbow trout fed diets that contained herring oil compared with a lipid source from swine. Thomassen & Røsjø (1989) identified milder flavours and odours in Atlantic salmon fed canola oil, while others have found no effect in Atlantic salmon fed canola oil (Koshio *et al*. 1994) or soybean oil and tallow (Hardy *et al*. 1987). However, Atlantic salmon are a relatively strong flavoured fish, which may make it more difficult to distinguish subtle flavour differences (Koshio *et al*. 1994). Catfish have a milder-tasting fillet and studies have shown that flavour in catfish is affected by dietary lipids (Lovell 1988; Johnsen & Dupree 1991; Eun 1993), although Morris *et al*. (1995) found no effect. The range of flavours in the present study suggest that diets that contain no fish meal can be used to produce traditional flavours in rainbow trout or milder flavours, depending on the ingredient composition of the diet. The observed yellowing of the fillet in fish fed the experimental diets was evidently caused by the xanthophyll content of the corn products (Schiedt *et al*. 1985; Hatlen *et al*. 1992); however, the extent of coloration was not measured in this study.

Although many plant byproducts are less expensive than fish meal, at high levels of incorporation several nutrients must be added again to the diet which results in additional costs. The plant byproducts used here had low levels of lipid, phosphorus, essential amino acids and minerals. In the SB40 diet, approximately \$30.00 ton^{-1} of nutrients were added to the diets that would have been supplied by fish meal. Partial incorporation of ingredients that contain higher levels of fat (e.g. full-fat oilseeds) or phosphorous (e.g. meat and bone meals) may increase the cost effectiveness of such diets.

In the present study, we observed favourable growth in rainbow trout fed diets that completely lacked fish meal. Additional research is needed to improve the quality of diets high in agricultural byproducts before they are to be cost effective. However, several formulae were identified in this study that might prove beneficial as prices for feedstuffs inevitably vary.

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