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Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*)

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ABSTRACT

The purpose of this study was to evaluate blends of alternate proteins as replacements for fishmeal in diets for rainbow trout (*Oncorhynchus mykiss*) and to use the results to develop and test alternate protein blends in diets for juvenile Atlantic salmon (*Salmo salar*). Nine experimental diets in which protein blends replaced 63%, 82% or 100% of fishmeal in the formulation (20, 10, and 0% fishmeal) were fed to rainbow trout (initial weight 19.5 g) for 12 weeks. Weight gains of trout fed diets containing the soy protein concentrate-based blend and the fishmeal control diet were similar, except at the 100% fishmeal replacement level, and significantly higher than that of trout fed diets containing the other blends. The soy protein blend and another based on wheat gluten meal were modified slightly and evaluated in early stage Atlantic salmon juveniles (initial weight 5.5 g). Protein blends replaced 50%, 66% or 84% of fishmeal (30, 20 or 10% fishmeal). Weight gains of early stage juvenile salmon after 18 weeks of feeding were significantly lower and feed conversion ratios higher when fed diets containing either blend compared to the fishmeal control diet, and gains decreased as level of fishmeal replacement increased. Blends were then modified further and tested in advance stage salmon juveniles (initial weight 31.5 g). These blends were solely either all-plant protein-based or contained poultry by-product meal. Both blends were evaluated with or without addition of *Spirulina* algae meal. Alternate protein blends completely replaced fishmeal in experimental diets. After 12 weeks of feeding, no differences in weight gain or feed conversion ratios were measured among groups fed experimental diets containing protein blends or the fishmeal control diet. Replacement of fishmeal with alternative protein blends in diets for early stage juvenile salmon is not recommended and the penalty in growth is severe. Fishmeal can be completely replaced in diets for late stage salmon over 30 g without compromising fish performance or using land animal protein ingredients in feed formulations.

1. Introduction

Commercial fisheries landings for both direct consumption and for fishmeal production have not increased for over a decade (FAO, 2010). The demand for seafood continues to increase, and aquaculture production has filled the shortfall associated with static wild fish landings (FishStat Plus, 2010). In fact, in 2012 aquaculture production is expected to exceed capture fisheries as a source of finfish products for consumption (FAO, 2010). Aquaculture production is expected to increase further and this will require higher production of aquafeeds. The inclusion of plant-protein sources in aquafeeds has increased due to the limited amount and increasing cost of fishmeal available for production of animal feeds (e.g., Gatlin et al., 2007; Glencross et al., 2005; Naylor et al., 2009). One of the greatest challenges for the aquafeed industry is to reduce fishmeal levels in feed further and increase the amount of plant protein and ingredient diversity in diets of carnivorous fishes.

The search for fishmeal replacements in rainbow trout (*Oncorhynchus mykiss*) Atlantic salmon (*Salmo salar*) feeds has been ongoing for many years and lately has received more attention as fishmeal prices and aquaculture production have increased (Gatlin et al., 2007). Many different plant-protein sources have been examined including plant-protein meals and plant-protein concentrates (Lim et al., 2008). Generally, levels of plant meals in salmon and trout feed formulations are limited in salmon and trout formulations by their composition (relatively low crude protein and high crude fiber content) and by the presence of anti-nutritional factors and non-soluble carbohydrates (Krogdahl et al., 2009; Lim et al., 2008; NRC, 2011). Plant-protein concentrates are more promising ingredients to replace fishmeal in aquafeeds than are plant meals. Canola, soy, pea,

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barley, rice protein concentrates, along with wheat gluten meal, have all been tested as fishmeal replacements with varying degrees of success (Forster et al., 1999; Thiesses et al. 2003; Barrows et al., 2007; Lim et al., 2008; Gaylord and Barrows, 2009). Protein digestibility of most plant proteins for salmon is generally similar to or higher than that of fishmeal; except for bacterial protein meal, extracted soybean, oat, rapeseed (canola), and sunflower meals (Storebakken et al., 2000; Glencross et al., 2004; Aas et al., 2006; Refstie et al., 2006; Aslaksen et al., 2007; Denstadli et al., 2007; Kraugerud et al., 2007). Amino acid profiles, however, are inferior to fishmeal and amino acid supplementation is needed to maintain growth performance of fish fed diets containing high levels of plant-protein concentrates (Gaylord and Barrows, 2009; Lim et al., 2008).

Commercial diet formulations use a combination of alternate protein sources to replace fishmeal to better balance amino acid levels, but many of the studies evaluating ingredients to replace fishmeal involve single ingredient substitutions (Gatlin et al., 2007; Lim et al., 2008. The purpose of this study was to test protein blends as replacements for fishmeal in diets for rainbow trout and to use these results to develop and test protein blends in diets for early and late stage juvenile Atlantic salmon.

2. Methods and materials

2.1. Rainbow trout trial

The rainbow trout trial tested three protein blends at three levels of substitution for fishmeal. The protein blends were formulated around three plant-protein concentrates, soy protein concentrate (SPC), corn gluten meal (CGM) and barley protein concentrate (BPC) (Table 1). SPC, BPC and CGM were combined with other protein ingredients and supplements to produce protein blends that contained digestible protein levels similar to menhaden fishmeal (Select grade). The rationale behind the protein-blend approach was that in commercial feed formulations, reducing fishmeal levels is best accomplished by combining several alternate protein sources to approximate the amino acid profile of fishmeal. Supplementing protein blends with amino acids further improves the nutritional profiles of blends in comparison with that of fishmeal (Cheng et al., 2003). Minerals shown to be important when feeding fishmeal-free diets were supplemented to each of the three blends (Barrows et al., 2010). Each of the three protein blends was included in experimental feeds at three dietary levels (nine experimental diets) such that 63%, 82% and 100% of the fishmeal was replaced (Table 2). A fishmeal control diet was also fed. The ten experimental diets were formulated to contain 39% digestible protein and 19% crude lipid, similar to commercial trout diets that contain 44% protein of which 87% is digestible. Vitamin and mineral premix levels remained constant in the feed formulations. Fish oil levels varied depending upon the lipid content of the blends. The feeds all contained levels of essential amino acids above minimum dietary requirements for rainbow trout (NRC, 2011).

All of the diets were produced using commercial manufacturing technology at the U.S. Fish & Wildlife Service Bozeman Fish Technology Center, Bozeman, MT, USA. All ingredients were ground to a particle size of <200 μm using an air-swept pulverizer (Model 18H, Jacobsen, Minneapolis, MN). The diets were processed using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with a ~25 s exposure to 127 °C in the extruder barrel (average across 5 sections). Pellets were dried with a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 20 min at 102 °C with a 10-minute cooling period, resulting in final moisture levels less than 10%. All added fish oil was top-coated after the pellets were cooled using a vacuum-coater (AJ Mixing, Ontario, CA). Diets were stored in plastic lined paper bags at room temperature until fed. Feeds were then shipped to the University of Idaho's Hagerman Station where they were analyzed to confirm that calculated proximate compositions were achieved and where the rainbow trout feeding trial was conducted. Diets were fed within four months of manufacture.

Juvenile rainbow trout (House Creek strain) from the University of Idaho broodstock were used in the feeding trial. The fish averaged 19.5 g at the start of the trial. Fish were stocked into 145 L tanks supplied with 4 L min^{-1} of constant temperature (14.5 C) spring water supplied by gravity in a single-pass water system. Water flow to each tank was increased to 8 L min^{-1} as the feeding trial progressed. Each tank contained 30 fish and each experimental diet was fed to three replicate tanks, arranged in a completely randomized design within the indoor fish rearing system. Fish were fed three times per day by hand to apparent satiation, and feed consumption was recorded. Photoperiod was maintained at a constant 14 h light:10 h dark with automatic timers. Fish in each tank were bulk-weighed and counted every three weeks during the 12-week trial. Average fish weight gain, feed intake per fish, percent feed intake, feed conversion ratio (FCR), thermal growth coefficients (TGC), and productive protein value (PPV) were calculated over the entire study. All experimental protocols involving rainbow trout rearing and sampling were approved by the University of Idaho's Institutional Animal Care and Use Committee.

Table 1
Protein-blend formulations (g kg^{-1}) used as fishmeal replacements in rainbow trout feeds.

Ingredient	SPC	CGM	BPC
Soy protein concentrate (SPC)[a]	241.2	–	–
Corn gluten meal (CGM)[b]	258.8	295.2	245.2
Barley protein concentrate (BPC)[c]	–	–	258.4
Poultry by-product meal[d]	143.5	279.5	233.5
Blood meal[e]	91.2	149.8	119.8
Soybean meal[f]	181.7	190.6	52.1
Lysine	29.3	32.3	36.9
Methionine	7.1	7.9	8.2
Threonine	7.1	8.9	10.1
Taurine	7.9	7.9	7.9
Mono-dicalcium phosphate	23.3	19.0	21.0
Sodium chloride	2.8	2.8	2.8
Potassium chloride	5.6	5.6	5.6
Magnesium oxide	0.5	0.5	0.5
Calculated composition, as-is basis			
Crude protein	629	637	630
Fat	26.5	46.1	38.8
Total phosphorus	12.1	14.2	13.5
Lysine	55.1	56.1	56.3
Methionine	17.6	18.3	18.1
Cystine	9.3	9.2	6.8
Threonine	27.6	27.0	27.3

[a] Solae, Pro-Fine VF, 693 g/kg crude protein.
[b] Cargill, 639.00 g/kg protein.
[c] Montana Microbial Products, 520 g/kg protein.
[d] Griffin Industries, 600 g/kg protein.
[e] IDF Inc., 832 g/kg protein.
[f] ADM Inc., 476 g/kg protein.

2.2. Atlantic salmon trials

2.2.1. Early stage juvenile trial

Based upon the results of the trial with rainbow trout, a feeding trial with early stage juvenile Atlantic salmon (5.5 g initial weight) was designed. Two protein blends were formulated using SPC, CGM and wheat gluten meal (WGM) to be equivalent to menhaden fishmeal (Select grade) in digestible protein content (Table 3). Amino acids were supplemented to ensure adequate levels of the three essential amino acids that were most limiting in the protein blends, lysine, methionine and threonine (NRC, 2011). Blends were also supplemented with minerals. The blends were then included in six experimental diets and a fishmeal control diet to replace 50%, 66% and 87% of fishmeal in the diets (Table 4). The experimental diets were manufactured as described above and shipped to the USDA, Agricultural Research Service National Cold Water Marine Aquaculture

Table 2
Diet formulations (g kg^{-1}) used in the protein-blend evaluation using juvenile rainbow trout (initial weight 19.5 g). Number after protein blend refers to percentage of fishmeal replaced.

Ingredient	FM control	SPC63	SPC83	SPC100	BPC63	BPC82	BPC100	CGM63	CGM82	CGM100
Menhaden fishmeal[a]	529.6	198.5	99.3	–	198.5	99.3	–	198.5	99.3	–
Blend 1 SPC	–	390.3	503.0	615.7	–	–	–	–	–	–
Blend 2 BPC	–	–	–	–	–	–	–	434.6	56.16	668.7
Blend 4 CGM	–	–	–	–	434.5	551.6	668.7		–	–
Wheat flour[b]	314.4	229.2	208.7	187.8	192.3	168.4	145.1	189.1	155.2	141.5
Menhaden fish oil[c]	136.0	162.0	169.0	176.5	154.7	160.7	166.2	157.8	163.9	169.8
Vitamin premix[d]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Stay-C (35% ascorbate)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Trace mineral premix[e]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Analyzed composition (as-is basis)										
Crude protein	429	453	455	451	483	482	498	493	471	481
Fat	174	196	185	178	151	165	160	169	180	150
Ash	93	70	62	52	75	68	63	587	61	50
Gross energy (joules/g)_	22.3	22.9	23.0	23.4	23.1	23.1	23.5	23.8	23.6	23.8

[a] Omega Proteins, Menhaden Special Select, 628 g/kg protein.
[b] Manildra Milling, 12 g/kg protein.
[c] Omega Proteins.
[d] Supplied the following per kg diet: vitamin A palmitate, 9650 IU; cholecalciferol, 6600 IU; DL-tocopheryl acetate, 132 IU; menadione sodium bisulfate 1.1 mg; thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine HCl 13.7 mg; DL-calcium pantothenate, 46.5 mg; cyanocobalamine 0.03 mg, nicotinic acid, 21.8 mg; D-biotin, 0.34 mg; folic acid 2.5; and inositol, 600 mg.
[e] Supplied the following per kg diet: copper, 3 mg as copper sulfate pentahydrate; manganese, 10 mg as manganese sulfate, monohydrate; iodine, 5 mg as potassium iodide; sodium selenate 0.960 g; and zinc, 37 mg as zinc sulfate, heptahydrate.

Center in Franklin, ME. The fishmeal control diet was fed to three replicate tanks and each experimental diet was fed to duplicate tanks of fish.

Early stage juvenile Atlantic salmon (St. John's strain) from the USDA ARS National Cold Water Marine Aquaculture Center's breeding program were used in the feeding trial. Fifty fish (mean weight 5.46 ± 0.06 g) were stocked into each 265 L tank supplied with 8 L min^{-1} of oxygen-saturated water from a recirculating biological filtration system at 2.0–3.0 g L^{-1} salinity. Dissolved oxygen and temperature were monitored continuously and ammonia, nitrite, nitrate, carbon dioxide, and pH monitored weekly to insure optimal water quality conditions. Fish were fed a commercial feed for one

Table 3
Protein-blend formulations (g kg^{-1}) used as fishmeal replacements in diets for early stage juvenile Atlantic salmon (initial weight 5.5 g).

Ingredient	Menhaden fishmeal	SPC	WGM
Menhaden fishmeal[a]	1000	–	–
Soy protein concentrate[b]	–	218.6	–
Corn gluten meal[c]	–	268.8	331.9
Wheat gluten meal[d]		95.4	185.4
Poultry by-product meal[e]	–	263.5	319.2
Blood meal[f]	–	61.2	59.7
Lysine	–	34.3	42.9
Methionine	–	7.1	7.1
Threonine	–	7.9	10.9
Taurine	–	15.0	15.0
Mono-dicalcium phosphate	–	19.3	19.0
Sodium chloride	–	2.8	2.8
Potassium chloride	–	5.6	5.6
Magnesium oxide	–	0.5	0.5
Calculated composition			
Crude protein	678.0	664.5	665.4
Fat	96.0	32.4	39.8
Total phosphorus	24.3	14.1	14.4
Methionine	19.9	18.4	18.5
Cystine	6.0	10.8	11.2
Lysine	50.4	53.8	54.1
Threonine	28.2	30.0	30.4

[a] Omega Proteins, Menhaden Special Select, 628 g/kg protein.
[b] Solae, Pro-Fine VF, 693 g/kg crude protein.
[c] Cargill, 639.00 g/kg protein.
[d] Manildra Milling, 753 g/kg protein.
[e] IDF Inc., 832 g/kg protein.
[f] Griffin Industries, 600 g/kg protein.

Table 4
Diet formulations (g kg^{-1}) used in the protein-blend evaluation with early stage juvenile Atlantic salmon (5.5 g initial weight). Number after protein blend refers to percentage of fishmeal replaced.

Ingredient	FM control	SPC50	SPC66	SPC87	WGM50	WGM66	WGM87
Menhaden fishmeal[a]	600.9	300.0	200.0	100.0	300.0	200.0	100.0
Soy protein blend	–	312.5	420.4	528.3	–	–	–
Wheat gluten blend	–	–	–	–	321.5	428.2	534.9
Wheat flour[b]	242.9	202.0	182.9	163.7	196.6	179.6	163.0
Menhaden fish oil[c]	136.2	153.0	158.5	164.5	150.2	155.1	159.9
Mono-dicalcium phosphate	–	12.5	18.2	23.5	11.7	17.1	22.2
Vitamin premix 702[d]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Stay-C (35% ascorbate)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Trace mineral premix[e]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated composition							
Crude protein	400	417	425	432	425	433	442
Fat	190	190	190	190	190	190	190
Total phosphorus	16	15	15	15	15	15	15
Methionine	9.7	10.6	11.0	11.3	10.8	11.1	11.5
Cystine	3.1	4.9	5.6	6.2	5.1	5.8	6.5
Lysine	26.1	29.9	31.3	32.8	30.4	31.9	33.3
Threonine	13.8	16.3	17.2	18.2	16.7	17.6	18.6

[a]Omega Proteins, Menhaden Special Select, 628 g/kg protein.
[b]Manildra Milling, 12 g/kg protein.
[c]Omega Proteins.
[d]Supplied the following per kg diet: vitamin A palmitate, 9650 IU; cholecalciferol, 6600 IU; DL-tocopheryl acetate, 132 IU; menadione sodium bisulfate 1.1 mg; thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine HCl 13.7 mg; DL-calcium pantothenate, 46.5 mg; cyanocobalamine 0.03 mg, nicotinic acid, 21.8 mg; D-biotin, 0.34 mg; folic acid 2.5; and inositol, 600 mg.
[e]Supplied the following per kg diet: copper, 3 mg as copper sulfate pentahydrate; manganese, 10 mg as manganese sulfate, monohydrate; iodine, 5 mg as potassium iodide; sodium selenate 0.960 g; and zinc, 37 mg as zinc sulfate, heptahydrate.

week after stocking prior to the start of the 18-week diet study. A natural photoperiod (14L: 10D initial, 9L:15D final) and ambient water temperatures (decreasing from 14.1 °C to 8.3 °C) were followed during the study. Fish were fed using automatic feeders such that feed was supplied at 110% of maximum expected intake to ensure that growth was not feed-limited. Fish in each tank were bulk-weighed and counted every four weeks during the trial. Average fish weight gain, feed intake per fish, percent feed intake, FCR, TGC, and PPV were calculated for the 18 week feeding period.

2.2.2. Late stage juvenile trial

A follow-up feeding trial with late stage juvenile Atlantic salmon (31.5 g initial weight) was designed based upon the results of the trials with early stage juvenile Atlantic. Protein blends were formulated using SPC, CGM, WGM, poultry by-product meal and *Spirulina* algae meal to be equivalent to menhaden fishmeal (Select grade) in digestible protein content (Table 5). Amino acids and minerals were supplemented as in the previous trial. The blends were then included in four experimental diets to completely replace fishmeal (Table 5). The experimental diets and a fishmeal control diet were manufactured as described above and shipped to the USDA, Agricultural Research Service National Cold Water Marine Aquaculture Center in Franklin, ME. Each of the five diets was randomly assigned and fed to three replicate tanks.

Atlantic salmon juveniles (St. John's strain) from the USDA ARS National Cold Water Marine Aquaculture Center's breeding program were used in the feeding trial. One hundred and twelve fish (mean weight 31.5 ± 2.9 g) were stocked into each 265 L tank supplied with 8 L min^{-1} of oxygen-saturated water from a recirculation, biological filtration system at 2.0–3.0 g L^{-1} salinity. Water quality was monitored as described above. Rearing conditions were the same as described above for small juvenile Atlantic salmon except that photoperiod (11.5 h light and 12.5 dark initially to 14 h light and 10 h dark, final) and ambient water temperature (8.3 °C to14.1 °C) increased during the study. Fish were fed using automatic feeders such that feed was supplied at 100% of maximum expected intake. Fish in each tank were bulk-weighed and counted every four weeks during the 12-week trial. Average fish weight gain, feed intake per fish, percent feed intake, FCR and TGC were calculated for the entire study. Samples of fish from the treatment groups were not taken at the end of this study because the fish were needed for a follow-up study designed to evaluate the performance of family groups fed the fishmeal control diet and the plant protein-based diet. All experimental protocols involving Atlantic salmon were approved by the National Cold Water Marine Aquaculture Center's Animal Care and Use Committee.

Table 5
Diet formulations (g kg^{-1}) used in the protein-blend evaluation with late stage juvenile Atlantic salmon fingerlings (31.5 g initial weight). FM = fishmeal, LAP = land animal protein, PP = plant protein and S = *Spirulina* algae meal supplementation.

Ingredient	FM control	LAP blend	LAP-S blend3	PP blend	PP-S blend5
Menhaden fishmeal[a]	386.2	–	–	–	–
Poultry-by-product meal[b]	–	319.2	258.1	–	–
Corn protein concentrate[d]	160.2	160.2	129.5	273.4	238.1
Soy protein concentrate[e]	–	–	–	216.4	149.4
Wheat gluten meal[h]	45.0	45.0	45.0	–	–
Spirulina[g]	–	–	112.5	–	112.5
Wheat starch[f]	127.5	156.1	118.3	88.0	73.7
Menhaden fish oil[c]	226.2	198.5	203.5	258.0	251.5
Vitamin premix 702[i]	15.0	15.0	15.0	15.0	15.0
Choline chloride	6.0	6.0	6.0	6.0	6.0
Stay-C (35% ascorbate)	3.0	3.0	3.0	3.0	3.0
Taurine	–	15.0	15.0	5.0	5.0
L-Lysine	14.5	12.5	16.9	45.0	25.7
DL-methionine	–	2.2	4.0	3.6	4.8
Threonine	–	0.8	3.0	2.1	4.9
Mono-dicalcium phosphate	14.8	56.0	59.4	53.1	55.9
Potassium chloride	–	5.6	5.6	5.6	5.6
Sodium chloride	–	2.8	2.8	2.8	2.8
Magnesium oxide	–	0.5	0.5	0.5	0.5
Trace mineral premix[j]	1.0	1.0	1.0	1.0	1.0
Astaxanthin	0.6	0.6	0.6	0.6	0.6
Calculated composition					
Crude protein	406	413	421	407	413
Fat	261	261	260	261	260
Total phosphorus	14	14	15	14	16
Methionine	10	12	12	12	12
Cystine	5.2	3.2	2.7	7.4	6.1
Lysine	31	32	32	32	32
Threonine	3.5	1.2	1.1	4.1	3.3

[a] Omega Proteins, Menhaden Special Select, 628 g/kg protein.
[b] IDF Inc., 832 g/kg protein.
[c] Omega Proteins.
[d] Cargill, Empyreal 75, 748 g/kg protein.
[e] Solae, Pro-Fine VF, 693 g/kg crude protein.
[f] Manildra Milling, 40 g/kg protein.
[g] Earthrise Nutritional Products, 727 g/kg protein.
[h] Manildra Milling, 753 g/kg protein.
[i] Supplied the following per kg diet: vitamin A palmitate, 9650 IU; cholecalciferol, 6600 IU; DL-tocopheryl acetate, 132 IU; menadione sodium bisulfate 1.1 mg; thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine HCl 13.7 mg; DL-calcium pantothenate, 46.5 mg; cyanocobalamine 0.03 mg, nicotinic acid, 21.8 mg; D-biotin, 0.34 mg; folic acid 2.5; and inositol, 600 mg.
[j] Supplied the following per kg diet: copper, 3 mg as copper sulfate pentahydrate; manganese, 10 mg as manganese sulfate, monohydrate; iodine, 5 mg as potassium iodide; sodium selenate 0.960 g; and zinc, 37 mg as zinc sulfate, heptahydrate.

2.3. Sample analysis

Feed samples and fish tissue samples were analyzed for proximate composition using AOAC (1990) methods. Frozen whole fish samples were partially thawed, then ground in an industrial food processor (Robot Coupe R2, Ridgeway, MS). Samples were dried in a convection oven at 105 °C for 8 h to determine moisture levels. Dried samples were finely ground by mortar and pestle and analyzed for nitrogen (N) using a LECO FP-428 nitrogen analyzer (LECO Instruments, St. Joseph, MI). Crude protein (CP) was calculated from sample N content (total nitrogen × 6.25 = CP). Crude fat was analyzed using an ANKOM XT15 extraction system (ANKOM Technology, Macedon NY) with petroleum ether as the extracting solvent, and ash was determined by incineration at 550 °C in a muffle furnace.

2.4. Statistics

Weight gain, FCR, TGC and PPV were subjected to one-way Analysis of Variance and Tukey's HSD Test for comparison of treatment mean values, using the Statistical Analysis System (SAS, 1985). Statistical significance was set at $P \leq 0.05$.

3. Results

Trout fed the fishmeal diet weighed 257 g at the end of the feeding trial, and had a FCR of 0.85 and a TGC of 0.287 over the course of the feeding trial (Table 6). Weight gains of trout fed the SPC blend diets at the 63% and 82% replacement levels were statistically similar to weight gain of trout fed the fishmeal diet (247 and 252 g, respectively), but performance (final weight, and TGC values) of trout fed the diet with 100% fishmeal replacement with the SPC blend was significantly lower (Table 6). Trout fed the BPC blend diets at 33% and 66% replacement levels exhibited significantly lower weight gain and TGC values than fish fed the fishmeal control diet not significantly different from values of fish fed the SPC blends at the same dietary level. Trout fed diets in which fishmeal was totally replaced with either the BPC or the CGM blends had significantly lower weight gain (15% lower), lower TGCs and higher FCRs than fish fed diets with lower levels of fishmeal replacement. Feed intake progressively decreased as replacement levels of fishmeal increased in the BPC group but was variable in the CGM groups, exhibiting no progressive decline (Table 6). There were no fish mortalities during the trial. Proximate composition of fish at the end of the study did not vary among dietary treatment groups (data not shown).

Early stage Atlantic salmon juveniles fed the fishmeal diet had significantly higher final weight and TGC values at the conclusion of the trial than fish fed diets containing either alternative protein blend at all levels of replacement. Fish fed diets containing protein blends weighed significantly less, 21.7 g for fish fed the SPC blend and 21.4 g for the WGM blend (Table 7). There were no significant differences in weight gain between juveniles fed diets containing either protein blend or among different levels of the protein blends, although fish fed diets with the highest level of protein blends gained the least weight (Table 7). FCR values were higher in all groups fed protein-blend diets compared to the fishmeal control group. Proximate composition of fish at the end of the study did not differ among dietary treatment group (data not shown) but PPV values were lower in all treatment groups containing protein blends compared to the PPV value of the fishmeal control treatment.

In contrast to results with early stage Atlantic salmon juvenile fish, late stage juveniles fed diets containing protein blends with no fishmeal did not differ significantly in final weight, feed intake, FCR, TGC or percent survival after 12 weeks of feeding from the fishmeal control diet (Table 8). Fish tripled their initial average weight over the course of the study. Addition of *Spirulina* algae meal to the diets had no statistical effect on fish performance. Notably, performance of fish fed diets lacking any animal or fish protein was statistically similar to that of fish fed diets containing land animal protein ingredients.

4. Discussion

The results of this study demonstrated differences in fish growth performance between rainbow trout and juvenile Atlantic salmon fed diets in which alternative protein blends provided most of the dietary protein, and also demonstrated that performance of juvenile Atlantic salmon fed diets in which protein was supplied from alternative protein blends improved with fish size/age. The effects of plant-protein meals and concentrates on growth of rainbow trout have been well studied (Gatlin et al., 2007; Lee et al., 2006; Lim et al., 2008). In most studies, rainbow trout fed diets containing a single alternate protein source or blends of protein sources exhibited reduced growth at high levels of replacement unless the diets contained 20–30% fishmeal (Adelizi et al., 1998; Glencross et al., 2010; Gomes et al., 1995). This is thought to be associated with differences between fishmeal and alternate protein sources in amino acid profile and availability, and, in the case of plant-protein ingredients, the absence of other essential nutrients and compounds such as macrominerals, trace minerals, sterols and taurine. In the present study, rainbow trout fed a diet in which the SPC blend replaced 63 and 87% of the fishmeal in the formulation grew as well as fish fed the fishmeal control diet (53% fishmeal). However, at 100% fishmeal replacement, fish weight gain was significantly reduced, despite the fact that limiting amino acids, macrominerals, trace minerals and taurine were supplemented to meet reported dietary requirements of the fish (NRC, 2011), Differences in feed intake account for a portion of the reduced fish weight gain, but differences in FCR and PPV suggest that other essential nutrients may be

Table 6
Final weight, feed conversion ratio (FCR), thermal growth coefficient (TGC) and productive protein value (PPV) for rainbow trout fed experimental diets for 12 weeks.[1]

Diet[2]	Final Weight (g)[3]	Total feed intake (g feed/fish)[3]	FCR[3,4]	TGC[3,4]	PPV[4]
FM control	257 ± 4.7^{A}	202 ± 4^{AB}	0.85 ± 0.01^{E}	0.287 ± 0.003^{A}	0.381 ± 0.015^{AB}
SPC63	247 ± 6.7^{ABC}	188 ± 5^{D}	0.83 ± 0.01^{E}	0.280 ± 0.004^{ABC}	0.393 ± 0.008^{A}
SPC82	252 ± 0.6^{AB}	192 ± 2^{CD}	0.83 ± 0.01^{E}	0.284 ± 0.000^{AB}	0.396 ± 0.029^{A}
SPC100	240 ± 3.6^{BC}	199 ± 3^{CB}	0.90 ± 0.01^{C}	0.277 ± 0.002^{ABC}	0.341 ± 0.007^{ABC}
BPC63	239 ± 2.3^{BC}	190 ± 1^{CD}	0.87 ± 0.01^{CDE}	0.275 ± 0.002^{BC}	0.329 ± 0.008^{ABCD}
BPC82	240 ± 3.2^{BC}	188 ± 4^{D}	0.85 ± 0.01^{E}	0.277 ± 0.002^{ABC}	0.362 ± 0.011^{ABC}
BPC100	218 ± 2.4^{D}	178 ± 2^{E}	0.90 ± 0.00^{CD}	0.261 ± 0.002^{D}	0.305 ± 0.005^{BCD}
CGM63	230 ± 4.8^{CD}	206 ± 2^{AB}	0.98 ± 0.03^{B}	0.270 ± 0.003^{CD}	0.296 ± 0.022^{CD}
CGM82	234 ± 0.5^{CD}	184 ± 1^{DE}	0.86 ± 0.01^{CDE}	0.272 ± 0.001^{CD}	0.396 ± 0.017^{ABC}
CGM100	220 ± 2.1^{D}	209 ± 5^{A}	1.04 ± 0.02^{A}	0.263 ± 0.002^{D}	0.258 ± 0.015^{D}

[1] Mean of three replicate tanks ± SEM. Initial fish weight was 19.5 g.
[2] FM is the fishmeal control diet. SPC, BPC and CGM are the soy protein concentrate, barley protein concentrate and corn gluten meal blends, respectively. Numbers denote the percentage of fishmeal replaced with the alternate protein blend.
[3] Values within columns having a common superscript letter do not differ significantly ($P > 0.05$).
[4] FCR = feed fed (g)/weight gain (g); TGC = (Final fish weight $^{1/3}$ – initial fish weight $^{1/3}$/degree days) * 100; PPV = (protein intake per fish/protein gain per fish).

Table 7
Final weight, feed intake, feed conversion ratio (FCR), thermal growth coefficient (TGC) and productive protein value (PPV) for early stage juvenile Atlantic salmon fed experimental diets for 18 weeks.[1]

Diet[2]	Final weight (g)[3]	Total feed fed (g/ fish)[3]	FCR[3,4]	TGC[3,4]	PPV[3, 4]
FM control	27.29 ± 0.16[A]	40.18 ± 0.55	1.85 ± 0.03[C]	0.075 ± 0.001[A]	0.236 ± 0.002[A]
SPC50	21.83 ± 2.17[B]	37.25 ± 2.31	2.31 ± 0.14[B]	0.062 ± 0.006[BC]	0.185 ± 0.009[BC]
SPC66	22.90 ± 0.17[B]	37.61 ± 0.41	2.18 ± 0.63[B]	0.065 ± 0.002[B]	0.195 ± 0.003[B]
SPC87	20.31 ± 1.48[B]	35.74 ± 1.44	2.42 ± 0.12[AB]	0.059 ± 0.005[BC]	0.173 ± 0.007[BC]
WGM50	22.11 ± 2.31[B]	35.31 ± 3.60	2.17 ± 0.09[B]	0.062 ± 0.008[BC]	0.196 ± 0.004[BC]
WGM66	22.97 ± 1.51[B]	37.92 ± 0.88	2.22 ± 0.13[B]	0.064 ± 0.004[BC]	0.186 ± 0.008[BC]
WGM87	19.23 ± 0.87[B]	35.44 ± 0.96	2.63 ± 0.06[A]	0.054 ± 0.002[C]	0.156 ± 0.004[C]

[1] Means of two replicate tanks ± SEM for plant-protein diet; three replicate tanks for the FM control treatment group. Initial average weight of the fish was 5.5 g.
[2] FM is the fishmeal control diet. SPC and WGM are the soy protein concentrate and wheat gluten meal blends. The number denotes the percentage of fishmeal replaced with the protein blend.
[3] Values within columns having a common superscript letter do not differ significantly ($P > 0.05$).
[4] FCR = feed fed (g) / weight gain (g); TGC = (Final fish weight $^{1/3}$ – initial fish weight $^{1/3}$/degree days) * 100; PPV = Productive protein value (protein intake/protein gain).

limiting or that utilization of dietary protein and/or protein turnover may be altered in trout fed diets containing the highest levels of plant-protein blend. Further research is required to identify the factor(s) responsible for these observations.

This is the first study to our knowledge that compared the effects of feeding diets with alternative protein blends on the performance of early stage Atlantic salmon juveniles with that of late stage juveniles. The results show that growth was reduced in early stage juvenile salmon even when the fish were fed diets containing 30% fishmeal despite the fact that blends contained approximately one-third land animal proteins (poultry by-product meal and blood meal) and met or exceeded the dietary requirements of the fish (NRC, 2011). The early stage juvenile salmon were purposely overfed at 110% of their expected intake so that feed availability would not limit fish growth. This resulted in higher FCRs than those reported in other studies and made it impossible to judge the effects of diet formulation on feed intake. PPV values were much lower in the early stage juvenile salmon than in the rainbow trout study. Growth rates of early stage juveniles in the present study were two to three times lower than rates reported in the literature (Austreng et al., 1987; Berge and Storebakken, 1996). This can likely be attributed to the fact that the published studies were conducted in the spring and summer where photoperiod was increasing whereas our study was conducted in during fall and early winter months as photoperiod and water temperatures were decreasing. Rainbow trout have been shown to have over a four-fold reduction in growth rate (from 2.0%/day to >0.5%/day) when the photoperiod was reduced to 8L:16D which was similar to the photoperiod at the end of our study (Taylor et al., 2005). Juvenile Atlantic salmon are similarly affected by photoperiod changes.

Late stage Atlantic salmon juveniles (31.5 g initial weight) in the second salmon study responded differently than early stage juvenile salmon, having similar weight gains and FCR values in all dietary treatment groups, including the fishmeal control group. The growth rates of the late stage juvenile salmon in the present study were equivalent to those reported by Austreng et al. (1987) and remained steady throughout the study. The late stage juvenile salmon in the second salmon feeding trial were exposed to an increasing photoperiod because the study started in late winter and concluded in late spring. This likely increased feed consumption, leading to faster growth in the second study compared to the first.

Our results suggest that even when fishmeal is included at 30% of the diet, early stage juvenile Atlantic salmon (5 g) will not grow as fast as fish fed a diet containing 60% fishmeal. However, growth performance of late stage juvenile Atlantic salmon fed fishmeal-free diets was equivalent to fish fed a fishmeal control diet. This results is in contrast to earlier published studies in which larger juvenile Atlantic salmon exhibited reduced growth when fed diets containing less than 30% fishmeal and high levels of plant proteins, supplied as blends or as single protein sources. Although Refstie and Tiekstra (2003) and Øverland et al. (2009) found that feeding Atlantic salmon (82 g or 160 g respectively) diets that contain at least 30% fishmeal resulted in similar growth to fish fed 60% fishmeal diets, Mundheim et al. (2004) found that growth was reduced when fishmeal constituted 29% or 19% of the diet for 130 g Atlantic salmon. Drew et al. (2007) and Torstensen et al. (2008) found similar results for Atlantic salmon with initial weights of 48 g and 350 g respectively. Refstie et al. (2001) reported that growth of Atlantic salmon fed diets containing soybean meal or soy protein concentrate was not significantly different from salmon fed a fishmeal diet; however, fishmeal levels their diets exceeded 36%. All of these studies used fish that were at least smolt size at the beginning of feeding trials, with the smallest fish in the studies initially weighing 48 g.

Post-juvenile Atlantic salmon with an initial weight of approximately 950 g did not exhibit any differences in growth when fishmeal was replaced with soybean meal (12.7% of the diet) or wheat gluten (19.7%) (Storebakken et al., 2000). However, fishmeal levels were either 43.4% (soybean meal diet) or 32.1% (wheat gluten diet). The effects of a plant-protein blend containing sunflower expeller, corn gluten meal, soy protein concentrate, and wheat gluten meal on growth and lipid composition of Atlantic salmon were examined by Pratoomyot et al. (2011). This study found that sub-adult salmon (1.3 kg initial weight) grown to harvest weight could tolerate this plant-protein blend as long as fishmeal was at least 25% of the diet. Fish growth was significantly lower for fish fed diets that contained 18, 11 or 5% fishmeal (Pratoomyot et al., 2011). When fishmeal was completely replaced in post-juvenile Atlantic salmon (330 g initial weight), the fish exhibited significantly lower weight gains compared to fish fed a diet containing 49% fishmeal (Espe et al., 2006).

Our results are the first to show that late stage juvenile Atlantic salmon fed diets containing alternate protein blends in place of fishmeal grow as fast as fish fed a fishmeal-based diet, whether or not the alternate protein blends contained land animal proteins or *Spirulina*, an beneficial ingredient in feeding trials with juvenile

Table 8
Final weight, apparent feed intake, feed conversion ratio (FCR) and thermal growth coefficient (TGC) for late stage juvenile Atlantic salmon fed the experimental diets for 12 weeks.[a]

Diet[b]	Final weight (g)	Total feed fed (g/ fish)	FCR[c]	TGC[c]	Survival (%)
FM control	106.3 ± 6.8	74.9 ± 5.8	0.86 ± 0.02	0.128 ± 0.011	94.3 ± 0.3
LAP blend	103.2 ± 6.0	75.8 ± 2.8	0.95 ± 0.02	0.120 ± 0.013	94.8 ± 1.5
LAP-S blend	109.9 ± 4.0	70.0 ± 3.7	0.94 ± 0.04	0.133 ± 0.015	96.3 ± 2.4
PP blend	109.7 ± 3.7	74.9 ± 5.8	0.86 ± 0.02	0.135 ± 0.011	94.3 ± 0.3
PP-S blend	101.7 ± 4.3	75.9 ± 2.6	0.93 ± 0.01	0.120 ± 0.005	92.4 ± 1.1

[a] Means of three replicate tanks ± SEM. Initial average fish weight was 31.5 g.
[b] FM is the fishmeal control diet. LAP is the land animal–plant-protein blend and PP blend is the plant-protein blend. Addition of *Spirulina* algae to the blend is designated by S.
[c] FCR = feed fed (g) / weight gain (g); TGC = (Final fish weight $^{1/3}$ – initial fish weight $^{1/3}$/degree days) * 100.

marine fish (R. Barrows, unpublished data). A likely explanation for the positive results found in the present study using fishmeal-free diets compared to results of other published research is that in the present study, diets were supplemented with essential amino acids, minerals and other compounds, including a vitamin premix specifically developed for use in plant-based feeds for salmonids (Barrows et al., 2010).

5. Conclusions

Rainbow trout fingerlings fed a diet in which up to 87% of fishmeal was replaced with an alternate protein blend containing SPC had similar growth to fingerlings fed a fishmeal diet, but trout fed other protein blends based on CGM or BPC had lower weight gains. Early stage juvenile Atlantic salmon (5 g) grew poorly when fed diets containing alternate protein blends compared to growth rates of fish fed a fishmeal control diet even when fishmeal was included at 30% of the diet. However, late stage juvenile Atlantic salmon (initial weight 31.5 g) grew well on diets containing plant-protein blends, even when fishmeal and land animal protein ingredients were completely replaced in the diet. These results demonstrate that advance stage juvenile Atlantic salmon can be reared using fishmeal-free diets, although further testing in commercial settings should be undertaken.

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