Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (Oncorhynchus mykiss) fed diets containing defatted soybean meal

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Abstract

The aim of this experiment was to study the effect of defatted soybean meal (SBM) on lipid digestibility, bile acid level and morphological changes in the distal intestine (DI) in rainbow trout, and to assess whether the lipid digestibility and bile acid level are affected by the morphological changes in the DI. Triplicate groups of 0.35-kg trout were fed a diet with fish meal as the only high-protein feed ingredient (FM diet) or a diet with 30% SBM (SBM diet) for 40 days. Faeces, blood, intestinal chyme and tissue from the DI were sampled during the experimental period. Lipid digestibility was not significantly affected by diet. Trout fed the FM and SBM diets had similar bile acid concentration in the distal part of the DI (DI 2), whereas trout fed the SBM diet had gradually decreasing bile acid concentrations in the pyloric region (PR) and mid intestine (MI) over the 40-day experimental period. The levels of cholesterol and triacylglycerols in plasma were slightly lower in trout fed the SBM diet than the FM diet, although this was only significant at day 40 and day 5 for cholesterol and triacylglycerols, respectively. Gradually enhanced trypsin activity was evident in the DI 2 of trout fed the SBM diet. The trout developed SBM-induced enteritis, but the progression was slower than reported for Atlantic salmon. The results show that the bile acid concentration in the intestinal chyme was gradually lowered by soybean meal, and indicates that this was not due to increased faecal excretion of bile acids or soybean meal-induced enteritis.

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Keywords: Soybean meal; Digestibility; Lipid; Bile acid; Enteritis; Rainbow trout Oncorhynchus mykiss

1. Introduction

Defatted soybean meal (SBM) has become a widely used protein-rich feed ingredient in diets for salmonids and other fish species, which is due to its moderate price, high availability in the market and the relatively well-balanced amino acid profile (reviewed by Storebakken et al., 2000). However, soybeans contain a large variety of both heat-stable and heat-labile biological active components which act as antinutrients. In salmonids, dietary SBM may reduce lipid digestibility (Olli and Krogdahl, 1994; Olli et al., 1994; Refstie et al., 1998, 1999, 2000, 2005; Storebakken et al., 1998; Krogdahl et al., 2003; Romarheim et al., 2006; Yamamoto et al., 2007), lower the level of bile acids (Romarheim et al., 2006; Yamamoto et al., 2007), and induces morphological changes in the distal intestine (DI) (Ingh and Krogdahl, 1990; Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Refstie et al., 2000; Krogdahl et al., 2003; Romarheim et al., 2006).

Some alcohol-soluble components contribute to the reduced lipid digestibility (Olli and Krogdahl, 1995) and morphological changes in DI (Ingh et al., 1996), and the severity of the morphological changes depends on the dietary inclusion level of SBM (Krogdahl et al., 2003). The exact agent(s) and mechanism for the SBM-induced enteritis have not yet been...
identified, and the impact on fish performance is not fully understood. However, it is clear that the enzymatic activity in the DI and nutrient transport across the intestinal wall is affected in salmon (Bakke-McKellep et al., 2000; Nordrum et al., 2000a; Krogdahl et al., 2003), and it is also an ethical problem that has to be solved if high levels of SBM are to be used in salmonid diets. Soy saponins have been suggested to be the active component responsible for the development of SBM-induced enteritis (Bureau et al., 1998; Knudsen et al., 2007), but Krogdahl et al. (1995) did not find any morphological changes in the DI of salmon when soy saponins were added to a diet. Hence, more studies with purified antinutrients are required to allow firm conclusions.

The progression of SBM-induced enteritis in Atlantic salmon has been well described by Baeverfjord and Krogdahl (1996), but has not been described in detail for rainbow trout. Refstie et al. (2000) suggested that rainbow trout develop a milder SBM-induced enteritis than Atlantic salmon, but the sampling was limited to the start of the feeding experiment and after 84 days of feeding. The objective of the present experiment was to follow lipid digestibility, bile acid concentrations and the development of morphological changes in the intestine of rainbow trout fed a SBM-containing diet.

2. Materials and methods

2.1. Experimental diets, fish and facilities

Two diets were formulated to have similar crude protein:lipid ratio; one with FM as the only protein-rich feed ingredient (FM diet), and one with partial substitution of FM by defatted, toasted SBM (SBM diet; Table 1). The diets were extruded with a five-section twin-screw extruder (BCTG 62/20 D, Bühler AG, Uzwil, Switzerland) at the Center for Feed Technology, Ås, Norway. The trial was carried out with rainbow trout (Oncorhynchus mykiss) kept in tanks (1×1×0.6 m) supplied with sea water (32.5 g l−1 salinity) at AKV AFORSK, Sunndalsøra, Norway. Each diet was fed to triplicate groups of 121 trout for 40 days. The fish were fed every 30 min 24 h a day by automatic disc feeders (Storvik AS, Sunndalsøra, Norway), and exposed to continuous light. The water temperature decreased from 8.2 °C at the start to 6.9 °C at the termination. Feed intake was quantified as described by Helland et al. (1996). The commercial feeds used at the research station may contain soy products, and all fish were fed the SBM diet at days 0, 2, 5, 7, 10, 20 and 40. The sampled fish at day 0 were fed the SBM diet at days 0, 2, 5, 7, 10, 20 and 40. The sampled fish at day 0 were randomly collected from the holding tank prior to allocation into the experimental tanks. All fish used for sampling were individually weighed, and they were not returned to the experimental tanks.

Faeces for determination of apparent digestibilities (n ≥ 15 fish tank−1) were sampled by stripping the trout after anaesthesia with MS 222. Faeces from each tank were pooled, stored frozen and freeze-dried prior to analysis. The whole gastrointestinal (GI) tract from four fish per tank was sampled for analyses of bile acids and trypsin in the intestinal chyme. The GI tracts were gently removed from the fish, packed in aluminium foil and frozen in liquid nitrogen. In a half-thawed state, the intestines were cut open longitudinally and the chyme from the following sections was divided into two parts: pyloric region (proximal region, PR 1; distal region, PR 2), mid intestine (proximal region, MI 1; distal region, MI 2), and distal intestine (proximal region, DI 1; distal region, DI 2). The chyme was freeze-dried prior to analysis. Tissue for histological examination of the DI was sampled from two fish per tank by gently opening the intestine longitudinally, and dissecting out a 5×5 mm piece of the intestinal wall from DI 1. The tissue was fixed in phosphate buffered formalin, and the intestines of all sampled fish for histological examinations were filled with intestinal chyme. Blood plasma was sampled from seven fish per tank by drawing blood from the
2.3. Chemical and biochemical analyses

Dry matter and ash were determined by drying at 105 °C and combustion at 550 °C, respectively. Crude protein and lipids in the diets were analysed according to methods 981.10 and 948.16 of the AOAC (2003), respectively. The analysis of crude lipids in faeces included pre-extraction with petroleum ether followed by hydrolysis with hydrochloric acid and subsequent petroleum ether extraction. Starch was determined as total glucose after enzymatic hydrolysis (Total Starch Assay Kit [AA/AMG], Megazyme International Ireland Ltd., Wicklow, Ireland). Dietary amino acid and taurine composition was analysed according to the EC (1998), and the concentrations of free amino acids and amino acid derivates in plasma were analysed by ion exchange chromatography in an automated amino acid analyser (Biochrom 30, Biochrom Ltd, Cambridge, UK) using S-(2-aminoethyl)-L-cysteine hydrochloric acid as internal standard. The analysis of free amino acids and taurine in plasma was described in detail by Karlsson et al. (2006). Yttrium oxide was determined by an ICP-spectrometer after the following treatment of the ashed sample: dilution with HCl and HNO3 (2:1, v/v), boiling until loss of colour, addition of 2.5 ml HNO3 and dilution to 50 ml with distilled water. Trypsin inhibitor activity (TIA) was analysed according to the method of Hamerstrand et al. (1981). Bile acids were analysed by enzymatic assay purchased from Bio-Stat Ltd (Stockport, UK). Trypsin activity was analysed as described by Holm et al. (1988) in 10% (w/v) water extracts of the freeze-dried samples with Na-benzoyl-DL-arginine-p-nitroanilide (BAPNA, Sigma Chemical) as substrates. Plasma cholesterol and triacylglycerols were analysed by Cobas® Integra enzyme kits and automatic analyser equipment (Cobas Mira, Hoffman-La Roche & Co., Basel, Switzerland).

Preparation of tissue for histological examination was done at the Section for Pathology of the National Veterinary Institute (Oslo, Norway). Formalin fixed tissues were routinely dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. The tissue samples were sectioned longitudinally (i.e. perpendicular to the macroscopically visible circular folds; approximately 5 μm thick) and stained with haematoxylin and eosin before examination under a light microscope. Two independent evaluations were performed. Tissue morphology was evaluated according to the descriptions of Amin et al. (1992) and Baeverfjord and Krogdahl (1996).

2.4. Calculations and statistical analyses

Daily feed intake, as percentages of the body weight, was calculated as: \((FI_n \times BW_n^{−1}) \times 100\), where \(FI_n\) and \(BW_n\) represent feed intake and estimated body weight at day \(n\), respectively. The \(BW_0\) was estimated by extrapolating the thermal growth coefficients (TGC) from the fish left at day 40 (44–47 fish per tank). The thermal growth coefficient was calculated as \((BW_0^{1/3}−BW_1^{1/3}) / \Delta t\), where \(BW_0\) represents the start weight, which was assumed to be equal to the mean weight at start, \(BW_1\) represents the final body weight, and \(\Delta t\) represents the thermal sum (mean daily temperature × days). Apparent digestibility (%) was calculated as: \(100 \times (1−(I_n / N_n \times N_d / N_f))\), where \(I_n\) and \(N_f\) represent the concentration of inert marker in diet and faeces, and \(N_d\) and \(N_f\) represent the concentration of nutrients in diet and faeces, respectively.

Effect of diet on feed intake, weight of sampled trout, nutrient digestibility and plasma values was compared by the Students’ t-test at each sampling. Statistical differences in the bile acid concentration and trypsin activity within intestinal segments of fish fed the same diet were separated by Ryan Enot Gabriel Welsch multiple range test. The Ryan Enot Gabriel Welsch multiple range test was also used to separate means of lipid digestibilities and plasma analyses from fish fed the same diet, but sampled at different days. The level of significance was \(P<0.05\). All statistical analyses were conducted using SAS statistical software (version 8.02, SAS Institute). The chemical composition of the diets, registrations of the state of the gall bladder and the evaluation of the histological parameters were not subjected to statistical analyses.

3. Results

3.1. Diets, feed intake and growth

The profile of the dietary indispensable and semi-indispensable amino acids, including dietary taurine level, revealed that the SBM diet was lower in methionine, threonine, lysine and taurine, but higher in phenylalanine, histidine and arginine than the FM diet (Table 1). Trypsin inhibitor activity was only detected in the SBM diet, which had an activity of 0.4 mg inhibited bovine trypsin g⁻¹ feed.

No fish died by accident or disease during the experiment. The day-to-day variation in feed intake was large, but the feed intake pattern was similar in fish fed both diets. Average feed intake during the 40 day period was significantly lower in fish fed the SBM diet than in fish fed the FM diet, on average 0.50±0.04% (mean ±S.E.M.) and 0.69±0.04% of BW per day for fish fed the SBM diet and FM diet, respectively. Feed intake on the specific days, however, was only significantly lower for trout fed the SBM diet at days 6, 9, 11–13, 24, 30 and 39 (Fig. 1). The sampled fish fed the SBM diet had significantly lower average body weight than fish fed the FM diet from day 10 and onwards, and fish fed the SBM diet had 13% lower weight than fish fed the FM diet at the end of the 40 day experimental period (Table 2).

![Fig. 1](image)

**Fig. 1.** Estimated daily feed intake given as % of body weight (±S.E.M.). Body weight was estimated from the thermal growth coefficients of fish sampled at day 40. Asterisk indicates significant differences between treatments, where * = \(P<0.05\), ** = \(P<0.01\) and *** = \(P<0.001\).
3.2. Apparent digestibility and plasma free amino acids, cholesterol and triacylglycerols

Apparent digestibility of lipids varied from 91.6% to 94.7% in fish fed the FM diet, and from 90.6% to 93.4% in fish fed the SBM diet, but the mean values were not found to differ significantly during the 40 day period or between fish fed the two diets (Table 3). Trout fed the FM diet had a crude protein digestibility at day 40 of 88.0±0.2% (mean ± S.E. M.) which was slightly, but significantly (P<0.05), higher than that of trout fed the SBM diet (87.0±0.3%).

The concentrations of plasma free amino acids and amino acid metabolites most affected by diets are presented in Table 4. Trout fed the SBM diet had, in general, lower plasma levels of free threonine, methionine, serine and glycine, and the metabolites cystathionine and taurine, compared to fish fed the FM diet. Plasma levels of the other amino acids were not significantly or only slightly influenced by time of sampling or diet, and are therefore not presented.

Plasma cholesterol in fish fed both diets varied among samplings (Fig. 2a). The highest plasma cholesterol level was found at the start of the experiment in fish fed both diets (10.7±0.2 mM), and the lowest level was observed at day 10 in fish fed the FM diet (7.0±0.7 mM) and at day 5 in fish fed the SBM diet (5.9±0.3 mM). The only significant difference between the two diets was found at day 40, when fish fed the SBM diet had a lower cholesterol level than fish fed the FM diet. Triacylglycerols in plasma of fish fed the FM diet did not differ significantly among samplings, whereas fish fed the SBM diet had a significant drop in the concentration of triacylglycerols from the start (8.2±0.3 mM) to days 5 (4.0±0.2 mM) and 10 (4.4±0.2 mM) (Fig. 2b). However, the triacylglycerol concentration in plasma increased to levels not significantly different from the start at days 20 and 40. The only significant difference between trout fed the FM and SBM diets was found at day 5.

3.3. Bile acids and trypsin activity

The concentration of bile acids along the intestinal tract in trout fed the FM diet was reduced from 66–85 mg g⁻¹ freeze-dried material in the PR and MI to approximately 20 mg g⁻¹ in the DI 2 (Fig. 3). Feeding the SBM diet increased the concentration of bile acids in the PR to

### Table 2
Weight of sampled fish, g fish⁻¹ (n=3; ±S.E.M.)

<table>
<thead>
<tr>
<th>Day</th>
<th>FM diet</th>
<th>SBM diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start¹</td>
<td>352±2.0</td>
<td>368±15.2</td>
</tr>
<tr>
<td>5</td>
<td>371±12.8</td>
<td>382±1.6⁶</td>
</tr>
<tr>
<td>10</td>
<td>356±1.3⁶</td>
<td>387±3.9⁶</td>
</tr>
<tr>
<td>20</td>
<td>417±7.4⁶</td>
<td>444±17.5⁵</td>
</tr>
<tr>
<td>40</td>
<td>508±3.8⁷</td>
<td>444±17.5⁵</td>
</tr>
</tbody>
</table>

¹Means in a row with different superscript letter differ significantly (P≤0.05).
²Fish fed the FM diet for a 4-week pre-adaptation period.

### Table 3
Apparent lipid digestibility (±S.E.M.)

<table>
<thead>
<tr>
<th>Day</th>
<th>FM diet</th>
<th>SBM diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start⁵</td>
<td>91.6±1.10</td>
<td>93.4±1.10</td>
</tr>
<tr>
<td>5</td>
<td>94.7±0.30</td>
<td>93.4±1.10</td>
</tr>
<tr>
<td>10</td>
<td>93.0±0.30</td>
<td>90.7±1.10</td>
</tr>
<tr>
<td>20</td>
<td>91.8±0.50</td>
<td>92.3±1.60</td>
</tr>
<tr>
<td>40</td>
<td>92.0±1.10</td>
<td>90.6±1.00</td>
</tr>
</tbody>
</table>

⁵Fish fed the FM diet for a 4-week pre-adaptation period.

### Table 4
Plasma content of selected amino acids and amino acid metabolites in rainbow trout, μM (n=3; ±S.E.M.)

<table>
<thead>
<tr>
<th>Day</th>
<th>Threonine</th>
<th>Methionine</th>
<th>Serine</th>
<th>Glycine</th>
<th>Cystathionine</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM diet</td>
<td>SBM diet</td>
<td>FM diet</td>
<td>SBM diet</td>
<td>FM diet</td>
<td>SBM diet</td>
<td>FM diet</td>
</tr>
<tr>
<td>Start²</td>
<td>304±18.3</td>
<td>268±22.0</td>
<td>1757±180</td>
<td>1298±88</td>
<td>3.4±0.1</td>
<td>3.8±0.0</td>
</tr>
<tr>
<td>5</td>
<td>346±15.8⁶</td>
<td>268±22.0</td>
<td>1757±180</td>
<td>1298±88</td>
<td>3.4±0.1</td>
<td>3.8±0.0</td>
</tr>
<tr>
<td>10</td>
<td>2319±32.2⁶</td>
<td>268±22.0</td>
<td>1757±180</td>
<td>1298±88</td>
<td>3.4±0.1</td>
<td>3.8±0.0</td>
</tr>
<tr>
<td>20</td>
<td>348±26.5⁶</td>
<td>268±22.0</td>
<td>1757±180</td>
<td>1298±88</td>
<td>3.4±0.1</td>
<td>3.8±0.0</td>
</tr>
<tr>
<td>40</td>
<td>414±22.6⁵</td>
<td>268±22.0</td>
<td>1757±180</td>
<td>1298±88</td>
<td>3.4±0.1</td>
<td>3.8±0.0</td>
</tr>
</tbody>
</table>

²Statistical differences within a diet over time are indicated by different capital letters, and differences between dietary treatments within a time point are indicated by different lower case letters (P<0.05).
Fig. 2. a) Cholesterol and b) triacylglycerols in plasma of trout fed the FM and SBM diets \((n=3)\) for up to 40 days (±S.E.M.). Statistical differences within a diet are indicated by different capital letters, and differences between dietary treatments are indicated by different lower case letters \((P \leq 0.05)\).

Fig. 3. Bile acids in intestinal chyme from trout fed the FM and SBM diets for up to 40 days. Statistical differences within intestinal segments from trout fed different diets are indicated by different letters \((P < 0.05)\). PR = pyloric region; MI = mid intestine; DI = distal intestine. 1 and 2 represent the proximal and distal region, respectively.
above 90 mg g\(^{-1}\), but the level was not significantly different from that of trout fed the FM diet. Further feeding of the SBM diet decreased the concentration of bile acids in the PR to 45–61 mg g\(^{-1}\) after 10 and 20 days, and to approximately 30 mg g\(^{-1}\) in trout fed for 40 days. However, the concentration of bile acids in the DI 2 was similar in trout fed the FM and SBM diets. The frequency of empty gall bladders in trout fed the FM diet was from one to four out of 21 fish at each time of sampling, but no clear pattern could be found (Table 5). In contrast, among trout fed the SBM diet there were six and seven out of 21 individuals with empty gall bladders at days 20 and 40, respectively.

The variation in trypsin activity in the PR and MI was in general high, both among samplings and among the different segments of the PR and MI (Fig. 4). The trypsin activity in the regions PR 1 to MI 1 was highest in trout fed the SBM diet for 5 days. No significant differences were found in the MI 2 and DI 1, whereas the lowest activity in the DI 2 was found in trout fed the FM diet and the activity in trout fed the SBM diet increased with increasing duration of feeding.

### 3.4. Intestinal histology

The DI samples from fish fed the SBM diet showed a development of enteritis with general progression of reduced mucosal fold height, increased lamina propria width with moderate leukocyte infiltration and reduced enterocyte supranuclear (absorptive) vacuolization over the 40 day experimental period, whereas trout fed the FM diet did not show any signs of SBM-induced enteritis at the end of the same period. The numbers of individual samples from trout fed the SBM diet categorized by degree of change of histological parameters are shown in Table 6. The time course of development and progression of the soybean associated enteropathy is described as follows:

#### 3.4.1. Day 0

Mucosal folds appeared tall with thin submucosa and lamina propria. Low to moderate numbers of intraepithelial leukocytes (IELs) were seen. Enterocytes were moderately to highly vaculated (supranuclear absorptive vacuolization) with basally located nuclei.

#### 3.4.2. Day 2

Few samples exhibited a slight reduction in mucosal fold height, and slight increases in lamina propria width with concomitant leukocyte infiltration at day 2. Additionally a reduction of enterocyte supranuclear vacuolization was observed. The samples with shorter mucosal folds came from the same individuals that had the lower vacuolization of enterocytes.

#### 3.4.3. Day 5

Most samples showed medium height of the mucosal folds. Some samples showed slight to moderate increases in lamina propria width with similar degrees of lamina propria infiltration. Enterocyte supranuclear vacuolization continued to decrease so that the majority of samples had low to moderate vacuolization.

#### 3.4.4. Day 7

The notable changes from day 5 to day 7 were continued increases in lamina propria width and progression in reduction of enterocyte vacuolization such that the majority of samples showed low vacuolization. Two of the six samples also showed apical displacement of enterocyte nuclei.

#### 3.4.5. Days 10 and 20

At days 10 and 20 nearly all samples showed to some degree the changes typically associated with SBM-induced enteritis, however, not

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### Table 5

<table>
<thead>
<tr>
<th>Day</th>
<th>FM diet</th>
<th>SBM diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filled bladder</td>
<td>Empty bladder</td>
</tr>
<tr>
<td>Start(a)</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
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</tr>
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<td>10</td>
<td>17</td>
<td>4</td>
</tr>
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<td>20</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

\(a\) Fish fed the FM diet for a 4-week pre-adaptation period.
as severe as seen in the fully developed condition. There was a progression in the reduction of mucosal fold height while the degree of widening and infiltration of the lamina propria remained slight to moderate with some variation between samples at day 10. Some samples showed high numbers of IELs. All samples showed moderate to low enterocyte vacuolization while the majority exhibited apical displacement of nuclei.

3.4.6. Day 40

All samples showed reduced mucosal fold height and moderate to marked increases in lamina propria width and leukocyte infiltration, whereas only half of the samples showed increased width of the submucosa. There was only one sample that showed marked widening of lamina propria and leukocyte infiltration. In general, high numbers of IELs and low enterocyte vacuolization were observed. Some samples showed vacuolization of the epithelial cells; however the vacuoles were dissimilar from the normal absorptive vacuoles. Rather, they appeared as large coalescing vacuoles unevenly distributed within the epithelial cells.

4. Discussion

Several studies have concluded that dietary SBM lowers the lipid digestibility in salmonids (Olli et al., 1994; Refstie et al., 1998, 1999, 2000, 2005; Storebakken et al., 1998; Krogdahl et al., 2003; Romarheim et al., 2006; Yamamoto et al., 2007). Alcohol-soluble components in soy may lower lipid digestibility in salmonids (Olli and Krogdahl, 1995), and Refstie et al. (2005) reduced the negative effect of SBM on lipid digestibility in Atlantic salmon by lactic acid fermentation prior to dietary inclusion. It was therefore surprising that the SBM diet supported similar lipid digestibility as the FM diet throughout the experiment since trout fed the SBM diet had lower concentration of bile acids in the intestinal tract. However, the lipid digestibility would most likely be lowered in the present study by extended duration of the feeding period.

Bile salts have a central role in the digestion and uptake of lipids, and dietary SBM may reduce the bile acid level (Romarheim et al., 2006; Yamamoto et al., 2007). In addition, Yamamoto et al. (2007) found that dietary inclusion of 1.5% bovine bile salts to a SBM-based diet enhanced total bile salts and digestibility of crude fat in rainbow trout, thus supporting the hypothesis that reduced lipid digestibility when feeding SBM is, at least partly, related to the bile acid level. The low feed intake in trout fed the SBM diet in the present study may have slowed down the passage rate such that the bile acid level was still sufficient to ensure proper emulsification and digestion of lipids even at the end of the experimental periods.

Taurine is a sulfur-containing amino acid derivate that is not incorporated into proteins by peptide bonds. However, taurine conjugated bile salts constitute most of the total bile salts in rainbow trout (Denton and Yousif, 1974; Goto et al., 1996), and low levels of plasma taurine might lead to decreased synthesis of bile salts. Fish meals are rich in taurine, whereas plant protein sources contain little or no taurine, thus explaining the higher taurine level in the FM diet compared with the SBM diet. It has been speculated whether or not taurine addition to diets based on plant protein sources is needed. Gaylord et al. (2006) got positive effects on growth and feed utilization in rainbow trout when 5 and 10 g kg\(^{-1}\) taurine was added to a plant protein based diet, whereas no significant effects of taurine additions were found on growth and feed utilization in a later study (Gaylord et al., 2007). However, taurine supplementation increased plasma taurine in both latter studies (Gaylord et al., 2006, 2007). Rainbow trout have the ability to convert cysteine into taurine (Yokoyama et al., 1997). Additional dietary methionine have also increased taurine in plasma of both rainbow trout (Cowey et al., 1992) and Atlantic salmon (Nordrum et al., 2000b), whereas Yokoyama and Nakazoe (1992) did not find such an effect in rainbow trout growing from 10 to 17 g. The

<table>
<thead>
<tr>
<th>Day</th>
<th>Height of mucosal folds</th>
<th>Submucosa</th>
<th>Lamina propria, width</th>
<th>Lamina propria, infiltration</th>
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<tr>
<td></td>
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<td>Thin &lt; Mod &lt; Wide</td>
<td>None &lt; Slight &lt; Mod &lt; Marked</td>
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*Fish fed the FM diet for a 4-week pre-adaptation period.
reduction in plasma levels of methionine, glycine, serine and cystathionine in trout fed the SBM diet may therefore be related to increased taurine synthesis provided by lower dietary taurine level. The lower plasma levels of amino acids when feeding a SBM diet may also be partly associated with lower feed intake, and reduced digestibility of sulfur-containing amino acids as shown by (Olli et al., 1994; Olli and Krogdahl, 1994; Romarheim et al., 2006). However, more research is needed on the long-term effects of reduced taurine concentration in plasma when substituting fish meal by soybean meal or other plant protein sources, and whether taurine should be included in diets containing soybean meal.

Some studies have reported a possible cholesterol-reducing effect in salmonids by substitution of FM by SBM (Kaushik et al., 1995; Refstie et al., 1999; Romarheim et al., 2006; Yamamoto et al., 2007), whereas Dabrowska and Wojno (1977) found no significant effect of dietary SBM on plasma cholesterol in rainbow trout. There is controversy among researchers on which components in soy that may influence the cholesterol and bile acid status, and possible regulatory effects of the cholesterol catabolism and bile acid synthesis have been discussed in several reviews (Potter, 1995; Demonty et al., 2003; Ricketts et al., 2005; Torres et al., 2006). Although few significant differences were found in plasma cholesterol and triacylglycerols between fish fed the two diets, the numerical values were always lower in trout fed the SBM diet. In addition, the plasma cholesterol level in trout fed the SBM diet was comparable with that of trout fed a diet with 25% SBM for 63 days (Romarheim et al., 2006), whereas the cholesterol level in trout fed the FM diet was somewhat lower than found in the previous study. The present findings also indicate that the reduction of bile acids was not caused by a lack of plasma cholesterol for bile acid synthesis, and dietary addition of cholesterol does therefore seem superfluous.

The morphological changes in the DI of trout fed SBM in the present study correspond well with those described by Baeverfjord and Krogdahl (1996) for 0.55-kg Atlantic salmon fed diets containing 330 g SBM kg^{-1} in salt water at 10.8 °C. Changes were observed as early as at days 2 and 5, which were indicative of later events. Baeverfjord and Krogdahl (1996) found that all the signs of the fully developed condition were present at day 7 in Atlantic salmon. This is in contrast to the present study where no samples showed all signs of SBM-induced enteritis at day 7, and the fully developed condition was observed in only one out of six samples at day 40. Refstie et al. (2000) compared the intestinal histology of DI in rainbow trout and Atlantic salmon fed a SBM-containing diet for 84 days in freshwater, and found that all rainbow trout sampled had developed SBM-induced enteritis at the termination of the experiment, but that the inflammation of the lamina propria and submucosa was less abundant in trout than in salmon. It is therefore likely that rainbow trout develop SBM-induced enteritis with a slower progression than Atlantic salmon.

Enhanced trypsin activity in intestinal chyme from the DI 2 of trout with developed SBM-induced enteritis was consistent with previous findings in both rainbow trout (Romarheim et al., 2006) and Atlantic salmon (Lilleeng et al., 2007). The results from the study with Atlantic salmon showed that trypsin mRNA was expressed throughout the gastrointestinal tract, and that fish with SBM-induced enteritis had an up-regulation of a trypsin-like activity in the DI tissue. They suggested that the higher trypsin activity in the intestinal chyme came from the intestinal wall, and that the enhanced trypsin activity in the DI wall may contribute to the development of SBM-induced enteritis. The development of SBM-induced enteritis and the increase in the trypsin activity in the intestinal chyme of the DI 2 coincided, thus supporting the hypothesis that trypsin enhances the severity of SBM-induced enteritis in the DI of salmonids.

The reduction in concentration of bile acids from the MI 2 to DI 2 in trout fed both the FM diet and the SBM diet may imply that the DI is an important site for re-absorption of bile acids. Thus, the present study indicates that analysing bile acids in the faeces from stripped fish may not give adequate information about the bile acid status in the gastrointestinal tract. Nordrum et al. (2006b) concluded that the DI is relatively more important for the absorption of cysteine and taurine than for other amino acids, and fully developed SBM-induced enteritis in the DI could therefore cause at least some of the reduction in plasma taurine or reduction of bile acids due to reduced re-absorption. In contradiction, Atlantic cod also exhibited reduced lipid digestibility when fed diets containing SBM (Førde-Skjærøvik et al., 2006), although SBM-induced enteritis has not been found in Atlantic cod (Refstie et al., 2006). Soybean fibres have also been suggested to bind to bile acids and thereby prevent them from being re-absorbed, and Romarheim et al. (2006) suggested that an elevated excretion of bile acids during the initial feeding with SBM diets could cause a loss of bile acids from the fish. However, this was probably not true in the present study since the concentration of bile acids was relatively constant in the latter part of the DI at all times of sampling.

The state of the gall bladder will vary with time after feeding and the simple classification (filled or empty) may therefore not fully reflect the effect of diets. However, all fish were fed continuously until sampling and proportions of fish with filled or empty gall bladder would be expected to be similar if there was no effect of diets. The observed lower number of trout with filled gall bladder at day 20 and 40, combined with the reduced concentration of bile acids in the GI tract, may indicate that the total bile acid pool was reduced in trout fed the SBM diet. It seems therefore likely that substances from the SBM lead to down-regulation of the bile acid synthesis, or that the level of available taurine was too low to ensure proper conjugation with bile acids to make bile salts.

In conclusion, the concentration of bile acid in the intestine was substantially reduced without affecting lipid digestibility. It was also concluded that the bile acid concentration along the PR and MI of rainbow trout fed SBM was gradually lowered throughout the feeding period, and that this was likely not caused by increased faecal excretion of bile acids. Development of SBM-induced enteritis was slower in rainbow trout than previously reported for Atlantic salmon, and the bile acid reduction did not seem to be caused by SBM-induced enteritis in this experiment. More work should be done on finding the underlying mechanisms for the bile acid reduction, and to study
the long-term effect of low bile acid levels when soybean meal is included in diet for salmonids.

Acknowledgments

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