Evaluation of barley protein concentrate (BPC) and fish protein concentrate, made from trimmings, as sustainable ingredients in Atlantic salmon (*Salmo salar* L.) feeds

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Abstract

Barley protein concentrate (BPC) was tested as a protein source in the diets of Atlantic salmon post-smolts. Fish were fed one of four experimental diets consisting of a fishmeal/soya protein concentrate control (CT) along with 2 feeds supplemented with increasing levels of BPC replacing the more costly SPC. A fourth diet partially replaced FM in the high BPC diet with a liquid fish protein concentrate (FPC) made from fish trimmings. No significant differences were observed in terms of growth at the end of the 12–week feeding period, although the protein efficiency ratio (g gain/g protein consumed) was significantly lower for the control compared to fish fed diets containing either BPC only or FPC and BPC. This suggests that diets containing BPC had a beneficial effect when compared to the control diet. Furthermore, the lack of any detriment to fish growth in diets containing BPC suggests there are no significant issues regarding any negative effects of potential anti-nutritional factors which can otherwise be the case with other plant origin products. The data presented in this study indicate that BPC and FPC are products which could be of benefit to salmon culture, and related species, in providing a valuable new raw material to the industry.

Key words: Atlantic salmon post-smolts, barley protein concentrate (BPC), fish protein concentrate (FPC), fish meal replacement, growth, fish meal

Introduction

Aquafeeds represent a significant part (30-70%) of the overall costs with respect to fish production (Cheng et al., 2003; FAO, 2010). The traditional marine-based protein sources derived from fishmeal (FM) are no longer available in quantities that meet the increased demands of modern intensive fish farm production and, as such, the future growth of aquaculture relies upon the development of a range of sustainable protein sources to replace FM (Gatlin et al., 2007). Numerous products have already been tested as potential FM replacements in aquafeeds, including animal by-products (Suigura et al., 2000; Yamamoto et al., 2002), single cell proteins (Lunger et al., 2007) and lupin seed meal (Gomes et al., 1995) among others. However, some proteins are less favoured, such as soybean meal, which can cause intestinal enteritis although this varies with both fish species and size and may be related to the presence of anti-nutritional factors (Olli et al., 1994). Soya protein concentrates (SPC) can provide a product of high protein content, good amino acid profile as well as good nutrient digestibility and palatability but are often less favoured due to cost fluctuations (Gatlin et al., 2007).

Recent studies where concentrated plant proteins were fed to rainbow trout (*Oncorhynchus mykiss*) provide good evidence that such products can replace FM with no or only minor reductions in growth (Gaylord et al., 2006; Barrows et al., 2009; Gaylord and Barrows, 2009). With FM prices continuing to rise, the aquaculture industry will need to test and validate a range of alternative protein ingredients to maintain high quality products acceptable to both fish producers and consumers alike.

Salmonids, such as Atlantic salmon (*Salmo salar*), have a high demand for protein in the region of 40-50% to maintain optimum performance (Hardy, 1996). In a recent study, Bell et al. (2010) successfully grew salmon smolts from 85 g to 3 kg over a 13 month period using a blend of plant proteins including extracted soybean meal, wheat,

SPC and corn gluten totalling 45% of the formulation and only 25% FM inclusion. Several plant proteins have been used for a range of salmonid species including rainbow trout, Arctic charr (*Salvelinus alpinus*) and Atlantic salmon (Reftsie et al., 2006; Overland et al., 2009; Davidson et al., 2013; Wolters et al., 2013) and digestibility studies in Atlantic salmon have shown a range of plant-based proteins to be similar to FM with exceptions for bacterial protein meal, extracted soybean meal, oats, canola and sunflower (Glencross et al., 2004; Reftsie et al., 2006; Denstaldi et al., 2007; Kraugerud et al., 2007). Furthermore, when the plant feeds were further processed to provide protein concentrates the digestibility remained unaffected (Glencross et al., 2004; Denstaldi et al., 2007). Barley Protein Concentrate (BPC) is a relatively new product, with production on a pilot scale level. However, current investigations suggest that the cost of BPC will be lower than for SPC while providing a similar protein content. Furthermore, BPC production uses a process that produces an ethanol co-product, improving the commercial viability of the process. Unlike the traditional distillers dried grains, this process is designed to protect the protein from heat damage as well as removing fibre and it is predicted that BPC will be priced competitively to the fermented SPC products. Replacing SPC with BPC should reduce the overall feed costs and provide an additional high quality ingredient to allow feed formulators to manage fluctuating ingredient prices. This new process produces a BPC product containing around 50-60% protein on a dry matter basis. However, limited studies using BPC have been performed in Atlantic salmon although results appear promising up to inclusion levels of 22% (Burr et al., 2013).

Fish protein concentrate (FPC) is a co-product of fishery processing, where the unused portion is processed into a high quality liquid product. FPC contains many of the

flavour compounds from the trimmings and may have beneficial effects with respect to feed intake when added to a high plant protein diet.

The primary aim of the present study was to test the BPC in combination with low levels of FM inclusion (15%) as a replacement for the more costly SPC in the diets of Atlantic salmon post-smolts to assess fish performance. In addition, FPC was tested as a partial replacement for FM together with BPC.

Materials and Methods

Fish populations and experimental set-up

Atlantic salmon post-smolts were sourced from the University of Stirling's ongrowing facility at the Niall Bromage Freshwater Research Unit (Buckieburn, Stirling) and transferred to the Marine Environmental Research Laboratory (MERL) at Machrihanish, Campbeltown, on the west coast of Scotland on the 6th May 2013. A total of 336 fish (90.5 ± 3.5 g, mean ± SD) were equally stocked into 12 circular 1 m diameter (500 L water volume) glass-reinforced plastic tanks (28 fish per tank), with conical bottoms, with seawater supplied by flow-through at a minimum rate of 1 L.min^{-1} per kg fish. Each diet was fed to triplicate tanks of fish. Fish were reared under natural photoperiod and temperature (range 13-14^{o}C) and at constant salinity (33 ppt). Fish were fed in excess of recommended feeding rates by hand with 3-4 meals per day. Uneaten feed was collected and recorded approximately 30 min after each meal. Fish were acclimated to tanks for a period of 6 days prior to the commencement of feeding the test feeds on the 13th May 2013.

Experimental diets

Four experimental diets utilising either SPC, BPC and FPC were formulated so that the three most limiting amino acids, lysine, methionine and threonine were supplemented to match the amino acid profile of salmon muscle at 3.3%, 1.2% and 1.7% of the diet respectively (see Table 1 for characterisation of test ingredients). All diets were formulated to be to be isonitrogenous (42% crude protein) and iso-lipidic 20% crude fat (Table 2) with poultry meal, corn protein concentrate and wheat gluten content held constant in all four diets. The commercial type diet (CT) contained 14.8% sardine meal and 20% SPC. For the next 2 diets the sardine meal content remained the same but BPC replaced half the SPC content in diet 2 (BPC50) and all SPC inclusion in diet 3 (BPC100) so that similar ingredients replaced each other. The BPC had 531 $g.kg^{-1}$ crude protein compared to 693 $g.kg^{-1}$ crude protein so slightly more BPC was required to replace the SPC. The fourth experimental diet contained the higher level of BPC (21.5%), no SPC and replaced part of the sardine meal with a liquid fish protein concentrate on an equal dry protein basis (BPC/FPC). All diets were supplemented with mono-dicalcium phosphate to maintain a dietary phosphorus content of 1.2%.

Diets were processed using a twin-screw cooking (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to 127^{o}C average across the 6 extruder barrel sections. The die plate was water cooled to an average temperature of 60^{o}C. Pressure at the die head varied from 27 to 31 bar depending on diet. Pellets were dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102^{o}C with a 10 min cooling period to provide a final moisture level of less than 10%. A top coating of fish oil was added to all feeds using a vacuum-assisted top-coater (A.J. Mixing, Ontario, Canada). The diets contained carotenoid pigment (Carophyll Pink; DSM Nutritional Products, Heerlen, Netherlands) at a concentration of 100 $mg.kg^{-1}$ diet (11% active product). Diets

(3 mm pellet size) were stored in plastic lined paper bags at room temperature until fed and used within 6 months from manufacture. All diets were formulated to satisfy the nutritional requirements of salmonid fish (NRC, 2011).

Sampling procedures

At the end of the trial all remaining fish were killed by an overdose of metacaine (MS-222) anaesthetic, length-weighed and the viscera removed and the carcass re-weighed to determine head-on dressed weight. The carcass and viscera from 5 fish per tank (15 per dietary treatment) were collected, frozen on dry ice and stored at -20^{o}C until proximate analysis was conducted. In addition, the fillet from the left side was removed from four fish per tank (12 per dietary treatment), frozen on dry ice and stored at -20^{o}C until analysed for pigment concentration. The specific growth rate (SGR) was calculated as: SGR (%bw.day^{-1}) = $100\times[\ln(W_F/W_I)/d]$, where W_F and W_I are the final and initial weights (g) respectively, and d is the number of days. The Thermal Growth Coefficient (TGC) was also calculated as TGC = $(W_F^{1/3} - W_I^{1/3}) \times (1000/DD)$, where W_F and W_I are as previously addressed for SGR and DD is the cumulative daily water temperature (^{o}C) in SW. Net protein utilisation (NPU) was determined as NPU (%) = $100\times[(PF-PI)/TPF]$, where PF and PI are final and initial body protein levels (g) and TPF is total protein fed (g).

Proximate compositions of diets and whole fish

Samples of diets were analysed using standard methods to determine crude lipid by acid hydrolysis of soxhlet samples (Tecator Soxtec method); moisture content by drying at 105^{o}C until constant weight (AOAC, 2000); crude protein (Kjeldahl, calculated as

N×6.25); and ash content by combustion in a muffle muffle furnace at 600^{o}C (AOAC, 2000).

Whole fish were analysed by first determining the moisture content of individual samples (AOAC, 2000) before samples were homogenized and analysed as described above for diets, with exception to crude lipid which was analysed by soxhlet without acid-hydrolysis. Moisture content was then used to convert results on a wet weight basis.

Pigment analysis

Flesh astaxanthin levels were determined using a modified method of Barua et al. (1993). Briefly, tissue samples (~1 g) were homogenised in 10 ml of absolute ethanol/ethyl acetate (1:1 vol) using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) with the homogenate centrifuged (1000 x *g*, 5 min) and the supernatant removed to a clean glass tube. The pellet was re-homogenised and centrifuged a further two times, first in 5 ml ethyl acetate followed by 5 ml isohexane, with the removal and combination of supernatants. The combined supernatant was dried under a nitrogen stream at room temperature and desiccated overnight *in vacuo* before resuspending in 2 ml isohexane prior to HPLC analysis. Samples were injected on a Thermo Scientific Ultimate 300 UHPLC system equipped with a 50 x 3 mm 1.7 µ Synchronis Silica Column (Thermo Scientific, UK) with detection at a wavelength of 470 nm. An isocratic solvent system was used consisting of isohexane/acetone/isopropanol (82:16:2 v/v/v) at a flow rate of 0.5 ml.min^{-1}. Astaxanthin and lutein were quantified using an external standard of astaxanthin obtained from DSM (Heerlen, Netherlands).

Statistical analysis

Statistical analyses were performed using Graphpad Prism™ (version 4.0) statistical package (Graphpad Software, San Diego, CA, USA). Data were assessed for normality with the Kolmogorov-Smirnov test and, where necessary, transformed using the natural logarithm or arcsine transformation. Data were compared by a one-way analysis of variance (ANOVA), with replicate tanks nested within their dietary treatments. Post hoc comparisons using Tukey's test. A significance of $P<0.05$ was applied to all tests performed.

Results

Diets

The analysed values for the proximate compositions of the experimental feeds used during the study are presented in Table 2. Moisture contents were 6.1, 5.5, 5.4 and 4.3% for (CT), BPC50, BPC100 and BPC/FPC respectively. Dietary lipid value content was 16.9% for CT, 19.3% for BPC50, 19.3% for BPC100, and 20.1% for BPC/FPC The protein contents were 42.8, 41.7, 42.8 and 43.2% and ash content values 7.0, 6.7, 6.4 and 6.3% for CT, BPC50, BPC100 and BPC/FPC respectively. Diets were close to the formulated values for crude protein and three of the diets were close for crude lipid, but the commercial type diet was approximately 2% lower in lipid compared to the other test diets.

Growth and fish quality parameters

At the end of the 12-week feeding period no significant differences were observed between the 4 dietary treatments for either final weight, length, head on dressed weight, or viscera weights (Table 3). Similarly, no significant differences were seen in total feed

211 consumption or FCR values, although diets containing BPC showed the lowest FCR
212 values (1.00 ± 0.05, 1.01 ± 0.03 and 1.03 ± 0.01, BPC/FPC, BPC50 and BPC100
213 respectively) relative to CT (1.17 ± 0.14). The SGR of fish ranged from 0.77 in BPC100
214 to 0.81% bw.day^{-1} in BPC50, although no significant differences were found between
215 treatments. There were no significant differences between other growth parameters
216 namely the average daily weight gain, protein intake and net protein utilization.
217 However, the protein efficiency ratio of CT fed fish was significantly lower than the
218 other dietary treatments containing BPC.

219 Data for moisture and crude lipid on whole fish analysis showed no significant
220 (P>0.05) differences between dietary treatments (Table 5). However, the ash content for
221 BPC50 fish was significantly lower than the ash content of fish from the other 3
222 treatments. Similarly, the protein content of BPC50 was also found to be lower than fish
223 fed CT, 17.54 ± 0.63 compared to 18.21 ± 0.48% respectively.

224 Mortality losses were generally low throughout the 12-week study (Table 3).
225 However, an outbreak of amoebic gill disease (AGD) on the 17th July 2013 affected one
226 of the tanks from the BPC100 treatment, resulting in a loss of 8 fish. The AGD problem
227 was quickly rectified by flushing tanks with freshwater for two hours, on a monthly
228 basis, and no further AGD related mortalities occurred thereafter. Despite the AGD
229 event affecting one tank from the BPC100 dietary treatment no statistical differences
230 between replicate tanks were recorded, thus all tanks from this treatment were included
231 for all statistical parameters measured.

232

233 *Carotenoid pigment levels*

234 Total carotenoid pigment levels, astaxanthin (Ax) and lutein from flesh fillets of
235 fish fed the experimental diets are provided in Table 4. Results for both total pigment

and Ax were highly variable within treatments and, as such, no significant differences were observed between treatments.

Discussion

The continued expansion and development of aquaculture production worldwide requires that sustainable alternatives for FM and fish oil (FO) are investigated, developed and introduced for use in feed formulations as a matter of urgency, due to the increasing global demand for farmed fish production (FAO, 2010). The results from this study indicate that BPC has the potential to replace the more costly SPC as a protein source in fish feeds without affecting the growth of Atlantic salmon. In addition, FPC could replace some of the FM to save costs and perhaps improve feed consumption.

Since FM and FO are currently exploited to the maximum levels, and no comparable marine-based alternatives are available, the industry has turned to plant-based products to fill the void left by declining FM availability. However, there are an increasing number of plant-based products on the market, which can contribute to the replacement of FM in feed formulations and can provide valuable sources of both protein and energy. These include grain products; principally corn and wheat glutens, and oilseeds including canola, cottonseed, soybean, and palm and sunflower meals. Additional products have included those derived from legumes such as lupins, beans, peas and peanuts, among others (NRC, 2011).

Nutrient profiles provided by FM products are largely devoid of anti-nutritional factors and are also endowed with high levels of essential amino acids that promote growth and development. By comparison plant-based feeds generally contain some anti-nutritional factors, although these can be reduced by appropriate pre-treatment processing methodologies as well as supplementation with appropriate amino acid

261 packages (de Francesco et al., 2004; NRC, 1993). One advantage of barley is that the
262 only known anti-nutrient it contains is phytate, which is relatively benign and readily
263 removed during the concentration process.

264 In this study, the BPC protein replacement product (Montana Microbial Products)
265 contained a high level of crude protein (53%) which has been shown to give 96%
266 protein digestibility in Atlantic salmon (Burr et al., 2011). The diets formulated
267 contained an equal amount of sardine meal (14.8%), with exception to the BPC/FPC
268 diet, which had a mixture of sardine meal and FPC (Table 1). Given that the main
269 nutritional components were similar among the 4 diets, with the exception of BPC
270 inclusion level, which increased from 0% in CT to 10.75% in BPC50 and 21.5% in
271 BPC100 and BPC/FPC diets, it is not surprising that there were no significant
272 differences between the 4 dietary treatments at the end of the 12-week study period. The
273 goal was to formulate practical type diets that would produce good growth if the
274 nutrients in BPC were readily available and it did not contain anti-nutrients.

275 Growth rates were not significantly different for the fish fed the four dietary
276 treatments at the end of the trial (Table 3). Under normal circumstances we would have
277 expected the smolts to double their weight over the 12-week trial period and at the end
278 of the study the smolts were just short of this target, although values were within normal
279 ranges expected for salmon grown under similar conditions within the same tank culture
280 systems (W. Roy, pers. comm). Numerically, the highest final growth was seen in fish
281 fed BPC50 closely followed by diets BPC100 and BPC/FPC respectively, with the
282 lowest growth in fish fed CT, which had no BPC supplement. This could be due to an
283 apparent mixing error resulting in the CT diet containing approximately 2% less lipid.
284 Similarly, the higher amount of wheat flour used in the CT diet (23.2%) compared to
285 BPC50 (21.9%) and BPC100 and BPC/FPC (20.7%) may have interfered with the

uptake of minerals and nutrients. This possibility is supported by the slightly higher FCR ($P = 0.08$) and the slightly lower NPU and PER values for the fish fed CT diet compared to all other diets. Including such levels of wheat flour provided adequate water stability (min. 6 hour) in order to collect intact uneaten feed as well as allowing for expansion of the feed to absorb the top-coated fish oil (~11%). This was achieved by including 4% wheat gluten which has 778 g/kg crude protein compared to 693 for SPC and 531 for BPC. Thus, diets with lower protein BPC had 20% wheat flour and diets with SPC had 23%, within range of wheat flour levels of 20-30% that have been used effectively in other studies (e.g. Barrows et al., 2007; Burr et al., 2013). Furthermore, diets containing BPC exhibited a lower FCR value than fish fed the CT diet, suggesting that diets containing BPC give better performance than the control. However, the higher levels of BPC in the BPC100 and BPC/FPC diets did not significantly improve growth compared to the lower BPC ration given in the BPC50 diet. It might be the case that no improvement in fish growth was seen in the BPC100 and BPC/FPC fed fish because there was no additional impact of anti-nutritional factors in these 2 diets which had twice the amount of BPC compared to the BPC50 diet. The data collected from diets containing BPC suggest that BPC was a useful addition to the total diet formulation and indicates that BPC, at levels up to 22%, could provide benefits for the culture of Atlantic salmon and related salmonid species (Burr et al., 2013), particularly with respect to replacing the more costly SPC alternative ingredient currently favoured by the industry. The other replacement product chosen for this study, FPC (Scanbio AS), is a co-product of fishery processing, used with the intent to further reduce the inclusion of fishmeal and enhance feed intake of fish fed the high BPC diet. The addition of FPC, while reducing fishmeal in the high BPC diet (BPC/FPC), did not affect the performance of the salmon post smolts during the 12-week study. Including FPC

311 improves the sustainability of the diet compared to the other three experimental diets.
312 As Fishery processing products are not considered by many environmental groups as a
313 "wild fish" input, there is positive effect of lowering the Fish-in Fish-out ratio when
314 FPC is included in the formulation.

315 The 3 diets based on BPC inclusion represent a significant potential benefit in terms
316 of future salmon feed formulations which is a requirement for the on-going
317 development of new aquafeeds to ensure aquaculture food security in the 21st century.
318 All four diets used in this study had the majority of their protein components presented
319 in the form of plant-based alternatives with 54.7, 54.2, 53.7 and 53.7% (CT, BPC/50,
320 BPC/100 and BPC/FPC respectively) of protein provided by plant products and 28% of
321 protein provided by FM, poultry meal and FPC. These diets are comparable with other
322 sustainable feeds including those developed for salmonids by Kaushik et al. (1995) and
323 Espe et al. (2006) where the latter showed lower growth performance when fed fish
324 meal-free diets, containing corn and wheat glutens, fish solubles plus a package of
325 crystalline amino acids. While early attempts to replace FM with plant proteins resulted
326 in reduced fish growth this was generally related to reduced feed intake and effects
327 related to gut integrity which left protein and amino acid digestibility's unaffected
328 (Kaushik et al., 2004; Espe et al., 2006; Aslaksen et al., 2007; Glencross et al., 2005).
329 More recent studies using plant protein and plant oils included a range of components
330 including extracted soybean meal, wheat, wheat gluten and soya concentrate. This
331 provided total plant protein levels of 45% in a 6mm pellet and 39.9% in a 9mm pellet
332 (Bell, et al., 2010), which is lower than the plant protein level in the current study and
333 reflects the advances made in feed formulations over recent years. At the same time,
334 fish oil was partially replaced with rapeseed, palm and Camelina oils. In a related study
335 with Atlantic salmon, a maximum plant based diet was tested, where 80% of FM was

336 replaced with a mixture of plant protein products including wheat, wheat gluten, corn
337 gluten, extracted soybean meal and krill meal plus a mixture of vegetable oils (linseed,
338 rapeseed & palm oils). In addition, two intermediate replacement diets contained either
339 half this amount of FM replacement and maximum fish oil replacement or one half
340 replacement of fish oil and maximum FM replacement (Torstensen et al., 2008). SGR's
341 were significantly reduced in the combined high replacement group compared to the
342 intermediate experimental groups such that fish weights were 17% lower in the high
343 replacement group but only 9% lower in the high plant protein group compared to a
344 marine control and intermediate plant protein group (Torstensen et al., 2008). In
345 addition, the maximum FM and fish oil replacement diet provided a net production of
346 fish protein with 2 kg of salmon protein produced per kg of FM protein fed.

347 The results of the current study are in agreement with Burr et al., (2013) where
348 BPC was found to be a nutritious ingredient that supported growth performance
349 equivalent to Atlantic salmon fed a practical type fishmeal based diet. In that study, as
350 the inclusion rate for BPC increased from 0 to 11% to 22% the inclusion rate of
351 fishmeal, poultry meal, soy protein concentrate and corn protein concentrate were
352 reduced equally.

353 The levels of measured carotenoid pigment astaxanthin and lutein showed a high
354 degree of variability between individual fish within treatments resulting in no
355 significant differences between dietary treatments (Table 4). Generally, Atlantic salmon
356 start depositing carotenoids at around 200 g, with retention around 10-15% of the
357 ingested carotenoid (Ytrestøyl et al., 2006). In the present study, the post smolts
358 weighed only 90.5 g at the onset of the trial reaching around 200 g at final sampling.
359 Thus, carotenoid deposition would have been limited and, as such, fish would only have
360 begun to start laying down significant levels of pigment towards the end of the trial as

body weights reached 200 g. Nevertheless, if a longer scale trial with BPC is conducted in the future then it is likely that better levels of pigment deposition would be expected with an increase in fish size.

Although there were no significant differences in most growth parameters the slightly improved feed utilisation efficiency, in terms of FCR, PER and NPU, shown by fish fed with BPC or BPC/FPC diets compared to CT fed fish provide good evidence that BPC can be a valuable new ingredient for use in aquaculture applications. Provided sufficient volumes of BPC and FPC can be made available to the industry, we are hopeful that BPC will prove to be a useful new aquafeed product in the near future.

It is essential for the future development of global fed aquaculture that we continue to develop new formulated feed products that are ethical, economical and sustainable. As FM-based feeds become more expensive, due to price increases, then the protein demand will need to be met using largely plant-based alternatives, and processing co-products from other industries. This study demonstrated that a newly developed protein concentrate from barley can effectively replace 20% SPC in a low fish meal, commercial type diet. While there were no significant differences between the four dietary treatments in terms of fish growth, the BPC product and the combination of BPC and FPC, clearly showed potential for further testing and development in salmonids and other species. Currently, salmonid feeds are using lower levels of FM, with levels of 30% in post-smolts reducing to 5% or even lower in larger fish in seawater. The application of BPC and other plant proteins as FM replacers should be tested in larger fish to investigate the potential for growth in low FM diets.

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References

AOAC, (2000) Official methods of analysis (17th ed.). Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Aslaksen, M.A, Kraugerud, O.F., Penn, M., Svilhus, B., Denstadli, V., Jørgensen, Hillestad, Krogdahl, Å., Storebakken, T. (2007) Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. *Aquaculture*, **272**, 541-555.

Barrows, F.T., Gaylord, T.G., Stone, D.A.J., Smith, C.E. (2007) Effect of protein source and nutrient density on growth efficiency, histology and plasma amino acid concentration of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquacult. Res*, **38**, 1747-1758.

Barua, A.B., Kostic, D., Olsen, J.A. (1993) New simplified procedure for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum. *J. Chromatogr.*, **617**. 257-264.

Bell, J.G., Pratoomyot, J., Strachan, F., Henderson, R.J., Fontanillas, R., Hebard, A., Guy, D.R., Hunter, D., Tocher, D.R. (2010) Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh

adiposity: Effects of replacement of dietary fish oil with vegetable oils. *Aquaculture*, **306**, 225-232.

Burr, G., Barrows, F.T., Gaylord, G., Wolters, W. (2011) Apparent digestibility of macro-nutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar*, and Arctic charr, *Salvelinus alpinus*. *Aquacult. Nutri.,*, **17**, 570-577.

Burr, G., Barrows, F.T., Wolters, W. (2013) Growth of Atlantic salmon, *Salmo salar*, fed diets containing barley protein concentrate. *Journal of Applied Aquaculture*, **25**, 320-328.

Cheng, Z.J., Hardy, R.W., Usry, J.L. (2003) Plant protein ingredients with lysine supplementation reduce dietary protein level in rainbow trout (*Oncorhynchus mykiss*) diets and reduce ammonia nitrogen and soluble phosphorous excretion. A*quaculture*, **218**, 553-565.

Davidson, J., Good, C., Barrows, F., Welsh, C., Kenney, P.B., Sumerfelt, S.T. (2013) Comparing the effects of feeding grain- or a fish meal-based diet on water quality, waste production, and rainbow trout, *Oncorhyncus mykiss*, performance within a low exchange water recirculating aquaculture system. *Aquaculture Engineering*, **52**, 45-47.

Denstaldi, V., Storebakken, T., Svilhus, B., Skrede, A. (2007) A comparison of online phytase pre-treatment of vegetable feed ingredients and phytase coating in diets for Atlantic salmon (*Salmo salar* L.) reared in cold water. *Aquaculture*, **269**, 414-426.

De Francesco, M., Parisi, G., Medale, F., Kaushik, S.J., Poli, B.M. (2004) Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhnchus mykiss*). *Aquaculture*, **263**, 413-429.

434 Espe, M., Lemme, A., Petri, A., El-Mowafi, A. (2006) Can Atlantic salmon (*Salmo*
435 *salar*) grow on diets devoid of fish meal? *Aquaculture*, **255**, 255-262.
436 FAO. (2010) The state of world fisheries and aquaculture. Food and Agricultural
437 Organisation of the United Nations (FAO), Rome, Italy, pp 218.
438 Gatlin, D.M. III. Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W.,
439 Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W.,
440 Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E. (2007) Expanding the
441 utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res*, **38**,
442 551-579.
443 Gaylord, T.G., Teague, A.M., Barrows, F.T. (2006) Taurine supplementation of all-
444 plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Journal of the World*
445 *Aquaculture Society*, **37**, 509-517.
446 Gaylord, T.G., Barrows, F.T. (2009) Multiple amino acid supplementation to reduce
447 dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*,
448 **287**, 180-184.
449 Gomes, E.F. Rema, P., Kaushik, S.J. (1995) Replacement of fishmeal by plant proteins
450 in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth
451 performance. *Aquaculture*, **130,** 177-186.
452 Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P.,
453 Hawkins, W.E., Mass, R., Sipsas, S. (2004) A comparison of the digestibility of a
454 range of lupin and soybean protein products when fed to either Atlantic salmon
455 (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **237**, 333-346.
456 Glencross, B.D., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Mass, R.,
457 Sipsas, S. (2005) Evaluation of the digestible value of lupin and soybean protein

concentrates and isolates when fed to rainbow trout (*Oncorhynchus mykiss*), using either stripping or settlement faecal collection methods. *Aquaculture*, **245**, 211-220.

Hardy, R.W. (1996) Alternative protein sources for salmon and trout diets. *Animal Feed Science and Technology*, **59**, 71-80.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M. (1995) Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic effects, cholesterolemia and flesh quality in rainbow trout. *Aquaculture*, **133**, 257-274.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D. (2004) Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*, **230**, 391-404.

Kraugerund, O.F., Penn, M. Storebakken, T., Refstie, Krogdhal, Å., Svihus, B. (2007) Nutrient digestibilities, gut function and morphology in Atlantic salmon (*Salmo salar*) fed diets where non-starch polysaccharides from soy replace cellulose. *Aquaculture*, **273**, 96-107.

Lunger, A.N., McLean, E., Craig, S.R. (2007) The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters of juvenile cobia (*Rachycentron canadum*). *Aquaculture*, **264**, 342-352.

NRC. (1993) Nutrient Requirements of Fish. National Academy Press, Washington D.C.

NRC. (2011) Nutrient requirement of fish and shellfish In: *Animal Nutrition Series* (Hardy, R.W. ed.), National Academy Press, Washington D.C., pp. 1-376.

Olli, J.J. Hjelmeland, K. Krogdhal, Å. (1994) Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar* L): effects on nutrient digestibilities and trypsin in

pyloric caeca homogenate and intestinal content. *Comp. Biochem. Physiol.* , **109A**, 923-928.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdhal, Å., Skrede, A. (2009) Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*) – effect on growth performance, nutrient digestibility, carcass composition, gut health and physical feed quality. *Aquaculture*, **288**, 305-311.

Refstie, S., Glencross, B.D., Landsverk, T., Sørensen, M., Lilleeng, E., Hawkins, W., Krogdhal, Å. (2006) Digestive function and intestinal integrity in Atlantic salmon (*Salmo salar*) fed kernel meals and protein concentrates made from yellow of narrow-leafed lupins. *Aquaculture*, **261**, 1382-1395.

Sugiura, S.H., Babbit, J.K., Dong, F.M., Hardy, R.W. (2000) Utilisation of fish and animal by-product meals in low pollution feeds for rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquacult. Res*, **31**, 585-593.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbo, R., Hemre, G.-I., Fontanillas, R., Nordgarten, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G. (2008) Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture*, **285**, 193-200.

USDA. (2005) National Agricultural Statistics Service. http://www.nass.usda.gov.

Wolters, W., Hardy, R.W., Barrows, F.T., Burr, G.S., Phillips, J.G. (2013) Family growth responses of Atlantic salmon (*Salmo salar* L.) fed fishmeal-based or fishmeal-free diets. *Aquaculture*, Accepted November 6th 2013.

Yamamoto, T., Shima, T., Furuita, h., Suzuki, N. (2002) Influence of feeding diets with and without fish meal by hand and by self-feeders on feed intake, growth and

nutrient utilisation of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **214**, 289-305.

Ytrestøyl, T., Struksnæs, G., Rørvik, K.-A., Koppe, W., Bjerkeng, B. (2006) Astaxanthin digestibility as affected by ration levels for Atlantic salmon, *Salmo salar*. *Aquaculture*, **261**, 215-224.

Zar, J.H. (1999) *Biostatistical Analysis*, 663 pp. Prentice-Hall International, New Jersey.

514 **Table 1.** Analysed composition of the different test ingredients.
515

g.100 g^{-1}	SPC[a]	BPC[b]	FPC[c]
Dry matter	95.19	94.01	100.00
Crude protein	69.30	53.10	70.10
Crude fat	0.53	0.53	8.01
Phosphorus	0.89	0.67	1.60
Arginine	5.50	3.12	4.20
Histidine	1.95	1.11	1.80
Isoleucine	3.54	1.77	2.80
Leucine	5.52	3.47	4.60
Lysine	4.68	1.88	4.81
Methionine	1.01	0.84	2.00
Threonine	3.04	1.77	2.82
Valine	3.77	2.50	3.42

516 [a] Solae, Pro-Fine VF, St. Louis, Missouri, USA, expressed on an as-is basis
517 [b] Montana Microbial Products, Missoula, Montana, USA, expressed on an as-is basis
518 [c] Scanbio AS, Bjugn, Norway, this was applied as a wet product with approximately 50% moisture but
519 data presented on a dry matter basis.
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Table 2. Ingredient and nutrient composition of the experimental diets fed to Atlantic salmon post-smolts.

Ingredient g.kg^{-1}	Experimental Diet			
	Commercial Type	BPC50	BPC100	BPC/FPC
Sardine meal[a]	148.0	148.0	148.0	98.0
Soya protein concentrate[b]	200.0	100.0	0.0	0.0
Barley protein concentrate[c]	0.0	107.5	215.0	215.0
Fish protein concentrate[d]	0.0	0.0	0.0	50.0[e]
Poultry meal[f]	132.0	132.0	132.0	132.0
Corn protein concentrate[g]	75.0	75.0	75.0	75.0
Wheat gluten meal[h]	40.0	40.0	40.0	40.0
Wheat flour[i]	231.8	219.3	206.8	206.8
Fish oil[j]	113.0	115.0	118.0	118.0
L-Lysine HCl	11.1	12.6	14.6	14.6
DL-Methionine	3.2	3.0	3.0	3.0
L-Threonine	1.1	1.8	1.8	1.8
Mono-dicalcium phosphate	24.8	25.8	25.8	25.8
Choline chloride	6.0	6.0	6.0	6.0
Vitamin premix[k]	10.0	10.0	10.0	10.0
Stay-C 35%[l]	2.0	2.0	2.0	2.0
Trace mineral premix[m]	1.0	1.0	1.0	1.0
Astaxanthin[n]	1.0	1.0	1.0	1.0
Analyzed Nutrient Composition[o] (as-is)				
Moisture (%)	6.08	5.45	5.41	4.31
Crude protein	42.82	41.67	42.87	43.22
Crude lipid	16.89	19.33	19.26	20.14
Ash	6.97	6.71	6.36	6.33
Energy (MJ.kg^{-1})	20.71	21.67	21.90	22.01

[a]Sardine meal, Peruvian prime, 668.8 g.kg^{-1} crude protein
[b]Solae, Pro-Fine VF, 693 g.kg^{-1} crude protein
[c]Montana Microbial Products, 531 g.kg^{-1} crude protein
[d]Scanbio, 701 g.kg^{-1} crude protein dry matter basis
[e]Product contains 50% moisture, addition performed on dry matter basis
[f]IDF Inc., 832 g.kg^{-1} protein
[g]Cargill, Empyreal 75, 756 g.kg^{-1} crude protein
[h]Manildra Milling 778 g.kg^{-1} protein
[i]Manildra Milling, 120 g.kg^{-1} protein
[j]Omega Proteins Inc., Virginia Prime menhaden oil
[k]ARS 702; contributed per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132IU; vitamin K3 1.1 mg; thiamine mononitrate 9.1 mg; riboflavin 9.6 mg; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5 mg; inostitol 600 mg
[l]DSM Nutritional Products
[m]Contributed in mg.kg^{-1} of diet: manganese 13; iodine 5; copper 9; zinc 40
[n]Carophyll Pink 10, DSM Nutritional Products
[o]Values are of triplicate analyses

544 **Table 3.** Growth and quality data of Atlantic salmon post-smolts fed one of four
545 experimental diets for the 12-week study period. Means (± sd) bearing different
546 superscript lettering are significantly different (P<0.05).
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Parameter	Experimental Diet				One-way ANOVA (P value)
	Commercial Type	BPC50	BPC100	BPC/FPC	
Initial weight (g)	90.9 ± 4.9	90.4 ± 2.8	90.7 ± 4.9	90.2 ± 3.5	0.997
Midpoint weight (g)	124.5 ± 10.0	122.3 ± 1.9	124.3 ± 10.4	127.0 ± 8.4	0.920
Final weight (g)	171.3 ± 7.2	177.6 ± 1.1	173.0 ± 11.4	176.5 ± 8.6	0.753
Final length (cm)	25.2 ± 0.3	25.7 ± 0.1	25.6 ± 0.7	25.6 ± 0.4	0.545
Dressed weight (g)	158.0 ± 38.7	164.5 ± 35.9	160.3 ± 38.2	163.1 ± 36.9	0.752
Viscera weight (g)	12.9 ± 2.9	13.1 ± 2.8	13.2 ± 3.3	13.3 ± 3.1	0.803
Total Feed Consumption (g)	2557.8 ± 209.1	2403.7 ± 164.6	2354.9 ± 282.4	2431.4 ± 103.8	0.658
FCR	1.17 ± 0.14	1.01 ± 0.03	1.03 ± 0.01	1.00 ± 0.05	0.081
SGR (%bw.day^{-1})	0.78 ± 0.06	0.81 ± 0.03	0.77 ± 0.06	0.80 ± 0.03	0.533
TGC	1.11 ± 0.08	1.19 ± 0.04	1.13 ± 0.09	1.18 ± 0.05	0.493
Av. Daily wt gain (g.day^{-1})	1.00 ± 0.07	1.00 ± 0.02	1.00 ± 0.11	1.00 ± 0.07	1.000
Protein efficiency ratio (g fish.g dietary protein^{-1})	2.0 ± 0.25^{a}	2.4 ± 0.06^{b}	2.3 ± 0.02^{ab}	2.3 ± 0.12^{ab}	0.040
Protein intake (g.fish^{-1})	40.1 ± 3.68	36.8 ± 0.72	36.3 ± 4.04	37.1 ± 2.17	0.439
Survival (%)	94.4 ± 0.00	94.0 ± 7.43	90.5 ± 16.5	97.6 ± 4.12	0.829
Net protein utilization (%)	29.1 ± 1.93	34.4 ± 1.98	32.9 ± 4.24	33.6 ± 4.72	0.317

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551 **Table 4.** Carotenoid pigment concentrations (mg.kg^{-1}; mean ± sd) from the fillet flesh
552 of Atlantic salmon post-smolts fed one of four experimental diets.
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Carotenoid Pigment Source	Experimental Diet				One-way ANOVA (*P* value)
	Commercial Type	BPC50	BPC100	BPC/FPC	
Total Carotenoid	0.93 ± 0.73	1.06 ± 0.67	0.55 ± 0.47	0.55 ± 0.50	0.093
Astaxanthin	0.87 ± 0.73	1.01 ± 0.67	0.50 ± 0.47	0.52 ± 0.50	0.108
Lutein	0.06 ± 0.04	0.06 ± 0.04	0.05 ± 0.02	0.04 ± 0.01	0.325

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Table 5. Proximate compositions of whole fish fed one of four experimental diets over the 12-week study period. Means (± sd) bearing different superscript lettering are significantly different ($P<0.05$).

Parameter	Experimental Diet				One-way ANOVA (*P* value)
	Commercial Type	BPC50	BPC100	BPC/FPC	
Moisture	70.42 ± 1.25	70.84 ± 0.77	70.64 ± 0.98	70.27 ± 0.99	0.440
Lipid	7.83 ± 0.99	8.26 ± 0.88	8.64 ± 0.76	8.50 ± 0.76	0.052
Protein	18.21 ± 0.48[a]	17.98 ± 0.32[a]	17.54 ± 0.63[b]	17.88 ± 0.34[a]	0.003
Ash	2.39 ± 0.09[a]	2.40 ± 0.10[a]	2.32 ± 0.13[b]	2.43 ± 0.09[a]	0.037