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Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals

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Abstract

Ten different plant protein and/or starch sources were studied in a 5-week experiment with triplicate groups of 0.7-kg Atlantic salmon in seawater, pre-adapted to a diet with fish meal, faba beans, sunflower cake, and wheat gluten as sources of protein and starch. The experimental ingredients were corn gluten, defatted soybean, defatted sunflower, dehulled lupin, defatted double-low rapeseed, whole field pea, whole and dehulled faba bean, whole wheat and naked oat, tested one at a time (14–24% inclusion). The diets were balanced by addition of pure wheat starch and/or pure cellulose, to obtain equal macro nutrient compositions. The control diet consisted of fish meal, wheat starch, cellulose and fish oil. The results showed reduced faecal dry matter content in fish fed the soybean diet and to a lesser extent in those fed the sunflower, lupin and rapeseed diets. Diets containing lupin and rapeseed resulted in a moderate increase in the viscosity of the digesta, while diets with wheat and oat increased viscosity of digesta more. Apparent digestibility of lipid decreased linearly with increasing dietary cellulose level. A significant reduction in the digestibility of crude protein was seen for the soybean, sunflower, rapeseed and oat diets, reflecting reduced digestibilities of most amino acids. The salmon fed the corn gluten, lupin, pea, bean and wheat diets had protein digestibilities comparable to the control group. The digestibility of phosphorus was highest for salmon fed the rapeseed diet and lowest for fish fed the oat diet. Faecal excretion of sodium was highly elevated for salmon fed the soybean diet, and moderately elevated for fish fed the corn gluten and sunflower diets. Faecal excretion of zinc was elevated in the fish fed the oat diet. None of these observations were significantly related to the dietary concentration of phytic acid. A histological examination of the stomach, mid- and distal intestine of all groups showed no other irregularities than enteritis in the distal intestine of salmon fed soybean meal. The present study demonstrated a potential for several plant ingredients, such as field pea and faba bean, partly replacing high-quality fish meal in diets for Atlantic salmon, based on nutrient digestibilities and absence of pathologies in the stomach and intestine.
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Keywords: Atlantic salmon; Corn gluten; Soybean; Sunflower; Lupin; Rapeseed; Field pea; Faba bean; Wheat; Oat; Viscosity; Digestibility; Pathology; Enteritis

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1. Introduction

Supply of fish meal has become a limiting factor for increase in fish production. Thus, increased use of plant protein in the feed is necessary to support rapid and sustainable growth in intensive fish farming. Different salmonid fish species respond differently when fish meal is replaced by plant feed ingredients (Refstie et al., 2000; Glencross et al., 2004a,b; Krogdahl et al., 2004), and responses, therefore, must be assessed with the actual target species.

Plant protein sources with a low degree of processing, like defatted rapeseed, soybean, sunflower, beans, legumes or peas, or crude gluten meals are inexpensive and readily available in high quantities. However, they may contain a variety of antinutrients (Francis et al., 2001). Some heat labile antinutrients, like protease inhibitors, lectins, and some bitter components, are easy to inactivate by moist heating. For example, protease inhibitor activity in soy can be reduced to nutritionally acceptable levels simply by the energy input provided by extrusion of the feed (Marsman et al., 1995; Romarheim et al., 2005). Inactivation of antinutrients that are not destroyed by moderate heating, such as alkaloids, some bitter components, glucosinolates, indigestible oligosaccharides and non-starch polysaccharides (NSP), phytates, and tannins (Francis et al., 2001) requires more specific methods. Lactic acid fermentation (Ibrahim et al., 2002; Skrede et al., 2002; Refstie et al., 2005) and enzyme treatment (Denstadli et al., 2006a) are examples of processes that can potentially be applied by modifications of the feed processing line. Several antinutrients affect nutrient digestibilities. Typically, lipid digestibility is normally reduced when salmon are fed diets with defatted soybean meal (review by Storebakken et al., 2000a). Nitrogen digestibility and faecal cysteine excretion (Olli et al., 1994) are closely associated with protease inhibitor activity in the feed. Faecal excretion of divalent and trivalent ions, like zinc, is elevated when salmon are fed diets containing phytic acid (Denstadli et al., 2006b) and faecal excretion of sodium is elevated in salmon fed soy products (Storebakken et al., 1998, 2000a). The first aim of this experiment, thus, was to study effects of partial replacement of high-quality fish meal with plant protein sources with a low level of processing on the digestibility of macronutrients and the faecal output of sodium and zinc.

Except for corn and wheat gluten meals, plant protein sources that contain starch rather than NSP, like peas

Table 1
Chemical composition of experimental ingredients (g kg^{-1})

Ingredient	Fish meal [a]	Protein-rich plant ingredients					Starch-rich plant ingredients				
		Corn gluten [b]	Soybean [c]	Sunflower [d]	Lupin [e]	Rapeseed [f]	Pea [g]	Whole bean [h]	Dehulled bean [i]	Wheat [j]	Oat [k]
DM	920	918	897	907	901	892	860	866	877	857	872
In DM											
Crude protein	752.2	674.3	500.6	384.8	402.9	371.1	243.0	272.5	296.5	150.5	130.7
Lipid	103.3	92.6	33.4	40.8	92.1	49.3	22.1	21.9	27.4	31.5	95.2
Ash	157.6	16.3	59.1	81.6	27.7	74.0	32.6	34.6	35.3	19.8	20.6
Starch	N.d. [l]	153.6	81.4	N.d.	N.d.	N.d.	523.3	426.1	497.1	658.1	644.5
Dietary fibre	N.a. [m]	49.0	324.4	345.1	370.7	361.0	220.9	244.8	120.9	145.9	119.3
Water soluble fibre	N.a.	N.d.	35.7	14.3	53.3	13.5	19.8	N.d.	N.d.	23.3	49.3
Organic matter	842.4	983.7	940.9	918.4	972.3	926.0	967.4	965.4	964.7	980.2	979.4

[a] Low-temperature dried, NorsEco, Egersund fiskeoljefabrikk, Egersund, Norway. The raw material had the following composition (g kg^{-1}): Blue whiting, 300; capelin, 220; herring, 20; herring filleting by-products, 360; horse mackerel, 110.
[b] Corn gluten meal, Felleskjøpet, Kambo, Norway.
[c] Deno-Soy F®, hexane extracted and toasted soybean meal with hulls, Denofa, Fredrikstad, Norway.
[d] Sunflower (*Helianthus annuus*) seed meal, solvent extracted and mildly heat treated (Rosenkrantz AS, Aalborg, Denmark).
[e] Low alkaloid dehulled white lupins (*Lupinus angustifolius*), reported alkaloid content of <200 mg kg^{-1} (Schouten Products B.V., Giessen, Netherlands).
[f] The rapeseed meal (double low; *Brassica napus*) had been flaked and defatted by hexane extraction at 60 °C and given a short-time heat treatment at 100 °C under pressure. The reported glucosinolate content was 3 mmol kg^{-1} (Karlshamns AB, Karlshamn, Sweden).
[g] Whole field pea (*Pisum sativum*) (Läntmannen, Stockholm, Sweden).
[h] Whole faba bean (*Vicia faba*) (Nordic Bulk Company A/S, Aarhus, Denmark).
[i] Dehulled faba bean (Nordic Bulk Company A/S, Aarhus, Denmark).
[j] Whole wheat (Felleskjøpet, Kambo, Norway).
[k] Naked oat (Felleskjøpet, Kambo, Norway).
[l] Not detectable.
[m] Not analysed.

and beans, have received limited attention in diets for salmon. There are, however, indications that these feed ingredients cause fewer problems than soybean meal, as previous works have shown no negative effects on fish performance (Booth et al., 2001; Pereira and Olivia-Teles, 2002). These protein sources have the potential to substitute fish meal for protein and wheat as a source of starch in the feed mix. A second aim of this study was therefore to see how such protein sources affected nutrient digestibility in salmon compared to more traditional starch sources and a pure wheat starch.

Morphological changes in the intestinal mucosa are observed in Atlantic salmon when their feed contains soybean meal. These changes are characterised by a combination of 4 features (Baeverfjord and Krogdahl, 1996): (1) a shortening of height of the mucosal folds; (2) a loss of the normal supranuclear vacuolisation of the absorptive cells of the intestinal epithelium; (3) a widening of the central stroma within the mucosal folds, with increased amounts of connective tissue; and (4) a profound infiltration of inflammatory cells in the lamina propria. These morphological changes are associated with impaired functionality of the distal intestine, as well as reduced immune response and resistance to infections (Bakke-McKellep et al., 2000; Krogdahl et al., 2000, 2003). There are no published studies investigating whether oilseeds other than soy, such as rapeseed and sunflower have similar effects on the gut of salmon. However, other non-marine protein sources, such as bacterial protein produced on natural gas (Berge et al., 2005), lupin kernel meals and protein concentrates (Refstie et al., 2006), or wheat gluten (Storebakken et al., 2000b) do not cause these lesions in the gut of the salmon. In an experiment with lupin products, however, ulcer-like lesions were observed both in salmon fed fish meal as the main source of protein and in fish fed lupins, the condition being exacerbated by the use of lupin in the feed (Refstie et al., 2006). A key objective of this experiment, thus, was to investigate the effects of a wide range of plant feed ingredients on the integrity of the stomach and intestine of the salmon.

2. Materials and methods

2.1. Feed ingredients and production

Chemical composition of the experimental ingredients is shown in Table 1. Eleven diets were formulated (Table 2): a control diet with fish meal as the sole protein source and ten fish meal based diets each containing one plant protein, protein/starch or starch source, partly replacing fish meal and/or wheat starch (see Table 3 for processing parameters). The diets were supplemented with cellulose to obtain the same NSP content, based on literature values for the feed ingredients (Bach Knudsen, 1997), assuming that cellulose is inert in the digestive system of salmonids (Hilton et al., 1983; Wiesmann and Pfeffer, 1986). Diets with starch-rich plant ingredients

Table 2
Formulation of the diets (g kg^{-1})

Diet	Control	Protein-rich plant ingredients					Starch-rich plant ingredients				
		Corn gluten	Soybean	Sunflower	Lupin	Rapeseed	Pea	Whole bean	Dehulled bean	Wheat	Oat
Macro ingredients											
Fish meal	522	353	387	394	391	427	462	440	449	491	493
Plant ingredient		204	206	229	241	183	181	220	187	144	145
Wheat starch [a]	94	62	77	94	93	88					
Cellulose [b]	109	97	45			22	78	58	83	89	93
Fish oil [c]	267	266	272	269	261	267	268	269	269	266	259
Micro ingredients											
Vitamin, mineral and astaxanthin premix [d]	3.62	3.63	3.61	3.57	3.58	3.61	3.57	3.56	3.60	3.58	3.57
MCP [e]	4.79	13.39	9.40	10.87	11.06	9.44	7.45	8.46	8.27	6.07	5.92
Yttrium oxide [f]	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11

[a] Wheat starch (in g kg^{-1}: starch, 873; moisture, 119; protein, 2; ash, 3; lipid, <10) (Raisio Plc, Raisio, Finland).
[b] Commercial cellulose MN 100 (in g kg^{-1}: moisture, 38; ash, 4; starch, <10; lipid, <10; protein, <2) (Macherey-Nagel GmbH & Co. KG, Düren, Germany).
[c] Havsbrun Norge AS, Leknes, Norway.
[d] Vitamin and mineral premix, composition proprietary to BioMar AS, Trondheim, Norway. Carophyll Pink, DSM Nutritional Products Ltd, Basel, Switzerland; 0.3 g kg^{-1}. In addition 1.3 g *D,L* methionine kg^{-1} (Feed grade, 99% purity, Degussa AG, Hanau, Germany) was by mistake added to the corn gluten diet.
[e] Monocalsiumphosphate, Bolifor MCP-F, Kemira Growhow Denmark A/S, Fredericia, Denmark.
[f] Yttrium oxide (Y_2O_3), >99.9% purity, Strem chemicals, Bischheim, France.

Table 3
Processing parameters during production of the experimental diets and protein dispersibility index (PDI)

Diet	Control	Protein-rich plant ingredients					Starch-rich plant ingredients				
		Corn gluten	Soybean	Sunflower	Lupin	Rapeseed	Pea	Whole bean	Dehulled bean	Wheat	Oat
Feed mash											
Lipid content [a] (g kg^{-1})	46.4	48.6	35.0	39.4	49.4	44.9	49.3	43.3	42.6	52.3	55.4
Moisture content [b] (g kg^{-1})	238	269	273	251	290	261	292	272	290	264	284
Conditioning											
Temperature (°C)	101	97	97	100	96	97	97	96	96	99	100
Retention time (s)	62	62	62	62	62	62	62	62	62	62	62
Extrusion											
Temperature [c] (°C)											
Section 1	72	76	86	73	71	59	76	77	77	77	62
Section 3	124	122	126	128	128	124	124	128	124	133	131
Section 5	114	120	121	116	121	115	119	121	119	117	121
Die	110	110	116	113	116	108	113	115	113	114	116
Screw speed (rpm)	222	178	241	267	273	216	242	248	242	273	273
Throughput (kg h^{-1})	200	200	200	200	200	200	200	200	200	200	200
Die pressure (bar)	23	26	31	32	20	30	25	26	29	28	29
Torque [d] (Nm)	325	397	391	408	288	375	332	324	345	314	318
SME [e] (Wh kg^{-1})	33	32	42	50	34	37	35	36	36	40	37
Drying											
Air temperature [f] (°C)	110–120	110–115	105–115	100–110	105–120	95–105	105–110	105–110	95–105	100–110	100–110
Retention time [g] (min)	30	30	30	30	30	30	30	30	30	30	30
PDI (%)	22.8	12.6	19.0	17.0	18.1	23.6	18.9	17.1	17.0	18.3	17.9

[a] Lipid content in feed mash prior to processing. No lipid was added before vacuum coating.
[b] Total moisture content, calculated based on analysed moisture content in raw materials and added amounts of steam and water in the conditioner. No moisture was added in the extruder.
[c] Measured by sensors embedded in the extruder barrel.
[d] Engine load, 100% torque is 435 Nm.
[e] Specific mechanical energy.
[f] Measured in inlet air.
[g] Calculated based on throughput.

were formulated so that the plant ingredient would provide all the starch in the diet. Other protein-rich plant ingredients were made to replace 20% of crude protein from high-quality fish meal. Diets were formulated to contain equal amounts of crude protein, lipid, starch and total dietary fibre by balancing the diets with pure wheat starch and/or pure cellulose.

Center for Feed Technology at the Norwegian University of Life Sciences, Ås, Norway, produced the diets. Dry ingredients were mixed in a Dinnissen twin shaft mixer (Pegasus Menger 400 l, Sevenum, Holland), milled in a Münch hammer mill (HM 21.115, Wuppertal, Germany) with a 1-mm screen, preconditioned in a Bühler double conditioner (BCTC 10, Uzwil, Switzerland), extruded in a five-section Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland) with a 4-mm die and dried in a Bühler fluid-bed dryer (OTW 50 05TSR2, Uzwil, Switzerland). The extrusion process was optimized to obtain a pellet density of 460 g l^{-1} and a pellet diameter of 5 mm. Varying amounts of pressure were released from extruder section 4 to obtain the desired pellet density. The lupin diet was produced with a different screw configuration than the other diets, due to insufficient pellet expansion and binding in the initial production; one backward screw element was added in extruder section 4. Fish oil was added in a Dinnissen (Sevenum, Netherlands) vacuum coater before the diets were cooled in a Miltenz counter-flow cooler (Millbank Technology, Auckland, New Zealand) and packed.

2.2. Biological experiment and sampling

The experiment was carried out with Atlantic salmon (*Salmo salar*) in seawater at GIFAS, Gildeskål, Norway, over a period of 5 weeks from mid November to mid December, 2005. Each diet was fed to three groups of 100 fish with an average initial weight of 705 ± 15 g (mean ± s.e.m). There were no significant differences in initial fish weight among cages. The cages were 5 × 5 × 5 m and the average water temperature was 8 °C. The diets were soaked in seawater prior to feeding, since some of the diets proved to have a high percentage of floating pellets. The fish were fed once or twice a day to satiation, including the days of sampling. No fish died during the experimental period. The fish had been fed an extruded diet with a diameter of 6 mm prior to the experiment, produced by BioMar, Karmøy, Norway,

containing the following energy-bearing feed ingredients (kg^{-1}): fish meal, 470 g; sunflower cake, 60 g; wheat gluten, 80 g; faba beans, 150 g; and added oil, 230 g.

Faeces were collected by stripping (Austreng, 1978) at the end of the experiment and pooled for each cage. Viscosity of the intestinal content was determined in the supernatant (centrifugation: 10 min, 1400 ×*g*; Labor-M centrifuge, Wifug Ltd., Bradford, England) of chyme from the pyloric region, distal small intestine and large intestine in pooled samples from three fish using a Brookfield viscometer (Brookfield, Stoughton, MA, USA) at 12 rpm. The material had a temperature of 7 °C during measurement. Samples of intestinal content for determination of viscosity were taken from fish that had been killed while anesthetized.

2.3. Chemical analyses

Faeces were freeze-dried and ground with a pestle and mortar, and fish scales were removed with forceps prior to analysis. Diets and faeces were analysed for dry matter (EU 71/393), Kjeldahl nitrogen (N) (EU 93/28), lipid by HCl hydrolysis followed by diethylether extraction (EU 98/64), starch (AOAC enzymatic method 996.11), ash (EU 71/250), minerals (ICP-AES/ICP-MS) (Nordic Comitee on Food Analysis (NMKL) method 161) and yttrium (ICP-AES) (NS-EN ISO 11885). Amino acids (except tryptophan) were analysed according to EC (1998) on a Biochrom 30 amino acid analyser (Biochrom Ltd., Cambridge, UK), while tryptophan was analysed according to EC (2000) on a Dionex Summit HPLC system, with a Shimadzu RF-535 fluorescence detector. Uncoated diets were analysed for dietary fibre (total and water insoluble) (NMKL enzymatic–gravimetric method 129, 2nd edition) on a Fibertec system E1023 (Foss, Hillerød, Denmark). Results shown from analysis of dietary fibre are therefore corrected based on fat addition during vacuum coating. Phytic acid was analysed according to Carlsson et al. (2001), trypsin inhibitor activity (TIA) according to Hamerstrand et al. (1981), and the Protein Dispersibility Index (PDI) was determined according to AOCS (Ba 10a-05). The PDI value was calculated as: PDI = 100 × (water dispersible protein (g kg^{-1})) × (crude protein (g kg^{-1})$^{-1}$).

2.4. Gastrointestinal histology

The gastrointestinal tract was dissected from two fish per pen, and surface fat and connective tissue as well as stomach and intestinal content carefully removed. The gastrointestinal tract was divided into three regions: 1) stomach; 2) mid intestine [from the last pyloric caecum to the distal intestine]; and 3) distal intestine [distinguished by the increased diameter, darker mucosa and macroscopically visible complex folds]. Tissue samples (ca 1 cm^2) from each region were fixed in phosphate buffered formalin (4%; pH 7.4) for 24 h, and then transferred to 70% ethanol for storage until processing. All formalin fixed tissues were routinely dehydrated in ethanol, equilibrated in xylene and embedded in paraffin according to standard histological techniques. Transverse sections (approximately 5 μm thick) of the stomach and longitudinal sections of the mid and distal (i.e. perpendicular to the macroscopically visible circular folds) intestines were cut and stained with haematoxylin and eosin (HE). Processing of the tissues was done at the Section for Pathology of the National Veterinary Institute (Oslo, Norway). Blinded histological examination was performed by light microscopy. Tissue morphology was evaluated according to Amin et al. (1992) and Baeverfjord and Krogdahl (1996).

2.5. Calculations and statistical analysis

Specific mechanical energy (SME) during extrusion was calculated as: $(2 \times \pi \times 60^{-1}) \times (S_{rpm} \times T_{kNm} \times T_{t/h}^{-1})$, where S_{rpm} is screw speed, T_{kNm} is torque and $T_{t/h}$ is throughput. Apparent digestibility of the main nutrients, amino acids and phosphorous were calculated according to Austreng (1978), using Y_2O_3 as an inert marker (Austreng et al., 2000). Crude protein was calculated as N × 6.25. Apparent digestibilities of ash and several minerals, such as Na, are underestimated due to uptake from seawater and intestinal excretion as part of ionic regulation. Apparent digestibility measurements of phosphorous are valid since it is a macro element, with low concentrations in seawater. However, for other minerals, faecal excretion was calculated as: $(-100 \times (1 - (D_m \times F_m^{-1}) \times (F_n \times D_n^{-1}))) + 100$, where D_m and F_m are the marker concentrations in the diet and faeces, respectively, and F_n and D_n are the nutrient concentrations in the diet and faeces, respectively. The content of organic material in the diets and faeces was calculated by subtracting ash content from dry matter content.

Statistical analyses were performed using the General Linear Model (GLM) and regression (REG) procedures in SAS (SAS, 1990). Significant differences ($P < 0.05$) among means were ranked with Duncan's multiple range test. Correlation analysis between dietary content of native fibre from the respective plant ingredients and viscosity of digesta, and between viscosity of digesta and nutrient digestibility were performed using Pearson product–moment correlations.

3. Results

3.1. Processing of the experimental diets

The target pellet density of 460 g l^{-1} during extrusion was reached in all diets. However, some diets, especially the lupin and corn gluten diets, had a lower density than the other diets after drying, resulting in a high percentage of floating pellets. The diets differed somewhat in lipid content in feed mash, with the soybean and sunflower diets having less lipid content and the wheat and oat diets having more lipid content than the other diets. Total moisture content of 238 g kg^{-1} in the feed mash during production of the control diet was lower than in the feed mash of the other diets. Extrusion temperatures did not vary much among diets. Specific mechanical energy (SME) during extrusion was approximately 35 Wh kg^{-1} for all diets, except the soybean, sunflower and wheat diets, which had SMEs of 42, 50 and 40 Wh kg^{-1}, respectively. The inlet air temperature in

Table 4
Chemical composition of the diets (g kg^{-1})

Diet	Control	Protein-rich plant ingredients					Starch-rich plant ingredients				
		Corn gluten	Soybean	Sunflower	Lupin	Rapeseed	Pea	Whole bean	Dehulled bean	Wheat	Oat
DM	970	961	939	951	955	928	947	953	946	961	953
In DM											
Lipid	321.6	317.4	321.6	336.5	331.9	336.2	331.6	332.6	342.5	324.7	334.7
Ash	86.6	71.8	85.2	91.5	78.5	85.1	85.5	88.1	86.7	88.4	87.1
Starch	97.6	87.8	75.5	85.1	84.6	96.4	84.6	76.9	81.2	87.6	81.9
Dietary fibre	120.2	125.2	100.8	89.2	89.0	94.7	116.0	122.7	99.0	119.2	130.6
Organic matter	913.4	928.2	914.8	908.5	921.5	914.9	914.5	911.9	913.3	911.6	912.9
Crude protein	383.5	401.7	394.0	388.0	396.9	378.2	394.9	396.6	393.2	387.1	386.1
Amino acids [a]											
Total	305.0	338.6	315.2	309.8	316.0	302.5	320.3	324.2	324.4	311.2	310.7
Tryptophan	3.3	3.0	3.6	3.5	3.5	3.4	3.3	3.4	3.6	3.3	3.3
Cyst(e)ine	3.2	4.2	3.6	3.4	3.4	4.0	3.3	3.4	3.3	3.3	3.4
Methionine	9.8	10.2	8.6	9.2	8.0	8.7	9.3	9.2	9.3	9.4	9.5
Aspargine	31.3	29.9	33.2	31.7	32.6	30.1	33.5	34.0	34.0	31.4	31.7
Threonine	13.6	13.6	13.4	13.4	13.3	13.5	13.9	14.0	14.0	13.5	13.6
Serine	14.3	16.3	15.2	14.5	15.2	14.0	15.1	15.3	15.3	14.6	14.8
Glutamine	45.8	57.0	50.9	50.3	53.5	47.8	49.6	50.2	50.0	49.4	47.8
Proline	12.8	19.0	14.2	13.0	13.3	13.6	13.3	13.6	13.6	13.8	13.2
Glycine	18.2	16.1	16.9	18.1	17.0	17.6	18.2	18.4	18.4	18.1	18.3
Alanine	19.1	22.8	18.3	18.3	17.7	17.7	19.3	19.4	19.5	19.1	19.3
Valine	16.6	17.2	16.7	16.9	16.3	16.5	17.2	17.4	17.6	16.7	16.6
Isoleucine	14.4	15.1	15.0	14.6	14.9	14.6	15.2	15.4	15.6	14.6	14.4
Leucine	24.1	35.2	25.5	23.8	24.8	23.8	25.3	25.7	25.8	24.6	24.5
Tyrosine	10.7	14.0	11.5	10.7	11.7	10.7	11.4	11.6	11.4	10.9	10.9
Phenylalanine	13.3	16.7	14.6	13.9	13.7	13.4	14.1	14.2	14.2	13.5	13.6
Histidine	8.4	8.5	8.5	8.7	8.9	8.6	8.9	8.9	9.0	8.4	8.4
Lysine	26.1	21.3	24.9	24.0	24.2	24.4	26.9	27.2	27.3	25.9	26.2
Arginine	20.6	19.3	21.8	22.7	25.4	20.4	23.1	23.5	23.4	21.2	21.5
Minerals											
Phosphorous	13.4	12.5	13.8	16.3	13.6	15.1	14.8	14.7	14.8	14.6	14.7
Sodium	6.4	4.5	5.1	5.2	5.0	5.5	5.9	5.8	5.8	6.0	6.2
Zinc	0.17	0.17	0.18	0.17	0.17	0.19	0.18	0.18	0.18	0.18	0.17
Yttrium	0.08	0.08	0.08	0.08	0.09	0.08	0.09	0.09	0.08	0.08	0.08
Antinutrients											
Phytic acid [b]	N.d. [c]	2.0	2.2	6.8	1.0	5.1	1.2	1.5	1.5	0.9	1.0
TIA [d]	0.1	N.d.	0.6	0.4	0.3	0.2	0.3	0.4	0.3	0.1	0.1

[a] Presented in dehydrated form.
[b] *myo*-Inositol hexaphosphate (IP6).
[c] Not detectable.
[d] Trypsin inhibitor activity, given as inhibited bovine trypsin.

the dryer proved to be difficult to control, causing variations of up to 15 °C within diets. The lowest air temperature noted at any point during production of the diets was 95 °C and the highest was 120 °C.

3.2. Diet characteristics

There were some differences in carbohydrate content among the diets (Table 4). Dietary fibre content in the soybean, sunflower, lupin, rapeseed and dehulled bean diets was lower than in the other diets. Dietary content of water-soluble fibre (data not shown) was below the analytical limit of 10 g kg^{-1} in all diets. The corn gluten diet deviated in content of several amino acids compared to the other diets, especially with lower contents of tryptophan and lysine. Phytic acid content was generally low, except in the rapeseed and sunflower diets, 5.1 and 6.8 g kg^{-1} DM, respectively. Trypsin inhibitor activity was low in all diets, ranging from 0 to 0.6 g bovine trypsin inhibited per kg of feed.

3.3. Digestibility experiment

3.3.1. Effect of cellulose inclusion on nutrient digestibility

Lipid digestibility (ADC_L) was reduced in a linear manner with increasing concentration of dietary cellulose (C) (Fig. 1; $ADC_L = 93.6 - 0.061 \times C$; $n = 11$, $R^2 = 0.57$, $P = 0.0070$). A step-wise regression analysis revealed that two diets, the soy and sunflower diets, deviated significantly from a linear pattern, both

giving significantly lower lipid digestibilities than predicted by the dietary cellulose level. When the soy diet, which had the largest distance to the predicted line, was removed from the analysis, the regression was $ADC_L = 94.5 - 0.066 \times C$; $n = 10$, $R^2 = 0.80$, $P = 0.0004$. When both soy and sunflower were removed, the regression became $ADC_L = 95.7 - 0.011 \times C$; $n = 9$, $R^2 = 0.88$, $P = 0.0002$. The regression of lipid digestibility on dietary fibre (DF) explained less of the variation than that on cellulose ($ADC_L = 104.1 - 0.129 \times DF$; $n = 11$, $R^2 = 0.40$, $P = 0.037$). The soy diet deviated significantly from this regression line, too. When the results from the soy diet was removed, the regression became $ADC_L = 107.0 - 0.151 \times DF$; $n = 10$, $R^2 = 0.62$, $P = 0.0068$. Results for the sunflower diet did not deviate significantly from this regression line. Since cellulose was supplemented to the diets based on estimated NSP content in the plant feed ingredients, the regression of lipid digestibility on NSP from the feed ingredients, estimated as the difference between dietary fibre and added cellulose, had a significant, positive slope. Digestibilities of protein and starch were not significantly affected by dietary cellulose level.

3.3.2. Diets with protein-rich plant ingredients

Feeding the sunflower, rapeseed, lupin, and especially the soybean diet, resulted in low DM content in faeces compared to the control diet and all diets with starch-rich plant ingredients (Table 5). Viscosity in the intestinal content decreased from the pyloric region to the large intestine for all diets with protein-rich plant ingredients. The lupin and rapeseed diets resulted in significantly higher average viscosity than the control diet.

As can be seen from Table 6, the sunflower, lupin, and rapeseed diets all resulted in higher lipid digestibility than the corn gluten, soybean, and control diets, while the latter diets did not differ significantly in lipid digestibility compared to each other. The diets containing protein-rich plant ingredients proved to divide into two groups concerning protein digestibility; the corn gluten and lupin diets performed at similar levels as the control diet, while the soybean, sunflower, and rapeseed diets all gave significantly poorer digestibility of protein and most amino acids than the former diets. However, lysine (89.7%) and tryptophan (76.9%) digestibility in the corn gluten diet was lower than in the control diet. The same effect on tryptophan (69.8%) was seen in the soybean fed fish, in addition to a low cysteine digestibility of 56.1%. No effects of diet on phosphorous digestibility were seen, except for a significantly higher digestibility in the rapeseed diet compared to the sunflower and control diets. All diets with protein-rich plant ingredients, except the lupin diet, resulted in significantly higher faecal excretion of sodium than the control diet, especially the soybean diet. No significant differences were found in excretion of zinc.

3.3.3. Diets with starch-rich plant ingredients

None of the diets with starch-rich plant ingredients resulted in significantly different faecal DM content than the control diet (Table 5). Viscosity in the intestinal content decreased from the pyloric region to the large intestine for all groups, except the oat fed fish, which along with the fish fed the wheat diet had significantly higher average viscosities than all other diets.

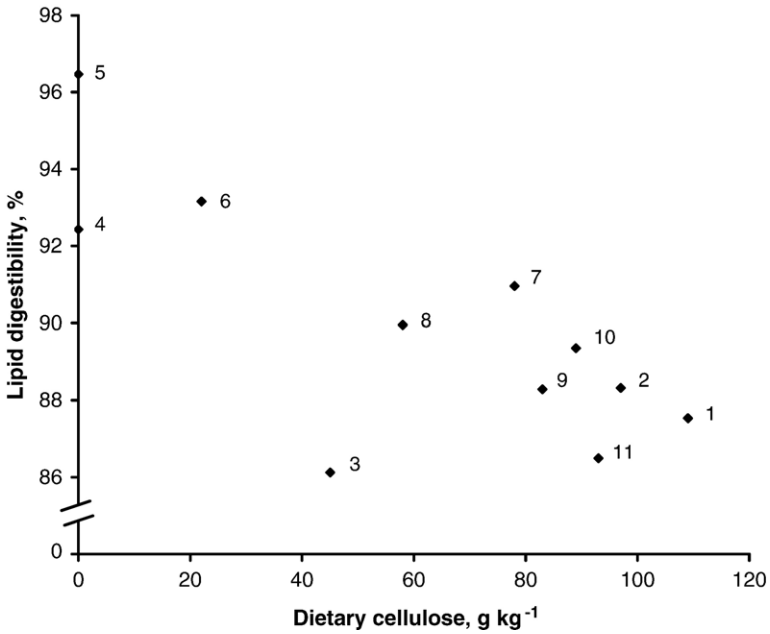

Fig. 1. Relationship between dietary cellulose inclusion and lipid digestibility. Numbers designate the experimental diets; 1, control; 2, corn gluten; 3, soybean; 4, sunflower; 5, lupin; 6, rapeseed; 7, pea; 8, whole bean; 9, dehulled bean; 10, wheat; 11, oat.

Table 5
Faecal dry matter content and viscosity of intestinal content in different parts of the salmon intestine

Diet	Control	Protein-rich plant ingredients					Starch-rich plant ingredients					SEM
		Corn gluten	Soybean	Sunflower	Lupin	Rapeseed	Pea	Whole bean	Dehulled bean	Wheat	Oat	
DM in faeces, g kg^{-1}	191.8 ^{ab}	190.8 ^{ab}	113.2 ^{d}	161.9 ^{c}	162.2 ^{c}	165.6 ^{c}	180.6 ^{b}	184.9 ^{ab}	185.9 ^{ab}	195.4 ^{a}	193.2 ^{ab}	4.3
Viscosity, cP												
Pyloric region	2.86 ^{b}	2.64 ^{b}	2.72 ^{b}	2.68 ^{b}	3.25 ^{ab}	3.21 ^{ab}	2.54 ^{b}	2.78 ^{b}	2.90 ^{b}	3.95 ^{a}	3.33 ^{ab}	0.23
Distal small intestine	2.37 ^{de}	2.37 ^{de}	2.43 ^{cde}	2.76 ^{bcd}	2.81 ^{bcd}	2.87 ^{abc}	2.22 ^{e}	2.24 ^{e}	2.26 ^{e}	3.14 ^{ab}	3.32 ^{a}	0.15
Large intestine	1.78 ^{cd}	2.07 ^{bcd}	1.89 ^{cd}	2.19 ^{bc}	2.27 ^{bc}	2.25 ^{bc}	1.76 ^{cd}	1.53 ^{d}	1.80 ^{cd}	2.45 ^{b}	3.77 ^{a}	0.17
All segments ^{f}	2.33 ^{c}	2.36 ^{c}	2.35 ^{c}	2.54 ^{bc}	2.77 ^{b}	2.78 ^{b}	2.17 ^{c}	2.18 ^{c}	2.32 ^{c}	3.18 ^{a}	3.47 ^{a}	0.12

^{abcde} Different letters denote significant ($P<0.05$) differences among diets.
^{f} Calculated average of the three sections.

No differences in lipid, protein or amino acid digestibility of the diets containing starch-rich plant ingredients were found compared to the control diet, with exception of the oat diet, which had significantly lower digestibility of protein and several amino acids than the pea, dehulled bean, and control diet (Table 6). Starch digestibility in the diets containing pulses was generally lower than in the diets containing cereals; the wheat diet resulted in significantly higher digestibility than the pea and both bean diets. The oat diet did not perform as well as the other diets with starch-rich plant ingredients concerning mineral uptake and excretion, with significantly lower digestibility of phosphorous and higher excretion of zinc. Sodium excretion was significantly elevated in the oat and whole bean diets compared to the wheat and control diets, and in the dehulled bean diet compared with the control diet.

3.3.4. Overall results

No significant correlation was seen between the inherent fibre content in the different plant sources and viscosity of intestinal content. No effect of viscosity could be seen on nutrient digestibilities.

Dietary phytic acid concentration did not prove to have any significant linear or non-linear relationships with the digestibility of phosphorous ($R^2=0.05$), or faecal excretions of sodium ($R^2=0.10$) and zinc ($R^2=0.06$).

Significant effects of TIA on digestibility of protein ($R^2=0.32$, $P=0.0006$) and total amino acids ($R^2=0.26$, $P=0.0022$) were observed. These effects were mainly driven by the observations for the fish fed the soybean diet, with the highest TIA. When this diet was excluded from the analysis, no significant pattern for effects of dietary TIA on digestibility was seen.

Results from the histological evaluation showed no diet related changes in any of the stomach or mid intestine samples. All samples were judged to be within normal limits regarding histological appearance. All distal intestine samples of the salmon fed the soy diet exhibited histomorphological changes. Distal intestine mucosal folds appeared shortened, and the lamina propria and submucosa appeared widened with increased cellularity. Enterocytes exhibited reduced to absent vacuolisation and apical displacement of nuclei. No histological changes in the distal intestine were noted for any of the other groups.

4. Discussion

The low total moisture content in the feed mash combined with the drying temperature of 110–120 °C during production of the control diet resulted in low moisture content in the finished feed that may potentially have increased the risk of over-drying, leading to heat damage of protein, as shown by Opstvedt et al. (1984). However, no such effect was indicated by reduction in digestibility of cysteine, or loss of lysine, both sensitive indicators of over-drying (Opstvedt et al., 1984; Ljøkjel

Table 6
Apparent digestibility of main nutrients, amino acids (AA) and phosphorous and faecal excretion of sodium and zinc in the experimental diets

Diet	Control	Protein-rich plant ingredients					Starch-rich plant ingredients					SEM
		Corn gluten	Soybean	Sunflower	Lupin	Rapeseed	Pea	Whole bean	Dehulled bean	Wheat	Oat	
Apparent digestibility, %												
Lipid	87.5 ^{def}	88.3 ^{def}	86.1 ^{f}	92.4 ^{bc}	96.5 ^{a}	93.2 ^{ab}	91.0 ^{bcd}	90.0 ^{bcde}	88.3 ^{def}	89.4 ^{cdef}	86.5 ^{ef}	1.1
Starch	55.1 ^{cd}	49.4 ^{e}	63.9 ^{a}	51.8 ^{de}	60.9 ^{ab}	54.2 ^{cde}	49.9 ^{de}	49.6 ^{e}	50.1 ^{de}	57.7 ^{bc}	54.4 ^{cde}	1.6
Organic matter	68.5 ^{b}	68.8 ^{b}	65.2 ^{c}	67.8 ^{b}	71.7 ^{a}	69.9 ^{ab}	69.0 ^{b}	68.6 ^{b}	68.3 ^{b}	69.8 ^{b}	65.2 ^{c}	0.6
Crude protein	84.6 ^{a}	84.8 ^{a}	80.4 ^{d}	81.4 ^{cd}	84.6 ^{a}	81.9 ^{cd}	84.6 ^{a}	83.7 ^{ab}	84.4 ^{a}	85.0 ^{a}	82.5 ^{bc}	0.5
Total AA	88.0 ^{a}	87.6 ^{a}	83.4 ^{c}	85.6 ^{b}	87.6 ^{a}	85.7 ^{b}	88.0 ^{a}	87.7 ^{a}	88.3 ^{a}	88.6 ^{a}	86.1 ^{b}	0.6
Tryptophan	82.3 ^{a}	76.9 ^{d}	69.8 ^{e}	79.8 ^{abc}	81.8 ^{ab}	79.7 ^{bc}	81.0 ^{ab}	81.9 ^{ab}	82.1 ^{ab}	82.0 ^{ab}	77.7 ^{cd}	0.8
Cyst(e)ine	68.7 ^{abc}	71.2 ^{a}	56.1 ^{e}	63.7 ^{cd}	67.1 ^{abcd}	65.7 ^{abcd}	64.7 ^{bcd}	64.8 ^{bcd}	68.2 ^{abcd}	70.3 ^{ab}	62.7 ^{d}	1.8
Methionine	90.0 ^{ab}	88.6 ^{bcd}	87.2 ^{d}	88.0 ^{cd}	88.8 ^{abc}	88.9 ^{abc}	89.3 ^{abc}	89.2 ^{abc}	89.9 ^{ab}	90.2 ^{a}	87.8 ^{cd}	0.5
Aspargine	78.3 ^{ab}	79.4 ^{ab}	74.3 ^{d}	75.4 ^{cd}	79.3 ^{ab}	75.3 ^{cd}	79.5 ^{ab}	79.8 ^{a}	80.6 ^{a}	79.1 ^{ab}	77.1 ^{bc}	0.7
Threonine	86.9 ^{a}	84.5 ^{cd}	77.8 ^{f}	83.1 ^{de}	85.3 ^{abc}	82.1 ^{e}	86.7 ^{ab}	86.5 ^{ab}	86.8 ^{ab}	86.9 ^{a}	84.9 ^{bc}	0.6
Serine	86.0 ^{a}	85.8 ^{a}	80.2 ^{c}	83.0 ^{b}	85.4 ^{a}	82.5 ^{b}	86.1 ^{a}	85.6 ^{a}	86.3 ^{a}	86.5 ^{a}	83.6 ^{b}	0.6
Glutamine	91.2 ^{abc}	90.7 ^{bcd}	87.6 ^{e}	90.1 ^{cd}	91.5 ^{ab}	90.4 ^{bcd}	91.4 ^{abc}	91.2 ^{abc}	91.5 ^{ab}	92.3 ^{a}	89.8 ^{d}	0.4
Proline	87.0 ^{ab}	88.7 ^{a}	81.7 ^{d}	84.0 ^{abc}	86.4 ^{b}	83.4 ^{c}	86.7 ^{b}	86.5 ^{b}	87.1 ^{ab}	88.2 ^{ab}	84.5 ^{c}	0.6
Glycine	84.3 ^{ab}	83.7 ^{ab}	79.5 ^{d}	80.7 ^{cd}	83.8 ^{ab}	82.5 ^{bc}	84.4 ^{a}	84.0 ^{ab}	85.1 ^{a}	84.7 ^{a}	81.5 ^{c}	0.6
Alanine	90.7 ^{a}	90.0 ^{ab}	87.2 ^{e}	87.9 ^{de}	89.3 ^{bc}	88.7 ^{cd}	90.1 ^{ab}	90.0 ^{ab}	90.5 ^{ab}	90.9 ^{a}	88.3 ^{cde}	0.4
Valine	88.9 ^{abc}	87.5 ^{cde}	83.3 ^{f}	86.7 ^{de}	88.0 ^{bcd}	86.2 ^{e}	88.9 ^{abc}	88.7 ^{abc}	89.1 ^{ab}	89.7 ^{a}	87.0 ^{de}	0.5
Isoleucine	90.3 ^{a}	88.0 ^{c}	85.4 ^{d}	88.3 ^{bc}	89.6 ^{ab}	88.1 ^{c}	89.9 ^{a}	89.8 ^{a}	90.1 ^{a}	90.8 ^{a}	88.3 ^{bc}	0.4
Leucine	91.7 ^{a}	91.3 ^{ab}	87.6 ^{d}	89.5 ^{c}	90.8 ^{abc}	89.6 ^{c}	91.4 ^{ab}	91.2 ^{ab}	91.6 ^{a}	92.1 ^{a}	90.2 ^{bc}	0.4
Tyrosine	85.2 ^{a}	86.0 ^{a}	82.1 ^{c}	82.8 ^{bc}	85.6 ^{a}	82.2 ^{c}	86.0 ^{a}	84.2 ^{ab}	84.8 ^{a}	85.2 ^{a}	82.6 ^{bc}	0.6
Phenylalanine	87.9 ^{a}	87.2 ^{ab}	83.4 ^{d}	86.1 ^{bc}	87.2 ^{ab}	85.9 ^{c}	87.7 ^{a}	87.3 ^{ab}	87.7 ^{a}	88.6 ^{a}	85.7 ^{c}	0.4
Histidine	86.6 ^{a}	86.0 ^{ab}	79.8 ^{d}	83.4 ^{c}	85.4 ^{abc}	85.4 ^{abc}	86.4 ^{ab}	85.9 ^{ab}	86.3 ^{ab}	86.7 ^{a}	84.2 ^{bc}	0.7
Lysine	92.5 ^{a}	89.7 ^{c}	87.8 ^{d}	89.4 ^{c}	91.2 ^{b}	89.6 ^{c}	92.1 ^{a}	91.7 ^{ab}	92.4 ^{a}	92.5 ^{a}	90.9 ^{b}	0.3
Arginine	92.4 ^{ab}	91.4 ^{bc}	89.8 ^{d}	91.5 ^{bc}	93.0 ^{a}	91.5 ^{bc}	92.4 ^{ab}	91.6 ^{bc}	92.2 ^{ab}	92.3 ^{ab}	90.6 ^{cd}	0.4
Phosphorous	26.4 ^{bc}	27.9 ^{abc}	27.5 ^{abc}	25.8 ^{bc}	30.7 ^{ab}	40.1 ^{a}	34.8 ^{ab}	36.0 ^{ab}	39.0 ^{ab}	37.0 ^{ab}	16.6 ^{c}	4.0
Faecal excretion, % ^{g}												
Sodium	92.2 ^{f}	150.6 ^{b}	214.1 ^{a}	149.3 ^{b}	106.6 ^{def}	126.1 ^{c}	106.4 ^{def}	120.0 ^{cd}	112.7 ^{cde}	99.0 ^{ef}	115.6 ^{cd}	4.9
Zinc	77.6 ^{bc}	76.6 ^{bc}	76.3 ^{bc}	82.7 ^{ab}	73.6 ^{bc}	73.4 ^{bc}	75.4 ^{bc}	75.0 ^{bc}	74.3 ^{bc}	71.4 ^{c}	87.4 ^{a}	3.0

^{abcdef} Different letters denote significant ($P<0.05$) differences among diets.
^{g} Faecal mineral excretion, % of dietary intake.

et al., 2000). Nor did PDI values indicate over-drying in the control diet. The high SME during extrusion of the soybean and sunflower diets may be ascribed to lower lipid content in the feed mash of these diets, since lipid reduces the mechanical energy due to its lubricating properties. Extrusion is known to increase the amount of free radicals, with heat, moisture content and SME being important factors (Schaich and Rebello, 1999). These free radicals can in turn form cross-links with proteins, making them poorly digestible. Digestibilities of cysteine and several other amino acids were low for the soybean diet, while the sunflower diet that had the highest SME, giving amino acid digestibilities higher than the soybean diet and similar to the rapeseed diet, indicating that elevated SME was not a main factor in explaining variations in amino acid digestibility. PDI values showed no correlation with SME, in accordance with Marsman et al. (1995).

It is common practice to use cellulose as inert filler in experimental diets for salmonids. Previous studies have indicated that "the digestibility of α-cellulose was not different from zero" (Hilton et al., 1983), that cellulose did not affect "efficiencies of utilization of dietary energy or protein" in rainbow trout (Wiesmann and Pfeffer, 1986), and that dietary cellulose did not influence digestibility of protein or starch in Nile tilapia (*Oreochromis niloticus*) (Amirkolaie et al., 2005). Even though the present experiment was not primarily designed to assess the effects of dietary cellulose, and specific effects of some ingredients were seen, the results strongly indicate that cellulose negatively affects lipid digestibility when processed together with other ingredients by extrusion of feed for salmon (Fig. 1). However, the results tend to support the previous conclusions that dietary cellulose does not influence digestibility of protein or starch.

The highly reduced faecal dry matter content in salmon fed the soybean diet is consistent with previous findings in rainbow trout and Atlantic salmon (Refstie et al., 1999, 2000). Also the sunflower, lupin and rapeseed diets, which contained high amounts of NSP other

than cellulose (Bach Knudsen, 1997), resulted in increased faecal water content, but to a much lesser degree than the soy diet. This is in keeping with previous observations that faecal water content was related to dietary NSP in salmon fed different soybean products (Refstie et al., 1999). The difference in faecal water content among salmon fed soybean and the other plant protein sources can be explained by the impaired ability of soybean fed salmon to absorb water in the damaged distal intestine (reviewed by Storebakken et al., 2000a), and/or differences in water binding capacity among the NSPs in these feed ingredients. However, the viscosity of the intestinal contents in salmon fed soy was not affected by dietary NSP content, in accordance with previous observations (Refstie et al., 1999). In contrast, the viscosity of the digesta was significantly elevated in the salmon fed the rapeseed and lupin diets compared to the control, while the sunflower diet showed a trend toward increased viscosity compared to the control. This supports the conclusion that not only impaired function of the distal intestine, but also dietary NSP, contributes to the elevated faecal water content in fish fed diets with soybean meal. Nutrient digestibilities did not seem to be affected by viscosity of the digesta, in accordance with Brinker et al. (2005).

The numerically lowest lipid digestibility was observed for the soybean diet although the distance to the control diet did not reach significance, somewhat in contrast to the previous markedly negative findings (reviewed by Storebakken et al., 2000a). Regression analysis (Fig. 1), however, showed that the soy diet gave a much lower lipid digestibility than predicted by the dietary cellulose level. A factor such as limited metabolic capacity of the distal intestine epithelium, or other components present in soy may have caused further reductions in lipid digestibility. Previously, NSP from soy has been suggested as one such factor (Refstie et al., 2005). The deviation of the sunflower diet from the pattern of lipid digestibility on dietary cellulose level indicates that additional factors other than soybean meal induced enteritis in the distal intestine contribute to reduced lipid digestibility, in keeping with our previous discussion on faecal dry matter content. The higher lipid digestibility of the lupin, sunflower, and rapeseed diets compared to the control diet can be explained by differences in dietary cellulose content, as these three diets had the lowest level of cellulose supplementation.

Two possible, though conflicting, explanations to the reduced amino acid digestibility of the soybean diet are: excessive heating causing protein damage, or too low heat treatment to properly inactivate protease inhibitors. Both excessive heating and too little heat of soy will give the same result, an overall reduction in apparent digestibility of amino acids, especially for cystein(e) (review by Storebakken et al., 2000a). The soybean meal had a TIA of 3.2 g kg^{-1}, and a PDI of 20.1%, both indicating that the toasting of the soy had been moderate. Even though TIA in the soy diet was higher than for the other diets, it was considerably lower than what is expected to cause significant reduction in protein digestibility in salmon, caused by a strong increase in endogenous loss (Olli et al., 1994).

Among the diets containing protein-rich plant ingredients, only the corn gluten and lupin diets gave similar digestibilities of crude protein compared to the control diet. The good protein digestibility of these diets is in accordance with Mente et al. (2003) and Glencross et al. (2004b), respectively. Corn gluten, however, has an unbalanced amino acid profile compared to fish meal, with low lysine and tryptophan contents, and the digestibilities of these amino acids were low compared to the control diet. This indicates that supplementation not only with lysine, but also with tryptophan should be considered when corn gluten meal is used.

The digestibilities of protein and most amino acids were low in the sunflower and rapeseed diets compared to the control diet in the present study. Tacon et al. (1984) observed decreasing protein digestibility with increasing sunflower inclusion in diets for rainbow trout (*Salmo gairdneri* R.). Even though sunflower contains one of the most potent trypsin inhibitors known, sunflower trypsin inhibitor-1 (Luckett et al., 1999), the TIA was low in the sunflower diet and does not explain the low protein digestibility observed in the present experiment. As mentioned earlier, the high SME during extrusion of the sunflower might have contributed to low protein digestibility. Rapeseed contains antinutrients such as tannins and sinapine, which could have affected feed uptake and utilization. Free tannins are also known to reduce protein digestibility (Fenwick, 1982). Neither the sunflower nor the rapeseed meal used in the present experiment seems to be suited for Atlantic salmon without further treatment to remove or inactivate the substances causing the reductions in protein and amino acid digestibility.

Reductions in protein digestibility in diets with plant protein sources are most often described to antinutritional factors, like those mentioned previously. However, the effect of the structural and functional properties of the globular storage proteins in plant protein sources has not been given as much attention. Thermal denaturation of globular proteins will rupture the quaternary structure, releasing the subunits, which after partial or complete unfolding will rearrange to form aggregates

(Kinsella et al., 1985). The mechanisms of such aggregation and the effects on digestibility have not been fully clarified, but negative effects have been shown (Carbonaro et al., 1997), especially with 7S globulins (Santoro et al., 1997; Cuadrado et al., 2002; Carbonaro, 2006). It is difficult to draw conclusions on whether such processes have negatively affected the digestibility of the multi-protein source diets in the present experiment. Effects of protein-related reactions caused by hydrothermal processing on the digestive process need further clarification, preferably in model systems.

The low values of starch digestibility in the present study are consistent with results from previous trials (reviewed by Krogdahl et al., 2005). The low degree of starch digestion may be due to limited functionality of α-amylase in salmon (Frøystad et al., 2006), leaving hydrolysis of starch largely to intestinal disaccharidases, thus being reduced in response to increasing dietary starch intake (Krogdahl et al., 2004, 2005). Native starch-rich plant ingredients probably contain varying amounts of α-amylase inhibitors, but extrusion has been shown to effectively reduce the activity of these inhibitors (Alonso et al., 2000).

Generally, the starch from cereals seemed to be better digested than starch from pulses in the present study. Native bean starch has a higher gelatinisation temperature than cereals (Betancur-Ancona et al., 2004). Bednar et al. (2001) indicated that the starch in legume granules is more resistant to hydrolysis than in cereal granules, supported by lower in vitro digestion of legume starches compared to cereal starches. In addition, X-ray diffraction studies of the crystalline parts of the starches give unequal diffractograms for legumes and cereals, supporting the theory of a different starch structure at a molecular level (Hoover et al., 1991; Gallant et al., 1992). Bean and pea starches have an amylose level of approximately 32% compared to approximately 25% for cereals (Betancur-Ancona et al., 2004). An increase in amylose–amylopectin ratio is known to decrease the digestibility of starch (Bednar et al., 2001; Svihus et al., 2005). These factors could explain the reduced starch digestibility for the legumes compared to the cereals.

In the present experiment, all diets with starch-rich plant ingredients, except the oat diet, resulted in good nutrient digestibilities. This was not unexpected since only 5–15% of fish meal protein was replaced with protein from the starch-rich plant ingredients. The nutrient digestibility of the pea diet was in accordance with previous results of pea meal for gilthead sea bream (*Sparus aurata*) (Pereira and Olivia-Teles, 2002), extruded dehulled pea meal for European sea bass (*Dicentrarchus labrax*) (Gouveia and Davies, 2000), and pea protein concentrate for Atlantic salmon (Carter and Hauler, 2000), where no detrimental effects of ~20% inclusion of the respective pea products were seen compared to fish meal-based control diets. Less work has been done with beans, but it has been shown to perform similarly as pea diets fed to Australian silver perch (*Bidyanus bidyanus*) (Booth et al., 2001). Both peas and beans seem to be well suited for salmon, when used as a starch source simultaneously partly replacing fish meal protein.

Faba bean hulls with high tannin content reduced protein digestibility in pigs (Jansman et al., 1995). In the present study, the dehulling of the beans did not significantly increase protein or amino acid digestibilities as found by Booth et al. (2001), who fed silver perch diets with 30% whole or dehulled faba bean. The lower inclusion level in the present experiment could explain the lack of effect. Nevertheless, dehulling of beans may be beneficial for salmon feed, especially if the aim is to find space for high inclusion levels of plant feed ingredients in the formula.

Determination of faecal sodium excretion in fish reared in salt water can be subject to relatively high margins of error, due to the high sodium content of the water. The coefficient of variation within diets for faecal sodium excretion ranged between 0.4 and 13.0% in the present experiment, which makes it difficult to make accurate conclusions. Storebakken et al. (1998) indicated that one of the possible factors affecting the faecal sodium excretion is NSP, due to the affinity polysaccharides have to cationic elements. The dehulled bean diet, however, did not result in significantly lower sodium excretion than the whole bean diet, even though the dietary fibre content was two percentage units lower in the dehulled bean diet. Additionally, the fact that two diets with similar cellulose inclusion and total dietary fibre content; the corn gluten and control diets, gave very different faecal sodium excretion, indicates that NSP and/or cellulose is at least not the sole factors explaining this effect. The elevated sodium excretion in the soybean fed fish is in accordance with previous works where Atlantic salmon were fed extracted soybean meal (Sugiura et al., 1998) and soy protein concentrate (Storebakken et al., 1998). The intestinal enteritis observed in these fish could be a contributor to the increased sodium excretion, due to the important role the distal intestine plays in ion absorption and regulation (Sundell et al., 2003).

The lack of an effect of dietary phytic acid concentrations from 0 to 5.1 g kg^{-1} on faecal zinc excretion is in contrast to the results obtained by Denstadli et al. (2006b), who observed increased faecal excretion of

zinc at dietary phytic acid levels above 2.1 g kg^{-1}. A possible explanation is that the phytic acid analysed in the feed was present as phytates already tightly bound to divalent or trivalent cations that became poorly dissociated in the stomach and gut. No studies have been conducted in order to determine whether phytic acid isolated from different plant ingredients exhibit different binding-efficacies towards different minerals. According to data from Teskeredžić et al. (1995) diets with 36% rapeseed protein concentrate containing 11 g phytic acid kg^{-1} diet had no significant effect on zinc bioavailability in rainbow trout compared to a control diet with herring meal. In contrast, Storebakken et al. (1998) reported significantly lowered zinc uptake and mineralization in Atlantic salmon fed soy protein concentrate (43%) diets with 9.3 g phytic acid kg^{-1}. Calcium phosphate from fish meal can also suppress mineral digestibility (Hardy and Shearer, 1985), but as seen from the formulation, the level of FM was almost similar in each diet. It is known that fibre structures, including cellulose, can impair the uptake of several minerals in the intestine (Partridge, 1978), and as the control diet in the present experiment contained approximately 10% cellulose, this could be a confounding factor when evaluating the mineral digestibilities. A low digestibility of P in fish fed the oat diet compared to fish fed the rapeseed diet could be rationalised by the difference in cellulose inclusion (93 and 22 g cellulose kg^{-1}, respectively). According to Partridge (1978), increased levels of cellulose reduced the absorption of P in pigs. Problems encountered using inert fibre as bulking agents have been studied by van der Aar et al. (1983), and in a trial with poultry, serum and tibia Zn was lower in diets where various fibre types replaced starch. The significant differences in zinc excretion may also be explained by binding to NSP from the feed ingredients (Ekholm et al., 2000). However, no systematic relationship was seen between faecal zinc excretion and dietary fibre content ($R^2=0.06$) or estimated NSP content in the feed ingredients ($R^2=0.002$). From this perspective it is not known if there is any additive effect of cellulose/NSP and phytic acid on availability of di- or trivalent minerals. Thus, the significant differences in faecal zinc excretion could be ascribed to other feed components, such as globular peptides, and proteins that facilitate zinc transport in the gut, discussed previously by Storebakken et al. (2000b).

Aside from soybean meal, there is scant evidence in the literature suggesting that the plant ingredients used in the present study cause alterations in the histomorphology of the gastrointestinal tract in fish. Currently no information is available on the effect of dietary oat, extracted sunflower, peas, faba beans or rapeseed meal in salmonids. Farhangi and Carter (2001) concluded that inclusion of up to 50% ground dehulled narrow-leaf lupin (*Lupinus angustifolius*) in the diet of rainbow trout did not affect proximal intestine morphology. The authors did report, however, that a non-significant trend of reduced villus height was observed with increasing levels of dietary lupin. No other regions of the intestine were examined. Glencross et al. (2004a) also reported a lack of effect on rainbow trout gastrointestinal histomorphology when dehulled yellow lupin (*Lupinus luteus*) meal was included in the diet up to 50%. However, the gastrointestinal somatic index was higher in fish fed diets containing 37.5 and 50% lupin meal compared to fish fed a fish meal based control diet, the significance of which is not known. Sitjà-Bobadilla et al. (2005) reported increased supranuclear protein droplets, lipidic vacuolisation of enterocytes and dilated/hypertrophied submucosa with eosinophilic infiltration in the distal intestine of juvenile gilthead sea bream (*Spaurus aurata*) fed diets in which 50, 75 or 100% of the fish meal was replaced by a combination of plant proteins (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin).

The changes observed in the distal intestine of soybean fed fish are consistent with previously described changes associated with soybean-induced enteritis (Baeverfjord and Krogdahl, 1996). The absence of pathological changes in the stomach and intestine of salmon fed the other experimental diets is highly promising for the selection of ingredients that require a low level of processing to be optimal in feeds for salmon. The healthy intestines of the salmon fed a variety of plant feed ingredients also narrows the search for the factor(s) causing soybean-induced enteritis to the soybean. Up to now it is known that these factors must be relatively heat stable since soybean meal is toasted, and that they are extractable by alcohol and water, since feeding the extract from production of soy protein concentrate manifests the intestinal enteritis (van den Ingh et al., 1996; Bureau et al., 1998). Limited information regarding effects of the individual soybean antinutrients is available. As neither trypsin inhibitors, lectins or phytic acid are removed from the beans by alcohol extraction these antinutrients are unlikely to be the causing agents in the inflammation of the gut. The findings in this study eliminate NSP from plant ingredients as a cause for the condition. The lack of histomorphological changes in the stomach of salmon fed the lupin diet confirms the conclusion of Refstie et al. (2006) that the formation of ulcers in the stomach was not caused by dietary lupin, but also emphasizes the need to carefully investigate health effects of new feed ingredients for carnivorous fish, as

they found that lupin worsened the condition once it was manifested.

5. Conclusion

The present experiment shows that several plant ingredients have potential for being used in diets for Atlantic salmon, based on nutrient digestibilities and absence of pathologies in the stomach and intestine. However, further work is needed to identify, and where possible, remove or inactivate the compounds that cause reduced digestibility, especially concerning plant protein sources like sunflower and rapeseed. Combined starch and protein sources, in this case pea and bean, seem to be well suited for fish feeds, even without specific pre-treatment of them as raw materials.

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