High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (Salmo salar L.)

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A R T I C L E   I N F O
Article history:
Received 2 September 2010
Received in revised form 21 October 2010
Accepted 31 October 2010

Keywords:
Atlantic salmon
Pea protein concentrate
Soy protein concentrate
Enteritis
Histology

A B S T R A C T
The current study investigated the effects of pea protein concentrate, soy protein concentrate and corn gluten, either singly at high inclusion, or in combination, each at lower inclusion, in diets for Atlantic salmon (Salmo salar L.). Growth performance, nutrient digestibility, intestinal brush border enzyme activity, and intestinal histology were studied in an 8-week feeding trial. Triplicate groups of Atlantic salmon (2.36 kg initial weight) were kept in sea water at winter temperature. Five diets were tested, including a control diet based on fish meal (FM; 250 gkg⁻¹ fishmeal) and four low fishmeal (100 gkg⁻¹) diets: a diet containing 350 gkg⁻¹ pea protein concentrate (PPC diet), a diet containing 300 gkg⁻¹ soy protein concentrate (SPC diet), a diet containing 300 gkg⁻¹ corn gluten (CG diet) and a combination diet containing 130 gkg⁻¹ pea protein concentrate, 105 gkg⁻¹ soy protein concentrate and 105 gkg⁻¹ corn gluten (CMB diet). Fish fed CG and PPC diets showed lower SGR than fish fed the FM diet and there was a trend (P = 0.09) towards a higher feed conversion (FCR) in the fish receiving the CG and PPC diets. Apparent fat digestibility was lower in fish fed SPC, PPC and CMB diets compared to FM. No difference in apparent crude protein digestibility was observed. Feeding the PPC diet resulted in reduced relative weight and inflammation in the distal intestine similar to those described for soy enteritis. Additionally, fish that received the PPC diet had reduced brush border enzyme activities in the distal intestine and increased trypsin activity in the digesta from the distal intestine region. In conclusion, pea protein concentrate at high inclusion was shown to induce an enteropathy in the distal intestine of Atlantic salmon and caution should be used when including it in formulated feeds for Atlantic salmon.

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1. Introduction

Fish meal is traditionally and nutritionally the most important protein source for the culture of carnivorous fishes. Alternative protein sources, such as soybeans, are being added to aquaculture feeds, but have limited inclusion because of negative effects at high inclusion levels. One significant health problem associated with full-fat or solvent extracted soybean meal (SBM) is the dose dependent enteropathy affecting the distal intestine (DI) in salmonids fed diets containing SBM. The condition has been described by Baeverfjord and Krogdahl (1996) as a non-infectious subacute enteritis.

Corn gluten, produced from maize (Zea mays L.), can replace a significant proportion of the fish meal in rainbow trout diets, although some studies have reported lower feed intake and growth using corn gluten as a fishmeal substitute (Fauconneau, 1988; Cowey and Cho, 1992). Another potential alternative protein source for fish feeds is pea protein concentrate. Field peas (Pisum sativum) have shown promise as a potential protein source for Atlantic salmon (Aslaksen et al., 2007; Overland et al., 2009), rainbow trout (Thiessen et al., 2003), and sea bass (Gouveia and Davies, 1998). Feeding 18% unprocessed field peas (Aslaksen et al., 2007) or 20% air classified pea protein concentrate (PPC) (Overland et al., 2009) to Atlantic salmon did not induce enteritis as seen when feeding soybean meal. Feeding PPC has been reported to support acceptable weight gain, feed intake, and feed conversion in both Atlantic salmon (Carter and Hauler, 2000) and rainbow trout (Thiessen et al., 2003). However, peas also contain several antinutritional factors (ANFs) which may reduce their nutritional value to fish, but the level of structural carbohydrates and ANFs in peas are generally lower compared with other legumes (Castell et al., 1996; Bach Knudsen, 1997; Kinjo et al., 1998; Francis et al., 2001). While many studies address the effect of soybeans on gut integrity, the information concerning the effect of PPC on gut health in salmon is scarce. The objective of the present experiment was, therefore, to investigate the effect of low fish meal, high plant protein (singly or in combination) diets for outgrowing Atlantic salmon on growth performance, nutrient digestibility, digestive physiology and histopathology in the gastro-intestinal tract.
2. Materials and methods

2.1. Fish, facilities and management

Triplicate groups of acclimated Atlantic salmon (Salmo salar L.), initial weight 2.36 kg (±0.05 kg; overall cage mean ± S.D.) were stocked into 125 m³ (5 × 5 × 5 m) sea pens (95 fish per pen), and kept under 24 h day⁻¹ light and ambient water temperature (5.2 ± 0.3 °C) for 58 days (February 27–April 27) at Gildeskål Research Station AS, Norway (67°N). Over this period the fish were fed manually one of five experimental feeds to satiation in 2 meals per day, only interrupted by fecal stripping that was conducted on April 11. Daily feed intake was assessed in each cage from the difference between the daily ration and the amount of feed waste collected after each meal in a ‘lift-up’ system fitted into each cage. One mortality was recorded during the experimental period. No specific health assessment was performed at the start of the trial but the fish were routinely checked by a veterinarian on a monthly basis prior to the trial. No diseases were detected and sea lice infestation was low. The very low mortality during the trial and the very high growth rates indicated that the fish were healthy and suitable for feeding experiments to begin.

2.2. Diets

Four dry extruded low fishmeal diets (9 mm diameter), and one high fishmeal control, were produced using feed formulations shown in Table 1. Equal amounts of two commercial high-quality fishmeals originating from anchoveta fisheries off the coast of South America was used across experimental diets and 1:1 ratio of double-low-rapseed oil and anchoveta fish oil comprised the added oils. The low fishmeal diets contained high levels (300–350 g kg⁻¹) of soybean protein concentrate, corn gluten meal or pea protein concentrate either singly, or as a combination of all three. All diets were supplemented with crystalline yeast and vitamin and mineral premix, hydrogenated vegetable oil for anti-fat leakage, soy lecithin, lysine, threonine, methionine, monocalcium phosphate, astaxanthin and yttrium oxide (Y₂O₃) as an indigestible marker (Austreng et al., 2000).

2.3. Sampling

On day 42, 30–40 fish in each pen were stripped for feces as described by Austreng (1978). Feces samples were collected by gently squeezing the hind gut of randomly selected fish. The hind gut content was collected in a plastic bowl on ice, stabilized with ethoxyquin solution (400 mg L⁻¹), and immediately frozen at ~22 °C. Feces were kept frozen and then freeze dried prior to analysis. Fish were counted and weighed in bulk at the end of the feeding trial (day 58) and weight gain, specific growth rate (SGR) and feed conversion rate (FCR) were determined. At this time eight fish were randomly sampled from each cage. The fish were individually measured and weighed, and blood was sampled before killing the fish. The body cavity was then opened and the gastrointestinal tract removed and cleared of surface fat and connective tissue. The tract was divided into three regions: pyloric intestine (PI, including pyloric caeca and section of attached intestine), mid intestine (MI, between the last pyloric caecum to the distal intestine) and distal intestine (DI, from the increase in intestinal diameter and presence of visible folds to the anus). The liver was also removed from these fish and weighed individually. From five of these fish the intestinal contents and tissue of each intestinal region were collected and frozen (separately) in ethanol cooled with dry ice. From the remaining three fish, tissue samples (approximately 5 mm × 5 mm) for histological analyses were taken from the central most pyloric caeca, mid intestine, and distal intestine. Tissue samples were fixed in 10% neutral buffered formalin (% formaldehyde; pH 7.0) for 24 h, then transferred to 70% ethyl alcohol and stored at 4 °C until processing. From all eight fish, the mid intestine, distal intestine and liver were weighed to calculate organosomatic indices.

2.4. Chemical analysis

Diets and feces were analyzed using standard methods for crude protein (Kjeldahl-N × 6.25; EU Dir 93/28/EEC), crude fat (method B in EU Dir 98/64/EC), dry matter (EU Dir 71/393/EEC), and ash (EU Dir 71/250/EEC). Starch was determined as glucose after hydrolysis with glucosidase. Yttrium concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave cooking and complete digestion in nitric acid.

2.5. Brush border enzyme activities

The activities of brush-border membrane bound alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and maltase activities were determined in mid and distal intestinal tissue homogenates as described by Krogdahl et al. (2003). Activities are expressed as mol substrate hydrolyzed h⁻¹ g tissue⁻¹, mg protein⁻¹ (specific activity) or kg BW⁻¹.

2.6. Trypsin activity and bile acid content of digesta

Trypsin activity and bile acid concentration were determined on pooled, freeze dried digesta from the PI, MI and DI. Trypsin activity was determined colorimetrically (Kakade et al., 1973) using the substrate benzoyl-arginine-p-nitroanilide (BAPNA) (Sigma no. B-4875; Sigma Chemical Co., St. Louis, MO, USA) and a curve derived from a standardized bovine trypsin solution. Bile acid concentrations were determined using the Enzabile® test kit (Cat. No. S50101, Bio-Stat Diagnostic Sysytems, Cheshire, UK). The concentration of 3α-hydroxy bile acids were calculated from a standard curve generated using standards containing taurocholic acid.

2.7. Histology

Tissues were routinely dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. The mid intestine was sectioned transversely, whereas the distal intestine was sectioned longitudinally (i.e. perpendicular to the complex folds). Tissue processing was performed at the Section...
for Pathology of the National Veterinary Institute (Oslo, Norway). Tissues were stained with haematoxylin and eosin (H&E). Tissue morphology was assessed by blinded evaluation. A continuous scale scoring system was used. Pre-selected tissue variables were scored using a continuous scale (see Table 2). The evaluator assessed each tissue variable and marked along the continuum where each sample warranted. After all evaluations were performed a numerical score was then determined based on the length along the continuum that the evaluator had assigned. The range for the tissue scores was arbitrarily set at 0–10.

### 2.8. Calculations

Specific growth rates (SGR, % body weight day$^{-1}$) were calculated as:

$$[\ln W_t - \ln W_0] \times (T-t)^{-1} \times 100$$

where $W_0$ and $W_t$ are weights in g at the start and the end of the growth period, respectively, and $T-t$ is the time in days between weighing. The feed conversion ratio was calculated from the amount of feed ingested and the total biomass gain:

$$\text{FCR} = \frac{\text{kg feed ingested}}{\text{kg final biomass - kg initial biomass + kg dead fish}} \text{ day}^{-1}.$$

Apparent digestibility coefficients (ADCs) were calculated from the measurements of the nutrient-to-indicator ratios in the feeds and feces:

$$\text{ADC} = 100 - \left[ 100 \times \frac{\text{marker in feed(%)}}{\text{marker in feces(%)}} \times \frac{\text{nutrient in feed(%)}}{\text{nutrient in feces(%)}} \right].$$

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Scoring system used to evaluate histomorphology of intestinal tissues.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score range</td>
<td>0 to 10</td>
</tr>
<tr>
<td><strong>Mucosal folds</strong></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>Short to Tall</td>
</tr>
<tr>
<td>Coalescence (bridging)</td>
<td>Distinct individual folds to Indistinct, markedly fused folds</td>
</tr>
<tr>
<td><strong>Submucosa</strong></td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>Thin to Markedly widened</td>
</tr>
<tr>
<td>Cellularity (leukocyte infiltration, connective tissue hyperplasia)</td>
<td>Low to Markedly increased</td>
</tr>
<tr>
<td><strong>Enterocytes</strong></td>
<td></td>
</tr>
<tr>
<td>Supranuclear absorptive vacuolization</td>
<td>Absent to Hypervacuolated</td>
</tr>
<tr>
<td>Nucleus position</td>
<td>Basal to Apical</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Growth performance of fish fed the experimental diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td>Initial individual weight (kg)</td>
<td>2.37</td>
</tr>
<tr>
<td>Final individual weight (kg)</td>
<td>3.41</td>
</tr>
<tr>
<td>Individual weight gain (kg)</td>
<td>1.05</td>
</tr>
<tr>
<td>Feed intake (g fish$^{-1}$ day$^{-1}$)</td>
<td>15.4</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>0.63</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>0.86</td>
</tr>
</tbody>
</table>

$^1$Standard error of the mean (pooled).

$^*Values with different superscripts within a row are significantly different (one-way ANOVA followed by REGW multiple range test, $\alpha=0.05$).

2.9. Statistical methods

Cage means (the average of sampled individuals within each tank) were calculated for each variable and used as the statistical unit in one-way ANOVA (SAS Enterprise Guide 4.1, SAS Institute, Inc.) to test for differences between dietary treatments. Results are presented as the mean for each treatment, and variance is expressed as the pooled standard error of the mean. When appropriate, differences between means were tested using the Ryan–Einot–Gabriel–Welsch multiple range test (REGWQ).

3. Results

3.1. Growth performance, whole body composition, and nutrient digestibility

The fish grew very well on all feeds given the low rearing temperature over the 2-month experimental period (overall SGR 0.58 ± 0.04; overall FCR 0.88 ± 0.04; mean ± SD), and only one mortality was recorded. At the end of the feeding period body condition factor (1.47 ± 0.09; mean ± SD) was within the normal range and did not differ between dietary treatments. Feed intake and growth performance variables are given in Table 3. There were significant differences among dietary treatments for weight gain and SGR, and a tendency ($P=0.08$) towards a lower FCR in the fish receiving the CG diet. Visually assessed feeding response was reported to be very good irrespective of feed treatment throughout the study. While not statistically significantly different, the absolute amount of feed eaten was lowest in the fish fed the CG diet and highest in the fish fed the FM diet. There were no differences in the digestibility of crude protein (Table 4). Lipid and starch digestibility did differ, with fish fed the SPC, PPC and CMB diets exhibiting lower lipid digestibility compared to the FM control, and SPC and CMB diets showing lower starch digestibility compared to FM control. Overall, starch digestibility was low in all diet groups (range: 45.1–28.5%). During stripping, the faecal matter from fish fed PPC was liquid in all replicates while fish fed on the other diets had faeces of more normal characteristics. Diarrhea was confirmed by dry matter (11%) in fish fed the PPC feed.
and no differences were observed in the other regions of the FM or PPC diets. The other diets (SPC and CG) were not different than distal intestine of fish fed the experimental diets. The activity of each enzyme is calculated for each intestinal region in three ways: 1) activity per gram of tissue, 2) activity per mg of protein in the sample and 3) activity per kg of body weight.

3.2. Organosomatic indices

Mid intestine, distal intestine and liver somatic indices are given in Table 5. Fish fed the PPC diet had a significantly lower distal intestine somatic index compared to all other dietary groups. The CMB diet group was intermediate between FM control and the PPC diet group. The changes were characterized by increased enterocyte histology for any of the diets. There were significant differences among treatments for all brush border enzyme activities in both the mid and distal intestines. The diets with plant ingredients generally had lower enzyme activities compared to the FM control. PPC fed fish consistently had lower enzyme activities in both regions of intestine compared to all other diets.

3.3. Digestive enzyme activities and bile acids concentration

Intestinal brush border enzyme (alkaline phosphatase, ALP; leucine aminopeptidase, LAP; and maltase) activities in the different intestinal regions, and bile acids concentration and trypsin activity in freeze dried feces are shown in Tables 6 and 7, respectively. There was a trend toward decreased bile acids in the pyloric and mid intestine of fish fed the PPC compared to FM, but no difference in the distal intestine. Contrarily, trypsin activity was significantly higher in the distal intestine of fish fed the PPC diet compared to FM. The CMB diet had an intermediate value, not statistically different from either the FM or PPC diets. The other diets (SPC and CG) were not different than FM, and no differences were observed in the other regions of the intestine for any of the diets. There were significant differences among treatments for all brush border enzyme activities in both the mid and distal intestines. The diets with plant ingredients generally had lower enzyme activities compared to the FM control. PPC fed fish consistently had lower enzyme activities in both regions of intestine compared to all other diets.

3.4. Intestinal histology

Histological changes were noted in pyloric caeca samples from all diet groups. The changes were characterized by increased enterocyte vacuolization accompanied by increased variation in vacuole size. No diet related histological changes were noted in mid intestine samples from any dietary group. No differences in histological scoring were found (data not shown).

Table 5

Relative weight (g 100 g 
-1 body weight) of the liver and different sections of the gastrointestinal tract of fish fed the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>FM</th>
<th>SPC</th>
<th>CG</th>
<th>PPC</th>
<th>CMB</th>
<th>SEM1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.2</td>
<td>1.27</td>
<td>1.23</td>
<td>1.27</td>
<td>1.24</td>
<td>0.03</td>
<td>0.58</td>
</tr>
<tr>
<td>Mid intestine</td>
<td>0.25</td>
<td>0.28</td>
<td>0.27</td>
<td>0.24</td>
<td>0.25</td>
<td>0.01</td>
<td>0.056</td>
</tr>
<tr>
<td>Distal intestine</td>
<td>0.63a</td>
<td>0.61ab</td>
<td>0.63a</td>
<td>0.43a</td>
<td>0.58b</td>
<td>0.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1Standard error of the mean (pooled).

*Values with different superscripts within a row are significantly different (one-way ANOVA followed by REGW multiple range test, α = 0.05).

Table 6

Activities of the epithelial brush border membrane enzymes alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and maltase in the mid and distal intestines of salmon fed the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>ALP activity</th>
<th>LAP activity</th>
<th>Maltase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid intestine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| μmol h
-1 g 
-1 tissue | 21.5a | 7.3ab | 0.42ab |
| μmol h
-1 mg 
-1 protein | 0.45a | 152.4 | 2.9 |
| μmol h
-1 kg 
-1 BW | 32.4a | 18.5ab | 1.1ab |
| Distal intestine | | | |
| μmol h
-1 g 
-1 tissue | 33.6a | 11.6a | 0.70d |
| μmol h
-1 mg 
-1 protein | 1.06a | 362.9a | 21.9a |
| μmol h
-1 kg 
-1 BW | 104.4a | 104.4a | 4.4a |
| Mid intestine | | | |
| μmol h
-1 g 
-1 tissue | 17.9bc | 5.7bc | 0.30bc |
| μmol h
-1 mg 
-1 protein | 0.38ab | 121 | 12.2ab |
| μmol h
-1 kg 
-1 BW | 24.6bc | 16.1bc | 0.8bc |
| Distal intestine | | | |
| μmol h
-1 g 
-1 tissue | 26.5b | 8.7bc | 0.36 |
| μmol h
-1 mg 
-1 protein | 0.77a | 295.8bc | 12.2ab |
| μmol h
-1 kg 
-1 BW | 24.1a | 80.1b | 2.2 |
| Mid intestine | | | |
| μmol h
-1 g 
-1 tissue | 19.8a | 8.1a | 0.47a |
| μmol h
-1 mg 
-1 protein | 0.39 | 105.1 | 13.3 |
| μmol h
-1 kg 
-1 BW | 30.2a | 101.5 | 22.9 |
| Distal intestine | | | |
| μmol h
-1 g 
-1 tissue | 11.1a | 9.6b | 0.33 |
| μmol h
-1 mg 
-1 protein | 0.77a | 277.3 | 14.1 |
| μmol h
-1 kg 
-1 BW | 24.1a | 80.9b | 4.4 |

Table 7

Trypsin and bile acids concentration in the digesta of pyloric, mid and distal intestines of salmon fed the experimental diets.

| Diet | Trypsin (U mg
-1 dry matter) | Bile acids (mg g
-1 dry matter) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyloric intestine</td>
<td>194.7</td>
<td>119.9</td>
</tr>
<tr>
<td>Mid intestine</td>
<td>102.1</td>
<td>103.7</td>
</tr>
<tr>
<td>Distal intestine</td>
<td>21.5a</td>
<td>17.6</td>
</tr>
<tr>
<td>Pyloric intestine</td>
<td>115.7</td>
<td>99.5</td>
</tr>
<tr>
<td>Mid intestine</td>
<td>66</td>
<td>63.1</td>
</tr>
<tr>
<td>Distal intestine</td>
<td>14.1</td>
<td>10.8</td>
</tr>
</tbody>
</table>

1Standard error of the mean (pooled).

*Values with different superscripts within a row are significantly different (one-way ANOVA followed by REGW multiple range test, α = 0.05).
Significant differences were observed in the distal intestine in several variables including mucosal fold height, fold fusion, lamina propria width, lamina propria infiltration, enterocyte vacuolization and displacement of enterocyte nuclei. Results of the statistical analyses of distal intestine histology scoring are shown in Table 8. In fish fed the PPC diet the simple mucosal folds appeared shortened, and the lamina propria appeared widened with concomitant leukocyte infiltration (see Fig. 1). Enterocytes exhibited reduced to absent vacuolization and apical displacement of nuclei. All samples in this diet group exhibited some degree of histological changes. No changes were observed in the other diet groups.

4. Discussion

The current study investigated the effect of low fish meal, high plant protein diets for outgrowing Atlantic salmon on growth performance, nutrient digestibility, digestive physiology and gut health. The negative effect of PPC on growth performance of salmon in the present experiment is in contrast with previous work with PPC in rainbow trout (Thiessen et al., 2003) and Atlantic salmon (Carter and Hauler, 2000; Overland et al., 2009). Overland et al. (2009) reported similar growth performance for fish fed a diet containing 200 g kg⁻¹ PPC compared to fish fed a FM control diet. However, this was less than in the current study, and the fish used were substantially smaller (385–432 g).

None of the diets adversely affected the apparent digestibilities of protein compared with the FM diet, but the SPC, PPC and CMB diets had lower lipid digestibilities compared to the other diets. The reduced lipid digestibility may be related to the lower bile acid content of the digesta in the PI and MI of fish fed the PPC diet. However, it appears that factors other than reduced bile acid content may be responsible for reduced lipid digestibility in other groups because the correlation between bile acid content and lipid digestibility is not consistent over all of the other diet groups, particularly SPC and CG fed fish. Reduced lipid digestibility is a common finding for diets with plant feedstuffs. The most severe effects are seen when soybean meal is used as a main ingredient (Olli and Krogdahl, 1994; Olli et al., 1994). Plant feedstuffs contain several components that may compromise various steps of lipid digestion such as emulsification, hydrolysis, incorporation of fatty acids into micelles, uptake and re-esterification in the enterocytes. Fibres, saponins, phytosterols and estrogens, and lipase inhibitors are examples (Krogdahl et al., 2010). Among these, both soy and pea protein concentrates contain substantial amounts of fibre (Bach Knudsen, 1997), as well as saponins if prepared by methods other than aqueous alcohol extraction (Anderson and Wolf, 1995). The SPC and PPC diets showed the lowest lipid digestibilities. The presence of such components may explain the negative effects of soy protein concentrate and peas observed in the present study. The protein digestibilities were similar in all diets, above 80%, and similar to those reported by Overland et al. (2009), but lower than those reported by Carter and Hauler (2000) in Atlantic salmon, which were above 90%.

Starch digestibilities in the current study were much lower than those reported in previous studies (Aslaksen et al., 2007; Overland et al., 2009). The low environmental temperature at sampling time in the present study may be a contributing factor and may have enhanced the effect of the specific molecular characteristics that have been observed in Atlantic salmon amylase. Atlantic salmon seem to have a low capacity for starch digestion due to low secretion of amylase from the pancreas and a defect in the substrate binding site for starch (Froystad et al., 2006). Enzymes in general show decreased activity with decreasing temperatures, a characteristic that has been observed also for fish enzymes (Chow and Halver, 1980). However, Papoutsoglou and Lyndon (2005) reported only slight differences in carbohydrate activity in Atlantic salmon over a wide temperature

<table>
<thead>
<tr>
<th>Table 8</th>
<th>Distal intestine tissue variable scores of the fish fed the experimental diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM SPC CG PPC CMB SEM</td>
</tr>
<tr>
<td>Mucosal fold height</td>
<td>7.4c 7.1a 7.9b 5.0a 7.2c 0.35</td>
</tr>
<tr>
<td>Mucosal fold coalescence</td>
<td>2.5a 1.9b 1.9b 3.8b 2.3a 0.23</td>
</tr>
<tr>
<td>Lamina propria width</td>
<td>1.6c 1.9a 1.5b 5.9b 2.1b 0.46</td>
</tr>
<tr>
<td>Lamina propria cellularity</td>
<td>1.7a 1.8a 1.2b 5.4b 2.0b 0.52</td>
</tr>
<tr>
<td>Submucosa width</td>
<td>2.3 2.6 1.6 4.3 3.7 0.58</td>
</tr>
<tr>
<td>Submucosa cellularity</td>
<td>2.3 2.6 2.0 4.6 2.9 0.75</td>
</tr>
<tr>
<td>Enterocyte vacuolization</td>
<td>7.5a 5.1b 7.2b 0.9b 6.0ab 0.52</td>
</tr>
<tr>
<td>Enterocyte nucleus position</td>
<td>1.35 2.5ab 2.0b 3.6b 1.8a 0.35</td>
</tr>
</tbody>
</table>

1Standard error of the mean (pooled).
*Values with different superscripts within a row are significantly different (one-way ANOVA followed by REGW multiple range test, α=0.05).
range. However, the results of the latter study should be interpreted with caution because only total carbohydrate activity was measured, in intestinal tissue, over the entire digestive tract after a period of starvation. As amylose in salmonids is primarily of pancreatic origin and secreted to be mixed with the intestinal content (Chow and Halver, 1980), it is more appropriate in such studies to measure amylose activity in digesta when the animals are in a fed state than in gut tissue in starved fish. Starch digestibility can also be influenced by starch source and/or combination of sources, ration size, dietary lipid level and quality, and diet processing conditions (reviewed by Krogdahl et al., 2005). In the present work starch digestibility differed quite substantially. The greatest difference was observed between diets with similar starch levels, the FM and the SPC diets. It is difficult to determine the reason for this difference. In the SPC diet, SPC substitutes a mix of fish meal and sunflower 40/60 as compared with the FM diet. Characteristics of the two plant ingredients, and differences in fiber qualities and/or contents of antinutrients may explain the difference in starch digestibility between the diets. An inverse correlation has been reported in Atlantic salmon between digestibility and starch levels, even within narrow ranges (Grisdale-Helland and Helland, 1997; Krogdahl et al., 1999). In the present experiment such correlation was not apparent. Again, differences in fiber qualities and antinutrient contents may be confounding factors masking such a relationship.

Feeding diets containing pea protein concentrate led to a reduction in the relative size of the distal intestine compared to FM fed fish. The effect was more pronounced in the fish fed the PPC diet than the CMB diet, suggesting that the effect is dose related. Feeding the PPC diet resulted in pronounced histological changes of the distal intestine. The changes observed in fish fed the PPC diet resembled the changes associated with soy enteropathy described by Baeverfjord and Krogdahl (1996) in Atlantic salmon. The reduction in the somatic index likely reflected the atrophied mucosal folds (Bakke-Mckelpe et al., 2007a,b) as observed histologically, which in turn may affect the function of this part of the intestine. No other dietary treatments affected the histological appearance of the intestinal tissues. The CMB diet also contained pea concentrate but in lower quantity and in combination with other plant source ingredients, and all diets contained field beans, leading to the conclusion that the high level of pea concentrate, or some other aspect unique to this diet was responsible for the observed changes. Additionally, the fish fed PPC had higher trypsin activity in digesta from the DI compared with the fish fed the FM diets. The morphological changes, increased trypsin activity and reduced brush border enzyme activities in the DI of the fish fed the PPC diet were consistent with changes associated with SBM induced enteritis in Atlantic salmon (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003; Lilleeng et al., 2007). Lilleeng et al. (2007) reported increased trypsin activity in the luminal contents as well as the wall of the DI, suggesting that locally produced proteases may contribute to the development of enteritis. Thorsen et al. (2008) have demonstrated the existence of protein activated receptor-2 (PAR-2) in salmon suggesting that the receptor may play a role in the inflammatory process. Whether locally produced proteases are cause or consequence of the inflammatory process remains to be determined. The presence of the morphological changes as well as the change in the relative weight and enzyme activities in the intestine of fish fed the PPC diet indicate that this protein source may be contraindicated for salmon at high dietary inclusion levels. In the current study, however, the reduction in mucosal fold height and increase in lamina propria width and infiltration were not as pronounced as the fully developed soy induced enteritis. This may be an effect of low water temperature. Immune responses in fish are influenced by ambient temperature, though adaptive responses may be more affected than innate responses (reviewed by Magnadottir, 2006). T-cells make up a large proportion of the cellular infiltrate observed in soy enteritis (Bakke-Mckelpe et al., 2007a,b) and thus may modify the inflammatory response. Uran et al. (2008) directly address the influence of temperature on the development and severity of enteritis in salmon fed diets containing soy, concluding that higher water temperature (12 °C compared to 8 °C) increases the rapidity of development and severity of the enteritis. However, the authors used analysis of variance to evaluate categorical data, a method that remains controversial for such data. Mean scores for many of the variables and the overall mean scores, statistically significantly different by ANOVA, often differed by less than one score category. Thus, the information regarding the relationship between temperature and development of soy enteritis is not conclusive. It is not clear whether the similarity of the changes in the current study and those previously described for the soy associated enteropathy suggests a similar etiology, or if the observed changes are a common consequence of disease processes that result in inflammatory conditions of some duration.

Several ANFs including protease inhibitors, phytic acid, oligosaccharides, lectins, tannins and saponins are present in peas (Wang and Daun, 2004; Vidal-Valverde et al., 2003). Some of these ANFs are heat-labile and may be inactivated during diet extrusion (Habiba, 2002; Thissen, 2003). Saponins appear to be a common constituent in leguminous beans, including soybeans, peas and field beans (Vicia faba), with soyasaponin I typically being the predominant form, though their total amounts vary (Kijno et al., 1998). The saponins reported in peas include soyasaponin I (Bb) and DDMP saponin, though DDMP is the predominant form as reported by Heng et al. (2006). Total saponin contents are reported to vary between 0.7 and 2.5 g kg⁻¹ (Davey et al., 1997; Heng et al., 2006). Interestingly, these levels are approximately up to one half the amounts of saponins found in soybean. Knudsen et al. (2006) suggest that commercial batches of soybean meal can be expected to contain 5–7 g kg⁻¹. While many of the other antinutrients may be inactivated by the processing methods used in diet production, saponin concentrations vary depending on the processing method. Saponins are resistant to heat inactivation but are removed by aqueous alcohol extraction (Ireland et al., 1986). Soy protein concentrate produced by aqueous alcohol extraction does not cause the enteropathy in salmonids that full-fat or solvent extracted soybean meal does (van den Ingh et al., 1991; Olli et al., 1994; Krogdahl et al., 2000). Air classification can be used to produce protein concentrates from pea meals by separating starch and protein particles based on size and density. However, saponin concentration may actually increase because they follow the protein fraction (Elkowicz and Sosulski, 1982; Anderson and Wolf, 1995; Lin et al., 2006). Presumably, aqueous alcohol extraction would remove saponins from pea meal or air-classified protein concentrates. Recently Knudsen et al. (2008) reported that a combination of saponins and lupin kernel meal in diets for Atlantic salmon was capable of inducing lesions similar to those described for soy enteritis, providing further evidence for the involvement of saponins in the soy associated enteropathy. Therefore it is reasonable to suspect saponins from peas may be involved in the inflammatory lesions observed in the current work.

5. Conclusion

The present experiment demonstrated that PPC at a high dietary inclusion level (35%) resulted in significant adverse effects on growth performance, nutrient digestibility, digestive physiology and gut health of Atlantic salmon. Air-classified PPC was shown to induce an enteropathy in the distal intestine of Atlantic salmon and caution should be used when including it in formulated feeds for Atlantic salmon. Low fishmeal diets containing high levels of vegetable proteins affected intestinal brush border enzyme activities but did not cause significant effects on performance or tissue histology. Further research is needed to identify the causative agent(s) of the
enteropathy. As with soybean meal, the pea protein model could be a very useful model in the study of food-induced enteropathy.

Acknowledgments

The authors would like to recognize technician Kjell-Atle Andreassen at Gildaeskål Research Station AS for the excellent feeding and husbandry of fish over the experimental period. We would also like to thank Ellen Hage, Elin Valen, Gunn Østby and Halvor Holm of the Norwegian School of Veterinary Science for their assistance during sampling and dedicated laboratory work.

References


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