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# Evaluation of a canola protein concentrate as a replacement for fishmeal and poultry by-product meal in a commercial production diet for Atlantic salmon (*Salmo salar*)

Gary S Burr[1*], William R Wolters[1], Frederic T Barrows[2] and Alan W Donkin[3]

* Correspondence:
gary.burr@ars.usda.gov
[1]ARS National Cold Water Marine Aquaculture Center, USDA, 25 Salmon Farm Road, Franklin, ME 04634, USA
Full list of author information is available at the end of the article

## Abstract

Atlantic salmon (*Salmo salar*) is an important cultured carnivorous species that, in the past, has not tolerated high levels of most plant protein feed ingredients in their diet. In order to increase efficiency and production to meet global demand, new sources of protein must be incorporated into the aquafeeds. A 38-week feeding trial was conducted at the National Cold Water Marine Aquaculture Center (Franklin, ME, USA) with juvenile Atlantic salmon (133 g per fish, initial weight) to determine the effect of feeding graded levels of canola protein concentrate (CPC) in a commercial-type diet. A commercial diet (Signature Salmon 3.5 mm, Northeast Nutrition, Truro, Nova Scotia, Canada) was modified and analyzed by the manufacturer to contain 10% or 20% canola protein concentrate, replacing fishmeal and poultry by-product meal. Fish fed diet containing 20% canola protein concentrate had significantly lower growth compared with those fed 0% canola protein concentrate diet ($p = 0.04$). There was not any significant difference in feed efficiency ($p = 0.22$) or protein efficiency ratio ($p = 0.21$). There was not a significant difference in growth comparing the salmon fed the 0% CPC and the 10% CPC diets ($p > 0.05$). Canola protein concentrate significantly depressed growth when included in the diet at 20%, but not at 10%, indicating that canola may be used as a minor feed ingredient when available.

## Background

Atlantic salmon (*Salmo salar*) is an important cultured carnivorous species that, in the past, has not tolerated high levels of most plant protein feed ingredients in their diet Gatlin et al. (2007). The inclusion of plant protein sources in aquafeeds is expanding due to the limited amount of fishmeal available for the production of animal feeds Glencross et al. (2005; Gatlin et al. 2007). Juvenile Senegalese sole (*Solea senegalensis*) fed diets that contain 5% fishmeal or less have been shown to have comparable growth to fish fed a 37% fishmeal diet when amino acids were supplemented Silva et al. (2009). For inclusion in the diets of rainbow trout (*Oncorhynchus mykiss*), many different plant protein sources have been examined, including plant protein meals and plant protein concentrates Lim and Webster (Lim et al. 2008). Canola protein concentrate (CPC), soybean meal and soy protein concentrates, pea protein concentrate, barley protein concentrate, rice protein concentrate, and wheat gluten meal have all been tested as fishmeal replacements with varying degrees of success Forster et al. (1999; Thiessen et al. 2003; Barrows et al. 2007; Lim et al. 2008; Gaylord and Barrows 2009). Many of these same protein sources have been tested in Atlantic salmon (Lim et al. 2008). Atlantic salmon was shown to have slightly reduced, but not significantly lower, performance when fed diets that were fishmeal-free and contained wheat gluten, corn gluten, fish soluble, and crystalline amino acids (Espe et al. 2006). Atlantic salmon fed a diet that contained 12% fishmeal had slightly decreased growth but produced 2 kg of fish for every 1 kg of fishmeal consumed (Torstensen et al. 2008). Stickwater, the water-soluble fraction that is produced in making fishmeal, has been shown to stimulate the growth of Atlantic salmon when fed low fishmeal diets (10% of the diet) and high plant protein diets (62% of the diet) (Kousoulaki et al. 2009). Amino acid supplementation is often needed to maintain the growth performance of fish fed plant protein-based feeds (Espe et al. 2007; Lim et al. 2008; Gaylord and Barrows 2009). A major challenge is to increase the amount of plant or other non-animal protein in the diet of carnivorous fishes while maintaining acceptable growth, feed conversion, and production costs compared to traditional diets.

Recently, multiple studies have been reported on the digestibility of various plant feed ingredients by Atlantic salmon (*Salmo salar*) (Storebakken et al. 2000; Glencross et al. 2004a; Refstie et al. 2005; Aas et al. 2006; Aslaksen et al. 2007; Refstie et al. 2008). The protein digestibility was not significantly lower for the plant feed ingredients, except for extracted soybean meal, oat, canola, and sunflower. Canola meal contains a high level of carbohydrates and other antinutritional factors, such as phytic acid, tannins, sinapine, and goitrogenic compounds, lowering the nutrient availability of this feed ingredient to aquatic species (Kousoulaki et al. 2009). Further processing into a protein concentrate should remove more of the antinutritional factors and carbohydrates; however, significant amounts of phytic acid could remain (Burel and Kaushik 2008). In rainbow trout (*O. mykiss*), experimental diets that contained 20% fishmeal and 19.4% canola protein concentrate significantly lowered growth (Drew et al. 2007).

Both existing and new aquaculture operations are trying to increase the sustainability and reduce the environmental impacts by optimizing water use and reducing waste. Recirculating systems for cold water fish have undergone significant improvements within the last decade and can provide culture systems with reduced environmental impact. Such systems have greater control of the rearing environment, minimize water use, produce a concentrated and relatively small volume effluent, and can have improved biosecurity measures to control disease (Summerfelt et al. 2001).

The purpose of this study was to determine the effect of feeding multiple levels of canola protein concentrate in a commercial-type diet on the growth and nutrient efficiency of Atlantic salmon in a recirculating aquaculture system.

## Methods

The experimental protocols and methods used in the research at the NCWMAC were in compliance with Animal Welfare Act (9CFR) requirements and were approved by the location's Institutional Animal Care and Use Committee. A 38-week feeding trial was conducted at the National Cold Water Marine Aquaculture Center (Franklin, ME, USA) with healthy juvenile Atlantic salmon. A commercial diet (Signature Salmon 3.5

mm, Northeast Nutrition, Truro, Nova Scotia, Canada) was used and modified by the manufacturer to contain 10% or 20% canola protein concentrate (insoluble canola protein concentrate, CanPro, Arborfield Saskatchewan, Canada). The three diets were formulated to contain 45% to 46% protein and 21% to 23% lipid. Diets were formulated and prepared by the manufacturer to ensure practical relevance, and the proportional composition is shown in Table 1. The 10% CPC diet contained only 75% of the fish protein ingredients, and the 20% CPC diet contained only 57% of the fish protein ingredients compared to the commercial diet. Amino acids and lipids were balanced by the manufacturer. The feed was prepared before the trial and was stored in a cool dry room (temperature, 4°C to 12°C).

Each experimental diet was randomly assigned to triplicate tanks (0.26 $m^3$ each) containing 20 Atlantic salmon (133.0 ± 7.8 g SD per fish, initial weight) at an initial stocking density of 10 $kg/m^3$. All tanks were connected to a common particulate (rotating drum filter) and a biological filter (fluidized sand) to maintain optimal water quality. The water in the system was brackish water (3%) and obtained from a ground source. Temperature (°C) and dissolved oxygen (mg/l) were continuously monitored

**Table 1 Nutrient composition of canola protein concentrate and proportional ingredient composition of reference and experimental diets**

| | Canola protein concentrate | 0% Canola protein | 10% Canola protein | 20% Canola protein |
|---|---|---|---|---|
| Ingredient[a] (g/kg) | | | | |
| Canola protein concentrate | | 0 | 100 | 200 |
| Plant protein ingredients (other than canola) | | 270.2 | 253.0 | 241.4 |
| Animal protein ingredients | | 398.4 | 336.8 | 260.3 |
| Fish protein ingredients | | 140.0 | 105.0 | 80.0 |
| Fish oil and lecithin | | 165.2 | 177.9 | 189.7 |
| Amino acids | | 4.4 | 5.5 | 6.8 |
| Additives (nutritional) | | 18.3 | 18.3 | 18.3 |
| Vitamin + trace mineral package | | 3.5 | 3.5 | 3.5 |
| Analyzed levels (as fed basis) | | | | |
| Dry matter (g/kg) | 950.6 | 950.5 | 947.5 | 961.7 |
| Protein (g/kg) | 604.1 | 459.6 | 449.6 | 455.7 |
| Fat (g/kg) | 24.2 | 228.6 | 209.4 | 211.3 |
| Ash (g/kg) | 54.1 | 66.1 | 61.9 | 57.1 |
| Calcium (g/kg) | 6.0 | 15.3 | 13.8 | 10.6 |
| Phosphorus (g/kg) | 8.7 | 10.9 | 10.1 | 8.5 |
| Sodium (g/kg) | 6.2 | 3.0 | 3.1 | 3.1 |
| Potassium (g/kg) | 3.6 | 6.1 | 5.5 | 5.2 |
| Magnesium (g/kg) | 4.5 | 1.2 | 1.5 | 1.7 |
| Manganese (ppm) | 57.25 | 143.46 | 194.83 | 128.95 |
| Copper (ppm) | 11.52 | 16.03 | 11.97 | 14.36 |
| Zinc (ppm) | 79.07 | 113.08 | 104.99 | 131.19 |

[a]The basal diet contained poultry meal, wheat, fishmeal, fish oil, blood meal, crab meal, feather meal, CPSP special G fishmeal (fish hydrolysate from fresh whole fish or fresh offals; Sopropêche, Cedex, France), lecithin, betaine, XPC yeast culture (Diamond V, Cedar Rapids, Iowa, USA), choline chloride, amino acids, vitamin, and mineral premixes. The CPSP special G fishmeal was omitted from the CPC-containing diets.

with stationary probes. The temperature averaged to 10°C using well water and varied seasonally, ranging from 8°C in January to 13°C in July. Fish were subjected to a natural photoperiod (9 h light (L):15 h dark (D) initial to 15L:9D final) maintained by artificial lighting controlled using a computer. Dissolved oxygen was maintained at or above saturation using an oxygen cone. The water quality of the system was monitored weekly and maintained within acceptable levels for Atlantic salmon.

The ArvoPro computer program (Arvo-tec, Huutokoski, Finland) was used to control the feed being dispensed using automatic feeders four times per day to the tanks, and the feed was adjusted daily with fish fed to apparent satiation. The computer program was developed from experimental growth models validated from commercial data for conditions and different genetic stocks (Ursin 1967; From and Rasmussen 1984; Ruohonen and Makinen 1992). The feed was slowly sinking. Fish were group weighed approximately every 4 weeks to confirm the projected weight (g) using the computer software. Throughout the trial, weight gain (g) was monitored. At the end of the trial, feed efficiency (weight gain/feed intake), protein efficiency ratio (g of weight gain/g of protein fed), and specific growth rate values were computed, and three fish were collected from each tank for subsequent measurement of whole-body composition according to established procedures (Webb et al. 2003).

## Results

Atlantic salmon fed the diet containing 20% canola protein concentrate showed the lowest weight gain compared to fish fed the diet containing 0% canola protein concentrate ($p$ = 0.04; Figure 1; Table 2). These fish fed the 0% canola protein concentrate diet had the highest weight gain but were not statistically different from the fish fed the diet containing 10% canola protein concentrate (Figure 1; Table 2). Fish fed the fishmeal reference diet had the highest average weight throughout the study (Figure 1). Feed efficiency and protein efficiency ratio did not differ among the treatments (Table 2), indicating that the fish fed the reference diet consumed the most feed. Fish fed the higher fish protein diet had a significantly greater specific growth rate compared to fish fed the 20% canola concentrate diet (Table 2).

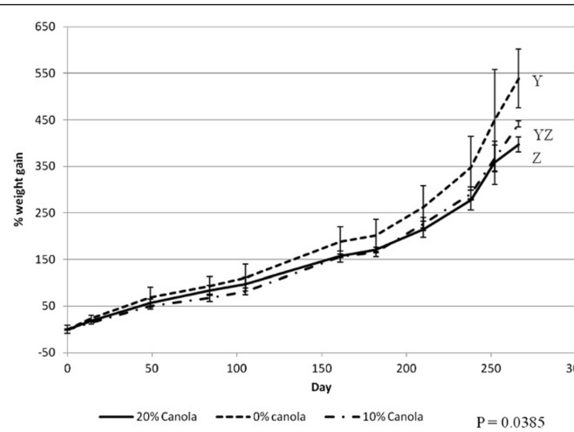


**Figure 1 Percent weight gain over 38 weeks of Atlantic salmon fed the three diets.** Values with common superscript letter do not differ significantly ($p$ > 0.05).

**Table 2 Feed efficiency (FE), protein efficiency ratio (PER), specific growth rate (SGR), and weight increase (%)**

| Diet | FE | PER | SGR | Weight increase (%) |
|---|---|---|---|---|
| 0 | 0.80 ± 0.07 | 1.74 ± 0.29 | 0.73 ± 0.05 Y | 539.6 ± 62.9 Y |
| 10 | 0.78 ± 0.01 | 1.74 ± 0.05 | 0.67 ± 0.01 YZ | 441.2 ± 6.8 YZ |
| 20 | 0.68 ± 0.01 | 1.50 ± 0.04 | 0.64 ± 0.02 Z | 397.3 ± 16.1 Z |
| ANOVA *p* | 0.2170 | 0.2081 | 0.0268 | 0.0385 |

Values within columns that have a common uppercase letter do not differ significantly ($p > 0.05$). ANOVA, analysis of variance.

Proximate composition of the whole fish lipid, moisture, or ash concentrations did not vary among the treatments ($p = 0.05$; Table 3). Fish fed with the control diet had significantly lower crude protein concentrations compared to fish fed with the canola protein concentrate diets ($p = 0.04$; Table 3).

## Discussion

Atlantic salmon fed the diet containing 20% canola protein concentrate had significantly lower weight gain and specific growth rate compared to fish fed diets containing higher levels of fish protein ingredients. However, feed efficiencies were similar, indicating that the increased weight gain of the fish on the fishmeal diet was the result of consuming more feed. There are several antinutritional factors associated with canola that could have influenced the consumption and resulting weight gain in this study, such as tannins, phytic acid, and glucosinolates (Kousoulaki et al. 2009). If antinutritional factors are indicated in future research, then each batch used in aquafeeds should be analyzed for antinutritional factors.

Several plant proteins have been evaluated in the diets of Atlantic salmon (Refstie et al. 2001; Refstie and Tiekstra 2003; Mundhein et al. 2004; Denstadli et al. 2007; Torstensen et al. 2008; Øverland et al. 2009). Potato protein concentrate (included up to 21% of the diet) and soy protein concentrate (included at 19.7% of the diet) did not inhibit the growth of Atlantic salmon compared to fishmeal-based diets (Refstie et al. 2001; Refstie and Tiekstra 2003). The growth of Atlantic salmon was inhibited by increasing plant proteins (full-fat soybean meal and corn gluten meal) up to 42% of the diet compared to a fishmeal-based diet (Kraugerud et al. 2007). Kraugerud et al. (2007) reported that a diet containing 46% soybean meal stunted the growth of Atlantic salmon (Torstensen et al. 2008). When included at 20% of the diet, canola protein concentrate also inhibited the growth of Atlantic salmon. These results indicate that a certain level of plant protein concentrates is tolerated in the diets of Atlantic salmon, but when the threshold is exceeded, either antinutritional factors or digestibility of the plant

**Table 3 Proximate composition of whole fish (g/kg of wet weight)**

| Diet | Protein | Lipid | Ash | Moisture |
|---|---|---|---|---|
| 0 | 140.1 ± 15.9 b | 107.5 ± 21.2 | 61.4 ± 36.3 | 716.0 ± 7.3 |
| 10 | 175.2 ±12.3 a | 108.6 ± 15.0 | 57.9 ± 12.6 | 689.2 ± 2.8 |
| 20 | 167.7 ± 11.4 a | 95.0 ± 3.6 | 67.8 ± 13.9 | 694.6 ± 3.0 |
| ANOVA *p* | 0.0401 | 0.5697 | 0.3961 | 0.1102 |

Values within columns that have a common lowercase letter do not differ significantly ($p > 0.05$).

proteins hinders the intake or uptake of nutrients and limit growth. This would indicate that the digestibility of plant proteins need to be improved or the formulae require further modification to compensate. The antinutritional factors need to be mitigated or removed from the plant protein product prior to feeding (Denstadli et al. 2007).

Canola meal and canola protein concentrate have been investigated in a variety of finfish (Burel and Kaushik 2008). Protein apparent digestibility coefficients for rainbow trout fed rapeseed meal are similar to fishmeal (Mwachireya et al. 1999; Burel et al. 2000). However, apparent digestibility coefficients for energy and dry matter are significantly lower compared to a fishmeal reference diet (Mwachireya et al. 1999). There have been contradictory results obtained for rainbow trout fed a canola protein concentrate. Rainbow trout grew only slightly slower when fed a diet containing up to 42% canola protein concentrate compared to fish fed a fishmeal-based diet (Forster et al. 1999). Drew et al. (2007) also found that the growth of rainbow trout fed a canola protein concentrate was not affected when the levels were below 29%. However, Teskeredžić et al. (Teskeredzić et al. 1995) reported that rapeseed protein concentrate included in the diet above 26% did significantly lower the specific growth rate of rainbow trout. Hajen et al. (1993) reported that Chinook salmon (*Oncorhynchus tshawytscha*) fed a diet containing 30% rapeseed protein concentrate had the same growth rate as fish fed a fishmeal diet. The growth of the juvenile Chinook salmon fed diets that contained up to 23% canola meal was similar to a fishmeal reference diet (Higgs et al. 1983). Canola meal inclusion rates could be increased up to 30% when the diet was supplemented with 5 ppm 3,5,3'-triiodo-L-thyronine (Webster et al. 1997). In channel catfish, fish fed a diet containing 48% canola meal had significantly lower growth compared to fish fed a fishmeal diet or diets containing 12% or 36% canola meal (Webster et al. 1997). Sunshine bass (*Morone chrysops* × *Morone saxatilis*) fed a diet containing 20% canola meal did not have a significant difference in growth compared to fish fed a fishmeal diet (Webster et al. 2000). The processing method affected the digestibility of canola meals for red seabream, and protein digestibility was similar to soybean meal (Glencross et al. 2004a). The growth of red seabream fed diets that contained up to 60% canola meal was not adversely affected compared to a fishmeal reference diet (Glencross et al. 2004b). These results along with the results in the present study indicate that the source and processing of the canola meal/protein concentrate and species of fish affect growth performance. However, due to limited published studies, more research is needed to determine the upper limit of canola meal/ protein concentrate that can be included in aquafeeds.

## Conclusions

Overall, Atlantic salmon does not appear to be able to tolerate canola protein concentrate above 10% when included in their diet. Canola protein concentrate could be a candidate for a diet if included in the diet at low levels. This study did not address the antinutritional factors associated with canola meal/protein concentrate, but the viability of canola as a feed ingredient could be enhanced if the antinutritional factors could be removed or the effects could be mitigated.

### Abbreviations

CPC: canola protein concentrate; CPSP special G: fish hydrolysate from fresh whole fish or fresh offals; XPC: fermented liquid and cereal grain ingredients with baker's yeast (*Saccharomyces crevisiae*).

**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

AD formulated and prepared the diets and assisted with the drafting of the manuscript. RB assisted with the diet formulation, study design, and drafting of the manuscript. WW assisted with study design, data collection, and analysis. GB performed the study, data collection, analysis, and drafting of the manuscript. All authors have read and approved the final manuscript.

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**Author details**

[1]ARS National Cold Water Marine Aquaculture Center, USDA, 25 Salmon Farm Road, Franklin, ME 04634, USA. [2]Agricultural Research Service, Fish Technology Center, USDA, Bozeman, MT 59715, USA. [3]Northeast Nutrition Inc, 494 Willow St, Truro, Nova Scotia B2N 6X8, Canada.

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