Canola protein concentrate as a feed ingredient for salmonid fish

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Running Title: Canola Protein Concentrate For Salmonids

Introduction

Historically fishmeal has been the most important protein source in commercial aquatic feeds. However, annual growth of greater than 10% per year has put increasing pressure on fish meal supplies. Currently world production of fishmeal is approximately 6 to 7 million tonnes per year and this level of production is expected to remain stable over the next 10 years (New, 1999). Since the supply of feed grade fish is limited, expansion of aquaculture production by 300% over the decade 2000-2010 (FAO 1995) will be dependent on lowering the inclusion rate of fishmeal in aqua feeds and replacing it with plant based protein sources.

Total replacement of fishmeal with plant proteins has proven difficult. Soybeans are the most commonly used plant protein source in the world accounting for approximately 75% of all plant and animal protein used in animal agriculture (Rumsey et al., 1994) and they are expected to be a major feed ingredient in aquaculture diets. For this reason they have been widely tested in diets for salmonids. However, the use of soybean meal in salmonid diets is limited by two problems: 1) the high protein level required in salmonid diets and d and 2) reduced feed intake when more than 25-30% of fish meal is replaced with soybean meal.

Murray D. Drew. 2004. Canola protein concentrate as a feed ingredient for salmonid fish. In: Cruz Suárez, L.E., Ricque Marie, D., Nieto López, M.G., Villarreal, D., Scholz, U. y González, M. 2004. Avances en Nutrición Acuícola VII. Memorias del VII Simposium Internacional de Nutrición Acuícola. 16-19 Noviembre, 2004. Hermosillo, Sonora, México

Soybean meal as an ingredient for salmonid fish

Salmonid fish are carnivorous and have nutrient requirements that are significantly different than terrestrial livestock species. Current recommendations for grow-out salmon diets are a digestible protein level of 35-45% (Cho and Cowey, 1991). Fish meal provides a palatable, nutrient dense source of protein containing from 60-75% crude protein with an amino acid balance that closely meets the requirements of the fish (Wilson, 1989; Sikorski et al., 1990). In contrast, the most commonly used protein source used in diets fed to terrestrial animals, soybean meal, contains only 48% crude protein making formulation of diets containing 45% crude protein difficult. The other major plant protein source used in aquaculture is corn gluten meal which contains approximately 60% crude protein. However, the use of corn gluten meal in fish diets is usually limited to less than 20% due to fears of undesirable pigmentation due to the high levels of xanthophylls in this product. Clearly there is a need for alternative plant proteins containing greater than 60% crude protein.

The second problem associated with the replacement of fish meal with soybean meal is a decrease in feed intake at high replacement levels. Numerous studies have shown that soybean meal can successfully replace up to 20-30% of fish meal protein with no negative effects on salmonid performance (Smith, 1977; Tacon et al., 1983; Smith et al., 1988, Pongmaneerrat and Watanabe, 1992, Refstie et al., 1998). However, at higher levels, fish growth performance and efficiency are decreased and intestinal pathology described as a non-infectious sub acute enteritis in the distal intestine occurs. (Baeverfjord and Krogdahl, 1996). Soybean meal contains a number of anti-nutritional factors which can be broken down into heat labile and heat stable factors. The heat labile factors, including trypsin inhibitor, lectins, goitrogens and anti-vitamins can be eliminated or reduced by a heat treatment such as extrusion during feed processing (Smith, 1977; Arndt et al., 1999). However, even after heat treatment, the growth performance and intestinal physiology of fish fed soybeans is adversely affected (Hendriks et al., 1990; Rumsey et al, 1993). Heat stable anti-nutritional factors include oligosaccharides, fibre, saponins phytates and protein antigens. Purification of soybean protein using ethanol extraction to form soy protein concentrate (SPC approximately 70% protein) or soy protein isolate (SPI approximately 90% protein) reduces the oligosaccharide, fibre and saponin content of soybean meal (Bureau et al., 1998). As a result of this processing, SPC and SPI have been shown to have fewer negative effects on the growth and physiology of salmonids than soybean meal, however, at high dietary inclusion rates, they still cause significant reduction in growth rate, intestinal morphology and immune status (Rumsey et al., 1994). This effect appears to be related to the antigenic properties of soybean protein.

Several papers have reported that soybean proteins cause hypersensitivity reactions in salmonids. Rumsey et al., (1994) fed rainbow trout, high antigen soybean meal or low antigen soy protein concentrate completely replacing fish meal. The β-congylcinin antigenicity index was 153 x 10^{-3} for the soybean protein concentrate compared to 800 x 10^{-3} units for soybean meal. After 26 weeks on these diets, the total weight gain for fish fed the soybean meal was 14 g compared to 43 g for fish fed soy protein isolate and 124 g for controls that were fed diets containing fish meal as the main protein source. Fish fed low antigenic soybean protein isolate also had significantly better feed efficiency and protein utilization ratios compared to those fed soybean meal.

Burrells et al., (1999) fed levels of soybean meal of from 0 to 89% in the diets of rainbow trout. Levels of 60-70% soybean meal caused a loss of integrity of the villus tips and increased inflammatory cell infiltration into the lamina propria. When dietary concentrations of soybean meal were increased to 80-89%, there was sloughing of the mucosa into intestinal lumen. In another study, soybean meal caused enteritis like changes in distal intestine and increased levels of lysozyme and IgM in mid and distal intestinal mucosa compared to SPC (Krogdahl et al., 2000).

The hindgut of the fish is sensitive to antigen stimulation and strong immune responses are achieved with antigens delivered to this site (Ellis 1995). Serum immunoglobulin levels are increased significantly in fish fed antigenic soybean meal, however, the specificity of the serum immunoglobulins is not directed against soy proteins (Burrels et al., 1999).

Processing of soybean meal to SPC by ethanol extraction has been shown to reduce antigenicity (Hancock et al., 1990). Feeding rainbow trout low antigenicity SPC significantly improves growth and feed efficiency (Adelizi et al., 1998; Mambrini et al., 1999). However, SPC still retains significant antigenicity and replacing more than 30% of the dietary protein with SPC reduces growth and feed efficiency.

Based on literature, soybean meal and soy protein concentrates are limited to replacing a maximum of 20-30% of fish meal in salmonid diets. A combination of the commonly used plant proteins, soybean and corn gluten meal can therefore replace only about 50% of fish meal. To achieve higher levels of replacement, clearly other plant proteins are required. Canola protein concentrate may be the ingredient that will fill this gap.

Canola meal as a protein source for salmonids

Canola/rapeseed ranks second behind soybeans in the global production of protein from oil cakes and meals. Canola is the name given to selected varieties of rapeseed that are low in glucosinolate (<30 μmoles of alkenyl glucosinolates per gram of oil-free dry matter of seed) and erucic acid (<2% of total fatty acids in the oil) (Bell 1993). In Canada, parts of Europe and Asia, canola has been of extensive interest as a protein source for animal feeds. The cost of canola protein is approximately half of that of fish meal per kg of protein basis and it has an excellent profile of essential amino acids. Canola storage proteins differ markedly from soybean proteins. Napin is the major storage protein in canola and is composed of 2 polypeptide chains with a total molecular weight of 13 Kd. Proteins with such small molecular weights are generally poor antigens. In support of this, there are no reports in the literature that canola protein causes hypersensitivity reactions when fed in animal diets. The low molecular weight of napin reduces its antigenicity after feeding compared to compare to the high molecular weight soybean proteins glycinin and β-conglycinin.

In addition to low antigenicity, canola protein has extremely high biological value compared to other protein sources. SPC has a protein efficiency ratio (PER) value of 1.60 compared to canola protein with a PER value of 3.29 (Friedman, 1996). In fact, canola protein has a PER value of similar to minced beef and bovine casein with PER values of 3.36 and 3.13 respectively. The combination of low antigenicity and high PER value make canola protein unique among plant proteins.

However, like soybean meal, canola meal contains a host of antinutritional factors including phytic acid, glucosinolates, phenolic compounds and particularly insoluble and soluble fibre. Canola meal contains approximately 14.5% cellulose, 5.0% hemicellulose and 8.3% lignin (Mwachireya et al. 1999). These chemical fractions can not be used by salmonid fish and may diminish the nutritional value of other dietary ingredients. The phytic acid content of canola meal ranges from 3.1– 3.7% (Higgs et al. 1995) and significantly reduces phosphorus digestibility, protein utilization and growth (Spinelli et al. 1983, Forster et al. 1999). An increased incidence of cataracts and structural changes in the pyloric ceca of rainbow trout have also been attributed to elevated levels of phytic acid (Higgs et al. 1995). While glucosinolate levels are considerably reduced in contemporary commercial varieties of canola, there is still concern over the effect this factor has on thyroid function, feed acceptance, liver and kidney function and growth performance in fish (Hardy and Sullivan 1983, Higgs et al. 1983, Hilton and Slinger 1986, Leatherland and Hilton 1988, McCurdy and March 1992). These antinutritional factors prevent the use of, canola meal at inclusion levels over approximately 10% of the diet. The solution to improving the nutrient utilization of canola meal is to produce canola protein concentrate (CPC) by aqueous extraction of protein. This process removes most of the antinutritional factors resulting in an excellent protein source for salmonids.

The high biological value of CPC has been confirmed in feeding trials with salmonids (Higgs et al. 1994, Mwachireya et al. 1999). Mwachireya et al., 1999 fed canola protein concentrate to rainbow trout and found that its apparent protein digestibility coefficient was 97.6%; the highest for any protein source ever tested including fish meal. In addition, an increase in the level of canola protein concentrate in the diet, with a relative decrease in the level of fish meal, led to an increase in protein digestibility of the diet, suggesting that canola protein was more digestible than fish meal protein (Stickney et al. 1996, Forster et al. 1999). Inclusion of an attractant into an enzyme dephytinized rapeseed protein concentrate diet allowed for replacement of 100% of the dietary protein derived from fish meal without a reduction in performance of trout (Higgs et al. 1995). Based on these observations, canola protein concentrate appears to be an extremely desirable ingredient for salmonid diets.

Studies on the Nutritional Value Commercial CPC in Rainbow Trout

Recently, CPC has become available in pre-commercial quantities for testing from MCN Bioproducts Ltd. located in Saskatoon SK. Our laboratory has performed a series of experiments to characterize the nutritional value of this product in rainbow trout. The chemical composition of the CPC used in these studies is shown in Table 1. The CPC used in these trials contains approximately the crude protein level as fish meal (South American super prime) and high levels of lysine and methionine relative to corn gluten and soybean meal. Phytate and glucosinolates are not concentrated during processing with the result that CPC is completely devoid of phytate and contains extremely low levels of glucosinolates. The nutrient digestibility of CPC and other commonly used protein sources is shown in Table 2. The crude protein digestibility was comparable among ingredients with the digestibility of key amino acids (lysine, methionine and arginine) greater than 90%. Apparent digestible energy of CPC was 4310 kcal/kg compared to 3360 for soybean meal.

Table 1. Chemical analysis of canola protein concentrate and other commonly used protein sources in salmonid feeds.

Fraction (%)	Fish meal	Corn gluten meal	Soybean meal	CPC
Crude protein	68.2	63.1	48.0	69.2
Lysine	5.46	1.02	3.02	3.55
Methionine	2.04	1.43	0.67	1.50
Arginine	4.01	1.93	3.48	4.56
Fat	7.93	0.96	1.33	0
Crude Fibre	0	0.52	2.15	2.83
Antinutritional Factors				
Phytate	0	1.2	1.7	0

Glucosinolates (umol/g)	0	0	0	3.44
Protein Antigenicity	Low	High	High	Low

Table 2. Nutrient digestibility and digestible energy contents of canola protein concentrate and other commonly used protein sources in rainbow trout. Values are apparent digestibility coefficients (%).

	Fish meal	Corn gluten meal	Soybean meal	CPC
Crude protein	89.6	91.1	95.0	89.9
Amino Acids				
Lysine	96.3	89.8	97.4	93.5
Methionine	94.9	95.3	98.4	95.4
Arginine	93.2	93.2	96.8	95.4
Energy				
Apparent Digestible Energy (kcal/kg)	4,550	5,050	3,360	4,310

An initial experiment was performed to examine the effect of 100% replacement of fish meal with CPC on the growth performance and feed intake of rainbow trout over a 12 week period (Drew and Thiessen, unpublished data). The diets fed in the trial (Table 3) were balanced for digestible crude protein and energy. The diets were cold extruded (3.0 mm die), dried in a forced air oven (55°C, 24 h), chopped and screened to obtain the appropriate size pellet. A total of 240 rainbow trout (Cangro Processors Ltd.; Lucky Lake, SK, Canada) weighing 13.4 g on average were randomly assigned to 12 tanks (20 fish per tank). Six replicates were used for each diet. The fish were maintained in 360 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 14 ± 1 ^{o}C and dissolved oxygen, nitrate, nitrite; ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. The fish were fed by hand to satiation twice daily. Daily feed intake and the total tank weight of the fish at 14 day intervals were recorded for the duration of the experiment. Growth performance was determined by weight gain (g/fish), total feed intake (g/12 weeks), feed conversion ratio (feed intake/wet weight gain) and protein efficiency ratio (PER) (wet weight gain/protein intake). Statistical analysis of the results was done using the General Linear Model procedure of SAS (SAS Version 8.2).

Table 3. Diets fed to determine the effect of replacing 100% of fish meal with canola protein concentrate.

	Fish Meal	**CPC**
Ingredient (g/kg)		
Fish meal	625	0
Wheat flour	155	64
Canola Protein Concentrate (CPC)	0	650
Whey	70	70
Fish oil	140	198
Lysine	0	8
Choline	5	5
Vitamin premix	2.5	2.5
Mineral premix	2.5	2.5
Total	1000	1000
Calculated Analysis		
Dig. Prot. G/kg diet (DM)	477.3	479.1
Dig. Energy MJ/kg diet (DM)	20.2	21.4
Dig. Protein/Dig. Energy g/MJ	23.6	22.3

The results of the experiment are shown in Table 4. Fish fed the CPC diet had significantly reduced feed intakes and growth rates compared to fish fed the fish meal diet ($P < 0.05$). However, feed efficiency and PER values were not significantly different between the diets. This suggests that although the palatability of the diets are reduced when CPC completely replaces fish meal, the inclusion of high levels of CPC does not impair the ability of rainbow trout to digest and absorb protein and energy from the diet. In contrast, Rumsey et al., (1994) fed diets where fish meal was replaced completely by either low-antigenicity soy protein concentrate or soybean meal. The SPC-fed fish gained 43 g compared to 124 g for the fish meal controls and feed: gain ratio was significantly higher for the SPC diets compared to the controls (1.3 vs 0.9 respectively).

Table 4. Growth performance of rainbow trout fed diets shown in Table 3 over a 12 week growth period.

	Fish meal	CPC
Feed intake (g/fish)	111.3^{a}	87.3^{b}
Weight gain (g/fish)	107.9^{a}	84.0^{b}
FCR[2] (g feed:g gain)	1.03^{a}	1.04^{a}
PER[3] (g gain: g protein Intake)	1.97^{a}	1.88^{a}

[ab]Means within rows with the same superscript are not significantly different (P < 0.05).

A subsequent experiment (Thiessen et al., 2004) was performed to determine whether the effect of appetite suppression at high inclusion levels of CPC could be overcome by the addition of a palatability enhancer (Finnstim™; Finnsugar Bioproducts, Helsinki, Finland). The diets used in the study are shown in Table 5. Diets were balanced for digestible protein, lysine and energy. The control diet contained fish meal as the primary protein source while the test diets contained CPC replacing 50% and 75% of fish meal protein in the control diet with and without the addition of 10 g/kg of a commercial palatability enhancer (Finnstim™; Finnsugar Bioproducts, Helsinki, Finland). The diets were cold extruded and screened to the appropriate pellet size. The experimental period was 63 days and used a total of 500 rainbow trout weighing 28 ± 3 g randomly assigned to 20 tanks (25 fish per tank). Four tanks were used for each diet. The total tank weight of the fish was recorded at 21 day intervals for the duration of the experiments. Feed intake was recorded daily. Performance was determined by weight gain (g/ fish), total feed intake (g/fish/63 days), feed conversion ratio (feed intake/wet weight gain) and protein efficiency ratio (wet weight gain/protein intake).

Table 5. Diets fed in trial to examine the effect of feeding high levels of CPC with and with out an attractant (Finnstim).

Ingredient (g/kg)	Fish meal control diet	CPC 50%[1]	CPC 75%[1]	CPC 50% + attractant	CPC 75% + attractant
Fishmeal	625	310	155	310	155
Wheat flour	155	105	87	95	77
CanPro-IP65	0	330	490	330	490
Whey	70	70	70	70	70
Attractant	0	0	0	10	10
Fish oil	140	170	180	170	180
Lysine	0	5	8	5	8
Choline	5	5	5	5	5
Vitamin premix	2.5	2.5	2.5	2.5	2.5
Mineral premix	2.5	2.5	2.5	2.5	2.5

There were no significant differences in any of the performance measures. However, there was a trend to lower growth as the amount of CPC in the diet increased and the addition of the attractant at the 75% CPC inclusion level resulted in an increase in feed intake and growth. Feed efficiency and PER values were of the control and the 75% CPC + attractant diet were essentially identical over the 63 day period of the experiment. These results confirm that CPC can replace up to 75% of fish meal protein with no significant decrease in growth or feed efficiency.

Commercially, CPC is likely to be used in diets with several plant protein sources including soybean meal and corn gluten meal, at inclusion rates under 20%. Therefore, 5 diets were formulated (Table 7) to examine the effect of adding 0, 6, 12 or 18% CPC (replacing 10, 20 and 30% of fish meal protein) to commercial-type diets containing 10% soybean meal and 17% corn gluten meal on the growth performance of rainbow trout. The diets were formulated to contain equal digestible crude protein, lysine and digestible energy levels. A fifth diet was formulated by adding an attractant (Finnstim) to the diet containing 18% CPC. Rainbow trout were fed the experimental diets for a 63 day period and maintained as described above. A total of 250 rainbow trout weighing 178.7 g on average were randomly assigned to 25 tanks (10 fish per tank) with five tanks assigned to each diet.

Table 6. Performance of fish fed the diets shown in Table 5 over a 63 day period.

	Fish meal control diet	CPC 50%	CPC 75%	CPC 50% + attractant	CPC 75% + attractant
Feed intake g/fish	89.8[a]	82.7[a]	81.4[a]	83.4[a]	87.6[a]
Weight gain g/fish	84.7[a]	81.4[a]	72.7[a]	82.2[a]	80.9[a]
FCR g feed/ g gain	1.06[a]	1.01[a]	1.12[a]	1.02[a]	1.08[a]
PER g gain /g protein intake	1.81[a]	1.94[a]	1.73[a]	1.92[a]	1.81[a]

[a]Means within rows with the same superscript are not significantly different (P < 0.05).

Table 7. Diets used in trial studying the replacement of fish meal with CPC in commercial-type diets.

Ingredients (g/kg)	Control	6% CPC	12 % CPC	18% CPC	18% CPC + Finnstim
Fishmeal	390	330	276	219	219
Corn gluten meal	170	170	170	170	170
Wheat flour	190	189.99	178.98	170.97	160.98
CPC	0	60	120	180	180
Soybean meal	100	100	100	100	100
Finnstim	0	0	0	0	10
Fish oil	140	140	145	150	150
Lysine	0	0.01	0.02	0.03	0.02
Choline	5	5	5	5	5
Vitamin premix	2.5	2.5	2.5	2.5	2.5
Mineral premix	2.5	2.5	2.5	2.5	2.5
Total	1000	1000	1000	1000	1000

The results of the experiment are shown in Table 8. There were no significant differences in feed intake during the experiment ($P > 0.05$). However, feed intake tended to decrease with increasing inclusion rates of CPC and the addition of the attractant to the 18% CPC diet resulted in increased feed intake and growth. Fish weight gains were highest in the 6% CPC-fed group and were significantly reduced in the 18% CPC-fed fish. Feed efficiency and PER values were best for the 6% CPC diets and lowest for the 18% CPC diets. Taken together the results suggest that the inclusion of 6% CPC, replacing 10% of fish meal protein in a commercial-type diet fed to rainbow trout may improve their overall growth performance and feed intake. At levels higher than this, the addition of a palatability enhancer has a positive effect on feed intake and growth.

Table 8. Growth performance of fish fed diets shown in Table 7 over a 63 day growth period.

	Control	6% CPC	12% CPC	18% CPC	18% CPC + Attractant
Feed intake (g/fish)	275.4^{a}	285.8^{a}	258.6^{a}	253.5^{a}	264.8^{a}
Weight gain (g/fish)	236.7^{ab}	262.1^{a}	217.3^{b}	202.9^{b}	224.2^{b}
FCR (g feed/g gain)	1.17^{ab}	1.09^{b}	1.19^{ab}	1.26^{a}	1.19^{ab}
PER (g gain/g protein intake)	1.77^{ab}	1.87^{a}	1.70^{ab}	1.62^{b}	1.70^{ab}

^{ab}Means within rows with the same superscript are not significantly different ($P < 0.05$).

The replacement of the majority of fish meal in salmonid diets by plant proteins appears to be feasible by employing the strategy of formulating complex diets containing many protein ingredients at low inclusion rates. To be successful, this strategy will require the availability of new plant protein sources with high nutrient densities and low levels of antinutritional factors. Canola protein concentrate is a promising new ingredient for the aqua feed industry that will allow the use of lower levels of fish meal in salmonid diets.

References

Adelizi, P. D., Rasati, R. R., Warner, K., Wu Y. V., Muench, T. R., White, M. R., Brown, P. B., 1998. Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss.* Aquaculture Nutr. 4, 255-262.

Arndt, R. E., Hardy, R. W., Sugiura, S. H., Dong, F. M., 1999. Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho salmon, *Oncorhynchus kisutch*. Aquaculture 180, 129-145.

Baeverfjord, F., Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon *Salmo salar* L. distal intestine: a comparison with the intestine of fasted fish. J. Fish Dis. 19, 375-387.

Bell, J. M., 1993. Factors affecting the nutritional value of canola meal: a review. *Can. J. Anim. Sci.*, 73, 679-697.

Bureau, D. P., Harris, A. M., Cho. C. Y., 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon and rainbow trout. Aquaculture, 161, 27-43.

Burrells, C., Williams, P. D., Southgate, P. J., Crampton, V. O., 1999. Immunological, physiological and pathological responses of rainbow trout to increasing dietary concentrations of soybean proteins. Vet. Immunol. Immunopathol. 72, 277-288.

Cho, C. Y., Cowey, C. B., 1991. Rainbow trout, Oncorhynchus mykiss. In R.P. Wilson (ed.), Handbook of Nutrient Requirments of Finfish. CRC Press, Boca Raton, pp. 131-143.

Ellis, A. E., 1995. Recent development in oral vaccine delivery systems. Fish Pathol. 30, 293±300.

FAO. 1995. Aquaculture production statistics. Food and Agriculture program of the United Nations. Rome.

Forster, I., Higgs, D. A., Dosanjh, B. S., Rowshandeli, M., Parr, J., 1999. Potential for dietary phytase to improve the nutritive value of canola protein and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss)* held in 11^{o}C fresh water. Aquaculture 179, 109-125.

Friedman, M., 1996. Nutritional value of protein from different food sources. J. Agric. Food Chem. 44, 6-29.

Hancock, J. D., Peo, E. R., Lewis, A. J., Moxley, R. A., 1990. Effects of ethanol extraction and heat treatment of soybean flakes on function and morphology of pig intestine. J. Anim. Sci. 68, 3244-3251.

Hardy, R. W., Sullivan, C. V., 1983. Canola meal in rainbow trout (*Salmo gairdneri*) production diets. *Can. J. Fish. Aquat. Sci.*, 40, 281-286.

Higgs, D. A., Fagerlund, U. H. M., McBride, J. R., Plotnikoff, M. D., Dosanjh, B. S., Markert, J. R., Davidson, J., 1983. Protein quality of Altex canola meal for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) considering dietary protein and 3,5,3'-triiodo-L-thyronine content. *Aquaculture*, 34, 213-238.

Hendiks, H. G. C. J. M., van den Ingh, T. S. G. A. M, Krogdahl, A., Olli, J., Koninkx, J. F. J. G., 1990. Binding of soybean agglutinin to small intestinal brush border membranes and brush border membrane activities in Atlantic salmon (Salmo salar). Aquaculture 91, 163-170.

Higgs, D. A., Prendergast, A. F., Dosanjh, B. S., Beames, R. M., Deacon, G., Hardy R. W., 1994. Canola protein offers hope for efficient salmon production. In: *High Performance Fish* (Mackinlay, D.D. ed), pp. 377-382. Fish Physiology Association, Vancouver, BC.
Higgs, D. A., Dosanjh, B. S., Prendergast, A. F., Beames, R. M., Hardy, R. W., Riley, W., Deacon, G., 1995. Use of rapeseed/canola protein products in finfish diets. In. Nutrition and utilization of technology in aquaculture. D.J. Sessa (ed), pp. 130-156. AOCS Press, Champaign USA.
Hilton, J. W., Slinger, S. J., (1986) Digestibility and utilization of canola meal in practical-type diets for rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci*., 43, 1149-1155.
Krogdahl, A., Bakke-McKellep, A. M., Roed, K. H., Baeverfjord, G., 2000. Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (Furunculosis), and lysozyme and IgM levels in the intestinal mucosa. Aquaculture Nutrition 6, 77-84.
Leatherland J. F., Hilton, J. W. (1988) Thyroidal compensation in rainbow trout (*Salmo gairdneri*) fed canola meal. *Fish Phys. Biochem*., 5, 199-207.
McCurdy, S. M., March, B. E., 1992. Processing of canola meal for incorporation in trout and salmon diets. *J. Amer. Oil Chem. Soc*., 69, 213-220.
Mambrinin, M., Roem, A. J., Cravedi, J. P., Lalles, J. P., Kaushik, S. J., 1999. Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss.* J. Anim. Sci. 77, 2990-2999.
Mwachireya, S. A., Beames, R. M., Higgs, D. A., Dosanjh, B. S., 1999. Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in fresh water. Aquaculture Nutr. 5, 73-82.
New, M., 1999. Global Aquaculture: current trends and challenges for the 21st century. World Aquaculture 30, 8-13.
Pongmaneerat, J., Watanabe, T., 1992. Utilization of soybean meal as protein source in diets for rainbow trout. Nippon Suisan Gakkaishi 58, 1983-1990.
Refstie, S., Storebakken, T., Roem, A. J., 1998. Feed consumption and conversioin in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162;301-312.
Rumsey, G. L., Hughes, S. G., Winfree, R. A., 1993. Chemical and nutritional evaluation of soya protein preparations a s primary nitrogen sources for rainbow trout (*Oncorhynchus mykiss*). Anim. Feed Sci. Technol. 40, 135-142.
Rumsey, G. L., Siwicki, A. K., Anderson D. P., Bowser, P. R., 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth and protein utilization in rainbow trout. Vet. Immunol. Immunopathol. 41, 323-329.
Sikorski, A. E., Kolakowska, A., Pan, B. S., 1990. The nutritional composition of the major groups of marine organisms. In Seafood: Resources, nutritional composition and preservation. Pp. 30-54 Z.E. Sikorski ed. CRC Press, Boca Raton FL.
Smith, R. R., 1977. Recent research involving full-fat soybean meal in salmonid diets. Salmonid 1, 8-18.
Smith, R. R., Kincaid, H. L., Regenstein, J. M., Rumsey, G. L., 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture 70, 309-321.
Spinelli, J., Houle, C. R., Wekell, J. C., 1983. The effects of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. *Aquaculture*, 30, 71-83.
Tacon, A. G. J., Haastler, J. V., Featherstone, P. B., Kerr, K., Jackson, A. J., 1983. Studies on the utilization of full fat soybean and solvent extracted soybean in a complete diet for rainbow trout. Nippon Suisan Gakkaishi, 49, 1437-1443.
Thiessen, D. L., Maenz, D. D., Newkirk, R. W., Classen, H. L., Drew, M. D., 2004. Replacement of fish meal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition (in press)
Wilson, R. P., 1989. Amino acids and proteins. pp.111-151 in Fish Nutrition 2nd ed., J.E. Halver, ed., NewYork: Academic Press.