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Disintegration stability of extruded fish feed affects gastric functions in Atlantic salmon (*Salmo salar*)

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ABSTRACT

In the present study, two salmon feeds with different disintegration stabilities were produced by impacting extruder viscous heat dissipation with the use of different lipid levels in the feed mix. The feeds were then dried and coated to similar final lipid levels (30-31%) and nutritional composition. Both feeds were produced as 4 and 6-mm pellets. Feeds extruded with 20% lipid had a lower disintegration stability compared with those with 8% lipid. The feeds were used in two feeding studies using Atlantic salmon (Experiments 1 and 2) and an *in vitro* gastric study (Experiment 3). In Experiment 1, salmon postsmolts (370 g starting weight) fed the 4-mm pellets showed the fastest gastrointestinal (GI) passage rate when fed feeds with a lower disintegration stability. This was due to a faster transport of content from the stomach to the gut at 30 min and 1.5 h after a meal, and faster passage through the distal gut 9–24 h after a meal. The 6-mm pellets were used in a 100-day feeding study (Experiment 2) in which salmon fed high and low disintegration stability feeds grew from 572 to 1542 and 1604 g, respectively. There was a higher total feed intake in salmon fed diets with lower disintegration stability, but only tendencies towards higher growth and final body weight compared with salmon fed diets with a higher disintegration stability. The *in vitro* gastric experiment showed that pellets with a lower disintegration stability required a larger supply of stomach acid to maintain a stable pH of 4.5, which also gave a lower content viscosity. The *in vitro* study also showed an increased release of water-soluble components from these pellets and hydrolysis of larger peptides increased the pool of intermediate-sized peptides (4–6 kDa) available for easy transportation, hydrolyzation, and absorption in the proximal intestine. Although lower pellet disintegration stability increases feed intake. The GI passage rate combined with an increased GI filling can affect the ability to hydrolyze and absorb nutrients. This may explain why an increased feed intake did not result in increased growth in Experiment 2, despite observing no significant differences in nutrient digestibility or feed conversion ratio. Our results highlight the importance of the quality of extruded fish feed.

1. Introduction

Key factors for shorter production time and reduced costs in the commercial production of Atlantic salmon (*Salmo salar*) include high feed intake, efficient feed utilization, and high growth rates (Einen et al., 1995). The stomach has several functions that are related to both the utilization and intake of feed. It is an important organ for short-term storage while food is effectively processed *via* disintegration and pre-digestion to a fluidized mass (chyme/digesta) that is transferred to the proximal part of the intestinal tract, thus determining the gastrointestinal (GI) passage rate, nutrient digestibility, and feed intake (Grove et al., 1978; Soengas, 2014). Feed entering the stomach and downstream transport of nutrients to the proximal intestine stimulate signaling pathways to the brain that regulate hunger and satiety in animals (Hoskins and Volkoff, 2012). Water-soluble components, particularly peptides and amino acids, stimulate feed intake (Aksnes et al., 2006; Dias et al., 1997; Kousoulaki et al., 2009). In the stomach, feeds are hydrated in a mixture of acid (HCl) and gastric enzymes (mainly pepsin; Koelz, 1992; Einarsson et al., 1996) that disintegrates the surface of the food items and releases smaller particles and water-soluble

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components into the liquid phase that are then transferred to the intestine (Andersen and Beyer, 2005). Thus, feeds comprising partly hydrolyzed nutrients and/or those with a low disintegration stability may be beneficial for faster emptying of the stomach and ensuring high feed intake.

Numerous studies have shown that the inclusion of free amino acids in feeds results in a higher feed intake (reviewed by Morais, 2017). Furthermore, greater feed intake has been shown in fish fed presoaked feeds or feeds with a low disintegration stability in water (Aas et al., 2011, 2021; Baeverfjord et al., 2006). Faster disintegration of feeds in the stomach, shorter gastric retention time, and the transit time of digesta through the GI tract have been suggested to contribute to higher intake of these feeds (Aas et al., 2017, 2021). In line with this hypothesis, Adamidou et al. (2009) showed that inclusion of faba beans and chickpeas in diets for seabass increased the hardness of extruded pellets and prolonged the GI passage rate. Jacobsen et al. (2018) found a negative correlation between pellet hardness and feed intake and growth of Atlantic salmon. However, Glencross et al. (2011) showed that the inclusion of lupin in the diets resulted in harder pellets, but also increased feed intake. These contradictory findings suggest that hardness is not the best parameter to predict fish performance. There may be a poor correlation between pellet hardness and water disintegration due to different technical properties of the ingredients of the feed mix (Samuelsen et al., 2018). Lower disintegration stability of feed pellets increased feed intake in rainbow trout (*Oncorhynchus mykiss*) and was correlated with increased growth and condition factor. Reduced nutrient digestibility in fish fed this feed compared to a feed with higher disintegration stability is also reported (Aas et al., 2011). The GI passage rate, in combination with increased GI filling due to increased feed intake, can affect the ability to hydrolyze and absorb nutrients. Thus, both pellet quality and nutritional availability must be considered for assessment of the optimal utilization of feeds.

Extrusion is the key process in fish feed manufacturing and is a high-temperature, short time process in which the dry feed ingredients are moistened and heated by the addition of steam and water and subjected to thermomechanical treatment in extruder screws. Cooked and transformed feed mass is expanded to form a porous structure over an extruder die. The extrudates are then dried and lipids are added during vacuum coating. The physical quality and expansion of the final product are controlled by various factors, such as water and steam injection, viscous heat dissipation, and extruder screw and die configuration. The processing and quality of the final product is also influenced by the physicochemical and rheological properties of the feed ingredients (Samuelsen et al., 2013, 2014, 2018). Limited market availability of fishmeal has led to the introduction of alternative protein ingredients, all with different technical quality characteristics (Samuelsen et al., 2018; Ahmad et al., 2019; Oterhals et al., 2019). The choice and inclusion of alternative protein ingredients in fish feed formulations is based on the nutritional value, technical quality, and availability and price in the commodity market (Gatlin III et al., 2007). In 2016, soy protein concentrate was the main protein ingredient used in Norwegian salmon feed production (Aas et al., 2019) and still dominates the market in 2021. This ingredient demands high moisture levels and temperature to obtain a satisfactory transformation in the extrusion process and durable pellets (Samuelsen et al., 2018; Ahmad et al., 2019). Lipid levels of the feed mix also impact ingredient transformation during processing. Increased fat levels reduce the extruder viscous heat dissipation due to the lubrication effect of lipids, resulting in poorer physical pellet quality and water disintegration stability (Samuelsen et al., 2018).

In the present study, two salmon feeds with different disintegration stabilities were produced by impacting extruder viscous heat dissipation with the use of different lipid levels in the feed mix. The feeds were dried and coated to similar lipid levels, resulting in a similar nutritional composition of the final feed pellets. The disintegration stability and protein hydrolysis of the feeds were studied using an *in vitro* gastric method and *in vivo* experiments using Atlantic salmon fed a meal of the feeds after a 48-h fast. Furthermore, stomach emptying rate, GI passage rate, digestion, feed intake, and growth were studied in a growth experiment to elucidate long-term effects of feeds with different disintegration stabilities.

2. Materials and methods

2.1. Feed production

Salmon diets were extruded at two lipid levels (8% and 20%) and oil coated to reach similar final lipid levels (30-31%; Table 1) at Nofima's Feed Technology Centre (Fyllingsdalen, Norway). The feeds were produced using a combined preconditioner and co-rotating twin-screw extruder system (TX-52, Wenger Manufacturing Inc., Sabetha, KS, United States), dried in a dual layer carousel dryer (Model 200.2; Paul Klockner GmbH, Nistertal, Germany), and oil-coated using a Dinnissen vacuum coater (Sevenum, The Netherlands). The feeds were formulated based on levels of ingredients used at Norwegian salmon feed producers in 2016 (Aas et al., 2019) and produced as 4 and 6-mm-diameter pellets for use in GI passage rate (Experiment 1) and growth (Experiment 2) experiments. Detailed information about the ingredients, feed formulation, extrusion settings, and feed composition of macronutrients,

Table 1
Ingredient diet composition, feed mix oil addition, and post extrusion oil coating (%).

Feed ingredients (% as fed)	Diet A	Diet B
Fishmeal	15.00	15.00
Soy protein concentrate	20.00	20.00
Wheat gluten	15.00	15.00
Corn gluten	8.35	8.35
Wheat	9.50	9.50
Additives[a]	6.15	6.15
Fish oil feed mash	0.00	0.78
Rapeseed oil feed mash	2.77	13.50
Fish oil coated	12.50	11.72
Rapeseed oil coated	10.73	0.00
Fat content (% as is)		
Prior to extrusion[b]	8.00	20.00
After coating[c]	30.90	30.30

[a] Soy lecithin, choline chloride, monosodium phosphate, carophyll Pink, vitamin and mineral mix, and yttrium oxide.

[b] Calculated fat content.

[c] Lipid extraction (Bligh and Dyer, 1959).

amino acids, and fatty acids are shown in Tables S1 and S2.

2.2. Fish experiments

The feeds were distributed to Atlantic salmon in two experiments. Experiment 1 examined the GI passage rate after a meal and Experiment 2 examined feed intake and growth during a 100-day study.

2.2.1. Experiment 1: GI passage rate study

Postsmolt Atlantic salmon (n = 120) were distributed into six 500-L fiberglass tanks (seawater, 13 °C, 20 L min^{-1} flow-through) and fed the respective feeds in triplicate for two weeks at Nofima's indoor tank facilities at Sunndalsøra (Norway). The fish were given one meal (*ad lib* feeding for 30 min) every morning. After the prefeeding period, the fish were fasted for 48 h and then fed one meal. Six fish from each dietary treatment group (average weight, 374 ± 35 g) were sampled at 0.5, 1.5, 3, 6, 9, 12, and 24 h after the end of the meal (detailed information about feeding and sampling times is listed in Table S3). Due to logistics and to prevent stress on the fish, each tank was only sampled two to three times (either 0.5, 6, and 24 h; 1.5 and 9 h; or 3 and 6 h after the meal). The fish were euthanized using a lethal dose of Finquel MS-222 (3 g L^{-1} tricaine methanesulfonate, Scan-Vacc, Hvam, Norway), and body length and weight were measured. GI sections of the stomach and proximal and distal intestines (Fig. 1) were removed after closing the ends with artery clamps. A small tissue sample was collected from between the proximal and distal gut (start of the midgut) for cholecystokinin (CCK) analysis in fasted fish prior to the meal, and at 1.5 and 9 h after the meal. The samples were collected in RNAlater solution (Invitrogen, Carlsbad, CA, USA) and stored at 4 °C overnight before storing at −80 °C prior to CCK analysis. The remaining GI sections and their contents were wrapped in aluminum foil, immersed in liquid nitrogen, and stored at −20 °C. The samples were later partly thawed and the contents from the different sections were collected, freeze-dried, and weighed. The stomach contents from 21 fish (4 and 4 fish 30 min after the meal, 4 and 3 fish 1.5 h after the meal, and 3 and 3 fish 3 h after the meal for fish fed Diets A and B, respectively) were used to analyze peptide size distribution of the water-soluble protein fraction.

2.2.2. Experiment 2: Growth study

Postsmolt Atlantic salmon (n = 360; 572 ± 4 g) were distributed into six 500-L fiberglass tanks and fed the respective feeds for 100-days. The water temperature was ambient and decreased from 12.3 °C during the first 30 days of the fall to 8.8 °C at final sampling during early winter. The fish were continuously (90 feedings per 40 s for 24 h) fed the two feeds using automatic feeders at a rate of 1.5% of fish tank biomass on day 1, and then adjusted according to the collected spill feed, aiming for 10%–20% feeding in excess. Feed intake and growth was monitored throughout the study as previously described by Helland et al. (1996). The total feed intake during the experimental period was calculated for each tank. The feed conversion ratio was calculated as total feed intake divided by the biomass growth in the tank. The specific growth rate was calculated as ((ln(final weight)-ln(start weight))/days in trial) × 100, and the condition factor was calculated as body weight divided by $length^3$ × 100. At the end of the experiment, 10 fish per tank were euthanized using a lethal dose of Finquel MS-222 as described in Experiment 1, and the contents were collected from the stomach and proximal intestine (mainly the content of the anterior gut, less from the pyloric caeca), as well as from four sections of the distal intestine, including midgut 1 and 2 (first and second half of the content from the pyloric sphincter attachment of the foregut ends to the increase in diameter of the intestine) and hindgut 1 and 2 the (first and second half of the content from the increase in diameter of the intestine to the anus). Samples of the GI segments were pooled by tank, freeze-dried, and used to analyze dry matter, crude protein, and yttrium(III) oxide to calculate the apparent digestibility in each GI section. The bile volume from the gallbladder was measured using a 5-mL syringe with 0.1-mL resolution in six fish per diet, and in 14 fish per diet after a 72-h fast.

2.2.3. Experiment 3: In vitro simulated gastric study

Feed pellets and seawater (33‰ salinity, distilled water with Tetra Marine sea-salt, Spectrum Brands Inc., Sulzbach, Germany) were mixed (1:2) in a 250-mL glass reactor. The solution was agitated with a propeller stirrer (92 rpm) at room temperature (22–24 °C). The volumes of 3 M HCl required to maintain the pH stable at pH 4.5 were determined by titration using a Titrando 906 titrator (Metrohm, Herisau, Switzerland). Acid consumption was recorded every 5 s using Titrando control software Tiamo. Experiments with and without added pepsin (porcine stomach pepsin, 0.7 FIP U/g; Merck Life Science AS, Oslo, Norway) were performed to study how pepsin affected protein hydrolysis in the gastric method. HCl titration volumes were monitored for 0 to 6 h, and samples were taken after 0.5, 1.5, and 3 h of reaction. Samples were stored at −80 °C prior to analyzing dry matter, crude protein, and peptide size distribution in the water-soluble protein fraction.

2.3. Analyses

2.3.1. Chemical analyses

Chemical analyses were carried out by accredited laboratories. Crude protein in the feeds and samples of GI content were analyzed using the

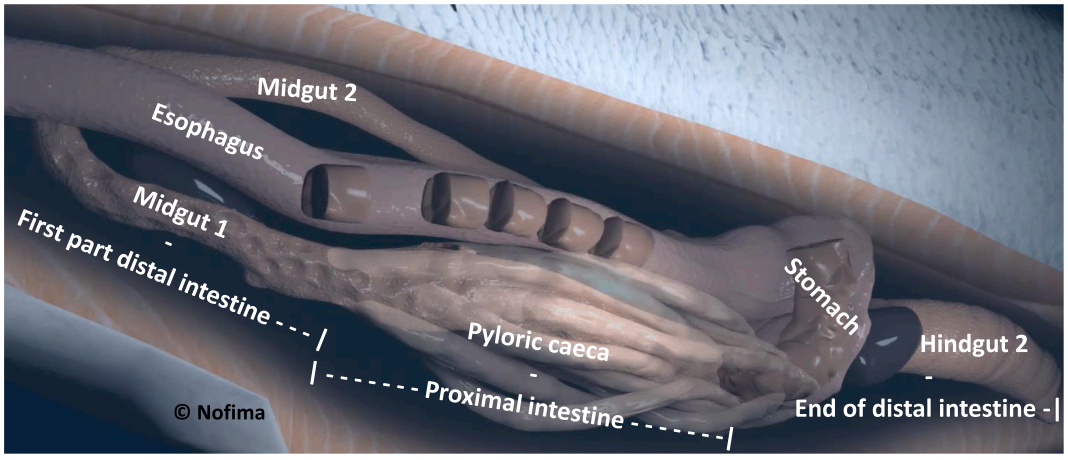

Fig. 1. Gastrointestinal tract of Atlantic salmon showing the esophagus, stomach, proximal intestine with pyloric caeca, and distal gut with midgut 1 and 2 and hindgut 1 and 2 (first part of the hindgut hidden in the present three-dimensional rendered model).

Kjeldahl method (N× 6.25; ISO 5983-1997). Moisture (ISO 6496-1999) and ash (ISO 5984-2002) were determined gravimetrically after drying preweighed samples in porcelain cups for 4.5 h at 103 ± 1 °C followed by incineration of the dried samples at 550 °C ± 20 °C for 16 h. Total lipid content in the diets and gut samples was quantified using the method previously described by Bligh and Dyer (1959). Yttrium was determined by inductively coupled plasma atomic emission spectroscopy (ISO 11885-1996). The water-soluble fraction was extracted with boiling water, the extract was then filtered using paper filter, and the crude protein content in the water phase was determined using the Kjeldahl method. Molecular weight distribution analysis was performed by size exclusion chromatography (1260 series HPLC Agilent Technologies) with a Superdex Peptide 10/300GL column (GE Healthcare, Uppsala, Sweden), acetonitrile with trifluoroacetic acid as eluent and ultraviolet detection at 190–600 nm (Aksnes and Asbjørnsen, 2003). The samples were solubilized in water containing 3 g/kg sodium dodecyl sulphate, centrifuged for 10 min at 1710.5 ×*g*, decanted, and filtered before being applied to the column. The following components were used to define the standard curve to relate molecular weight (MW) of peptides to the elution time: carbonic anhydrase (MW of 29,000 Da), lysozyme (MW of 14,300 Da), Cyt C (MW of 12,400 Da), aprotinin (MW of 6500 Da), alberta 4 (MW of 3249.38 Da), insulin A (MW of 2531.64 Da), alberta 3 (MW of 2441.54 Da), gastrin I (MW of 2126.28), alberta 2 (MW of 1633.7 Da), polymyxin (MW of 1470 Da), substrate P (MW of 1347.63 Da), [Val 4]-Ang III (MW of 917.06 Da), alberta 1 (MW of 825.86 Da), (Leu)3 (MW of 357.49 Da), and Gly (MW of 75.07 Da). Total starch and degree of starch gelatinization were measured using the glucoamylase method as described previously by Samuelsen and Oterhals (2016).

2.3.2. Disintegration properties in water

The disintegration properties in water were determined using the method previously described by Baeverfjord et al. (2006) with slight modifications. Triplicate samples of each diet (20 g each) were placed in custom-made steel-mesh buckets placed inside 1000-mL glass beakers filled with 500 mL of distilled water. The beakers were incubated in a thermostat-controlled water bath (23 °C) and shaken (145 rpm/min) for 0.5, 1.5, 3, 6, or 24 h. The remaining pellets in the steel-mesh buckets were dried at 104 °C overnight (>12 h) and losses of crude protein, total lipid, and dry matter were determined. Pellet stability was calculated as the percentage remaining pellets of the incubated pellets on a dry matter basis.

2.3.3. Computed tomography (CT) scanning

Selected samples of freeze-dried stomach content 30 min after a meal in Experiment 1 were micro-CT scanned. Scanning was performed using a SkyScan 1275 X-ray microtomograph (Bruker MicroCT, Kontich, Belgium). The scan parameters were adjusted for each sample to optimize the pictures. Typical scans were performed with no filter, a source voltage of 26–40 keV, and maximum source current. The scans were high resolution with a pixel size of 10–12 μm.

2.3.4. CCK analysis

Intestinal tissue samples were thawed on ice and rinsed by stirring the samples while being held with forceps in ice-cold 1× phosphate-buffered saline solution (PBS; Cat. No. P5493, pH 7.2–7.6, Sigma-Aldrich, Saint Louis, USA) to remove any remaining digesta. Epithelial tissue was scraped off the intestinal wall (muscle layers) and placed in preweighed 1.5-mL Eppendorf tubes, weighed, and then diluted using an appropriate volume of 1× PBS [1:10 (w/v)]. Samples were then homogenized using a motorized pellet pestle and centrifuged at 4 °C at 5000 ×*g* for 15 min. Supernatants were stored at −20 °C until processed. CCK concentration was quantified using Competitive inhibition Fish CCK enzyme-linked immunosorbent assay kits (cat. no. CSB-E13054Fh; Cusabio Biotech, Hubei, China) according to the manufacturer's instructions. Samples were measured in duplicate and concentrations were calculated using a standard curve (200–5000 pg mL^{-1}) and expressed as ng/g tissue.

2.3.5. Measurement of pepsin activity

The stomach contents from continuously fed salmon (Experiment 2) or the *in vitro* gastric study (Experiment 3) were mixed with cold (4 °C) purified water [1:10 (w/v)] and vortexed for 1 min. The solution was then centrifuged at 30,000 ×*g* for 30 min at 4 °C (Sorvall, LYNX 6000, Thermo Scientific, Waltham, MA). The supernatant was collected (enzyme extract) and stored (−80 °C) prior to pepsin activity analysis. Pepsin activity was measured by adding 1 mL of enzyme extract to 5 mL of 2.0% (w/v) bovine hemoglobin solution (Sigma Aldrich, Saint Louis, USA), pH 3.0 and 5.5 at 37 °C. After a 10-min incubation, 10 mL of 5% (w/v) trichloroacetic acid (6.1 M, Sigma Aldrich, Saint Louis, USA) was added to terminate the reaction. The mixtures were filtered through a 0.45-mm syringe filter before the absorbance was measured at 280 nm (Evolution 220, Thermo scientific, Waltham, USA). Pepsin activity was measured in the extracts using the following equation:

$$\text{units} \Big/ \text{g DM} = \frac{(A_{280}\text{Test} - A_{280}\text{Blank}) \times \text{df}}{10\ min \times \frac{\text{g}}{\text{mL}} \times 1.0\ \text{mL} \times 0.001}$$

Where df is the dilution factor, g/mL is g dry matter (DM) per mL of diluted stomach extract, and 0.001 is the unit definition for pepsin units at ΔA280.

2.4. Data analysis, calculations, and statistics

All data were calculated in Microsoft Excel. The apparent digestibility of nutrients was calculated as follows (NRC, 2011): $\left(1 - \frac{\text{MD}\times\text{NF}}{\text{MF}\times\text{ND.}}\right) \times 100$. Where M_D and M_F represent the concentration of digestibility indicator in diets and feces, respectively, and N_D and N_F represent nutrient concentration in diets and feces, respectively.

All data are presented as mean ± standard error (SE). All statistical analyses were performed using Statistica for Microsoft windows 10 (TIBCO software Inc., Palo Alto, USA). The effects were analyzed by one-way analysis of variance (ANOVA) and two-way ANOVA. Analyses by ANOVA were followed by Tukey's *post hoc* test to rank significant differences between the treatments. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Experiment 1: GI passage rate study

A total of 84 fish were sampled. Fish with total GI content < 0.05% of their body weight (n = 18) were excluded from the data set (similar size as fish with GI content, 1–3 fish/sampling). No or few fish had content in all three GI segments at 0.5, 1.5, 12, and 24 h after the meal. At the first sampling 30 min after the meal, only two fish fed Diet A had digesta in the proximal gut, while all fish fed Diet B had content in this region (Fig. 2). In addition, one fish fed Diet A had content in the distal gut 30 min after the meal, which was most likely undigested food from the meal before the fasting period. After 1.5 h, two fish fed Diet A and one fish fed Diet B had content in the distal gut. Similarly, 12 h after the meal, only one of the sampled fish had content in the stomach. On the other hand, 24 h after the meal, none of the sampled fish had content in the stomach and only three of the SAMPLED fish fed Diet B were included as the others had total GI content <0.05% of their body weight (Fig. 2). Thus, the total sampled GI content decreased with time after the meal. The dry GI content relative to the body weight decreased from 0.4%–0.5% at the first sampling to <0.1% at the final sampling. There appeared to be a dietary effect on total GI content at 1.5–9 h after the meal, although this was not significant. However, major differences were observed in the

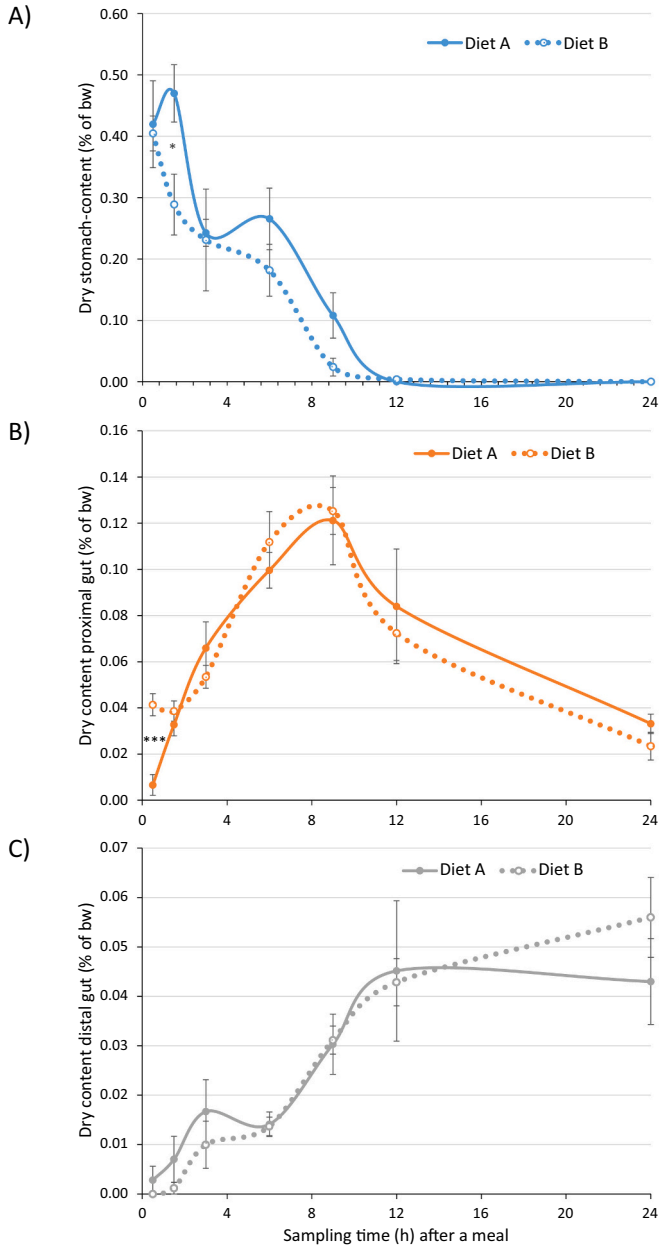

Fig. 2. Relative dry content (mean ± SE) in (A) stomach, (B) proximal gut, and (C) distal gut to body weight (bw) in fish sampled 0.5, 1.5, 3, 6, 9, 12, and 24 h after a meal of either Diet A or Diet B. Analysis only included fish with total GI content > 0.05% of body weight (mean ± SE). *$P < 0.05$; ***$P < 0.001$ (Tukey's *post hoc* test).

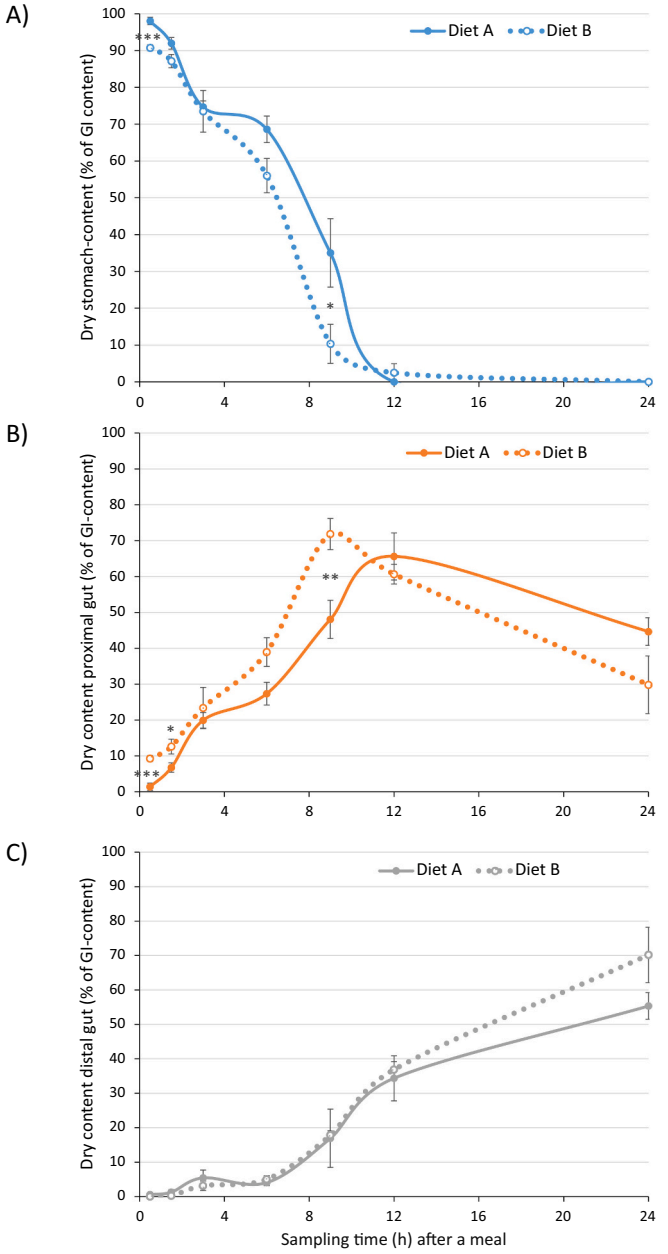

Fig. 3. Relative dry content (mean ± SE) in (A) stomach, (B) proximal gut, and (C) distal gut to total GI content in fish sampled 0.5, 1.5, 3, 6, 9, 12, and 24 h after a meal of either Diet A or Diet B. Analysis only included fish with total GI content > 0.05% of body weight (mean ± SE). *$P < 0.05$; ***$P < 0.01$ (Tukey's *post hoc* test).

stomach content. The relative stomach to total GI content was higher in salmon fed Diet A compared with those fed Diet B, and showed significant or nearly significant differences at 30 min ($P = 0.001$), 1.5 h ($P = 0.082$), 6 h ($P = 0.088$), and 9 h ($P = 0.050$) after the meal (Fig. 3A). This was also reflected by a numerically higher content of digesta in the proximal intestine in salmon fed Diet B compared with those fed Diet A at 0.5–9 h after the meal, whereas no dietary differences were observed in the amount of indigested content in the distal intestine (Fig. 3B and C). Furthermore, the dry matter content in stomach decreased with time after the meal, from an average of 50% at 30 min to an average of 30% at 9 h after the meal. There was also a tendency towards lower stomach dry matter content in fish fed Diet B compared with those fed Diet A. In contrast, the dry matter content in the proximal and distal gut was unaffected by diet or time after the meal (Fig. 4).

The content of water-soluble proteins in the feeds increased from 5.9% and 6.2% to 7.9% and 8.7% in the stomach content sampled 30 min after feeding a meal of Diet A or B, respectively. The water-soluble protein content in the stomach declined at 1.5 and 3 h after a meal. The peptide size distribution of the water-soluble proteins also changed as a function of time. Levels of peptides <500 and >6000 Da decreased in the stomach content after the meal, while peptides sized between 2000 and 6000 Da increased in fish fed Diet B after 30 min and then appeared to be the largest group of peptides in the stomachs of fish fed both diets (Fig. 5).

Different stomach feed disintegration was analyzed using CT scanning 30 min after a meal (Fig. 6). Pellets from Diet B were disintegrated into a more homogenous mass compared with the clearer pellet structure of Diet A.

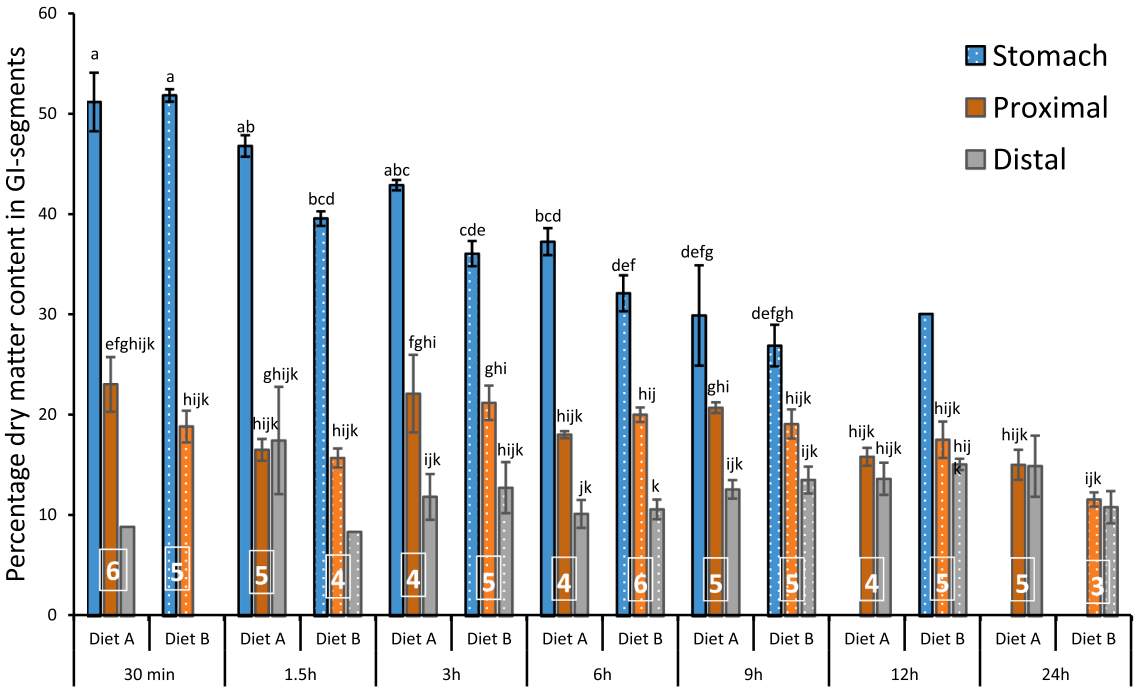

Fig. 4. Dry matter content (% of wet content; mean ± SE) in different GI segments. Stomach, proximal gut, and distal gut content from fish sampled 0.5, 1.5, 3, 6, 9, 12, and 24 h after a meal of either Diet A or Diet B. Numbers in squares represent the number of fish sampled with total GI content >0.05% of body weight (mean ± SE). Three-way ANOVA, mean effect of time after meal ($P = 0.02$), feed ($P = 0.50$), and GI segments ($P < 0.01$). Superscripts with different letters indicate significant differences ($P < 0.05$) between GI segments, feeding groups and time after a meal as determined by Tukey's *post hoc* test.

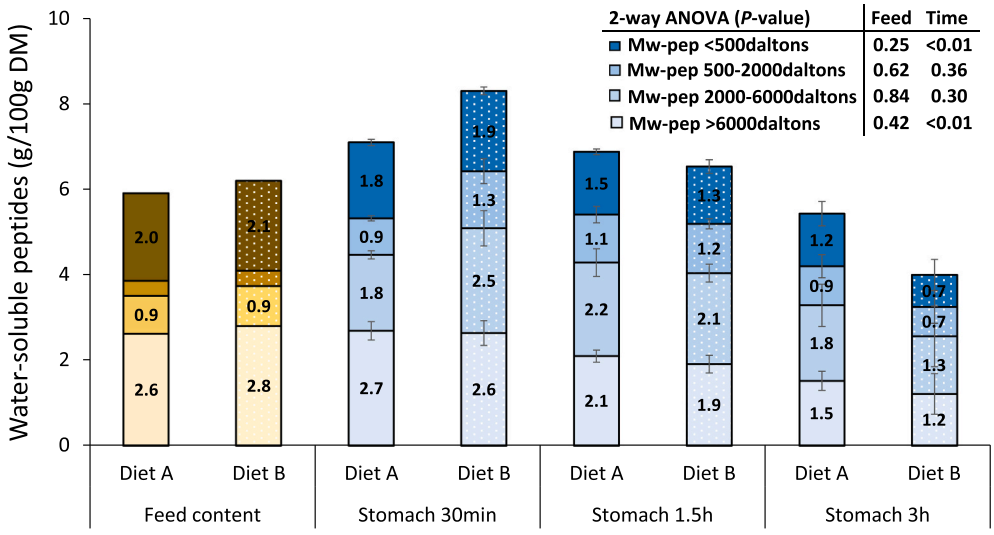

Fig. 5. Total content of water-soluble proteins and peptides within feeds and stomach content from salmon sampled 0.5, 1.5, and 3 h after a meal of either Diet A or Diet B (mean ± SE; n = 3). Two-way ANOVA mean effect of feed and time after the meal were examined for the different peptide fractions.

The CCK content in proximal gut tissue was higher in salmon fed Diet B compared with those fed Diet A at 1.5 and 9 h after the meal; however, the differences were not statistically significant. The values for Diet A tended to be lower compared with those for fasted fish sampled just prior to the meal (Fig. 7).

3.2. Experiment 2: Fish growth study

The average body weight increased from 572 to 1542 and 1604 g in salmon fed Diets A and B, respectively, with a specific daily growth rate of 1% during the 100-day experiment. There were no significant differences between the dietary treatments on final body weight, length, condition factor, or growth rate. The average feed intake per fish during the experimental period was significantly different between the dietary treatments, and was 800 g for fish fed Diet A and 905 g for fish fed Diet B. However, the feed conversion ratio was not significantly different between the dietary groups (Table 2).

Total GI content in the sampled fish comprised 1.8% and 0.7% of their body mass on a wet and dry weight basis, respectively. Seven fish fed Diet A and six fish fed Diet B had empty stomachs, but had the same level of GI filling as the other fish, and no significant differences were observed between the dietary treatments. On a wet weight basis, continuously fed fish had the highest filling in the proximal gut section (0.7% of their body weight), followed by the hindgut sections, and equal

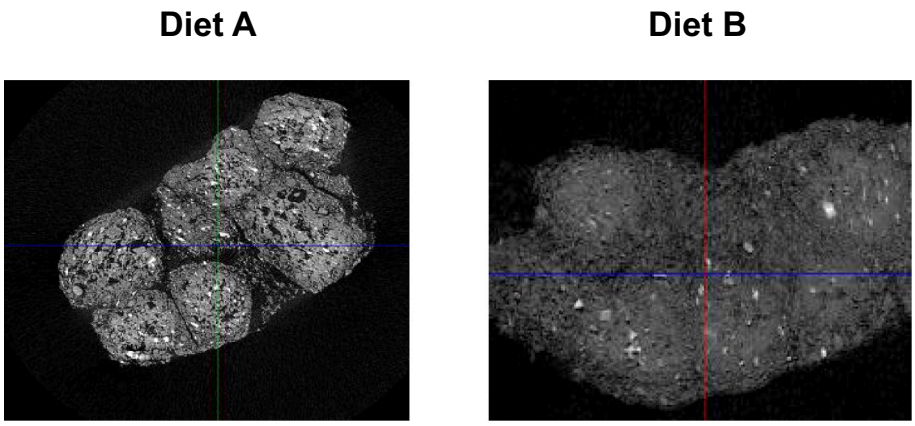

Fig. 6. CT scan of stomach content from salmon sampled 30 min after a meal of either Diet A or Diet B.

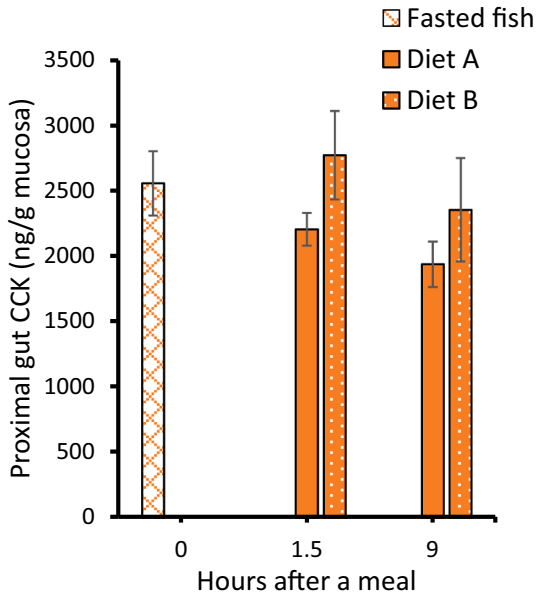

Fig. 7. CCK content in the proximal gut mucosal tissue in fasted salmon at 0 (immediately prior to the meal), 1.5, and 9 h after a meal of either Diet A or Diet B (mean ± SE, n = 4). No significant differences were observed by two-way ANOVA mean effect of feed ($P = 0.11$) and time ($P = 0.25$).

amounts in the midgut and stomach, while the weights of the dried samples were more equally distributed in the GI tract due to differences in dry matter content (Fig. 8). The dry matter content in the pooled samples (n = 3 per diet) of the GI sections appeared to be lower in fish fed Diet B compared with those fed Diet A, although this was not significant. This was most pronounced in the stomach (41.7% and 43.4%) and proximal gut (15.2% and 16.9%), but this was not seen in the distal gut sections with dry matter content in the last section (14.1% and 14.7%) for fish fed Diet B and Diet A, respectively.

The pH in the GI segments did not appear to be affected by the dietary treatments, although the stomach content from salmon fed Diet A (pH 4.7) had a lower pH compared with salmon fed Diet B (pH 5.0) in the pooled samples per tank (Table 3), although this was not significant. Stomach contents from individual fish sampled in Experiment 1 also showed no dietary effect on measured pH, although pH changed with time after the meal (pH 5.8 after 30 min, pH 5.5 after 1.5 h, and pH 5.2 after 3 h). Pepsin activity in the stomach content of continuously fed salmon in Experiment 2 revealed low pepsin activity at pH 5.5 and increased activity with lower substrate levels at pH 3.0, although no dietary differences were observed (Fig. 9).

Table 2
Fish performance data after 100 days of feeding postsmolt Atlantic salmon with Diets A or B in triplicate tanks (mean ± SE, n = 3).

Measurements	Diet A	Diet B	*P*-value
Start weight (g)	574.00 ± 1.68	570.89 ± 3.23	0.441
Final weight (g)	1542.33 ± 23.62	1603.88 ± 34.93	0.218
Final length (cm)	47.47 ± 0.13	47.77 ± 0.38	0.499
Condition factor	1.44 ± 0.02	1.46 ± 0.03	0.468
Specific growth rate (% per day)	0.99 ± 0.02	1.03 ± 0.02	0.203
Total feed intake/fish	799.95 ± 19.61	904.68 ± 18.15	0.017[a]
Feed conversion ratio	0.83 ± 0.02	0.88 ± 0.02	0.111

[a] One-way ANOVA for dietary differences at $P < 0.05$.

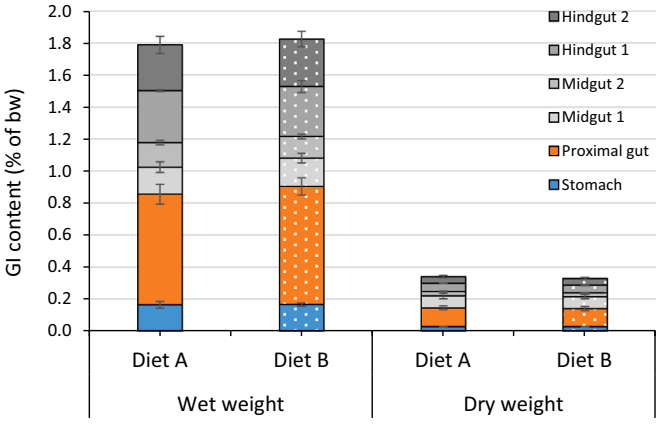

Fig. 8. Relative wet and dry weight contents in the stomach, proximal gut, and distal gut sections (midgut and hindgut) to body weight (bw) in sampled Atlantic salmon fed either Diet A or Diet B in triplicate tanks for 100 days (mean ± SE; n = 3).

Table 3
Measured pH in pooled samples of Atlantic salmon (n = 10) fed either Diet A or Diet B in triplicate tanks for 100-days (mean ± SE).

	Diet A (pH)	Diet B (pH)	*P*-value[a]
Stomach			0.301
	4.7 ± 0.2	5.0 ± 0.2	
Proximal gut			0.833
	7.4 ± <0.1	7.4 ± 0.1	
Distal gut			0.684
	7.8 ± 0.1	7.8 ± 0.1	

[a] One-way ANOVA for dietary differences at $P < 0.05$.

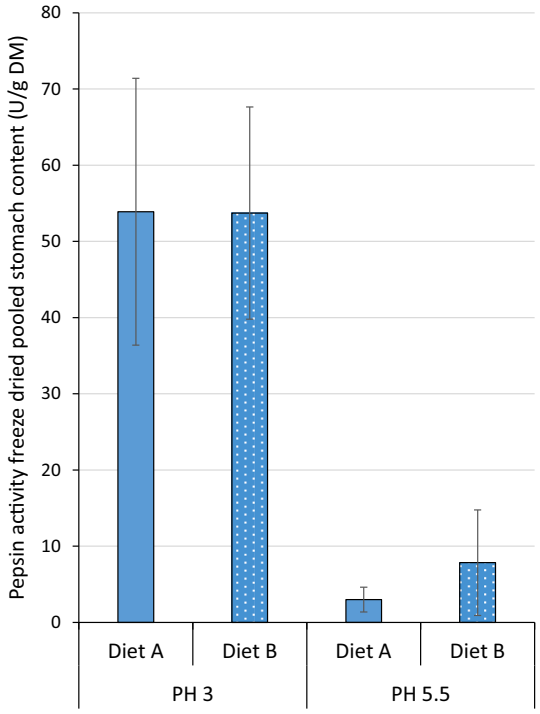

Fig. 9. Pepsin activity in freeze-dried stomach content from Atlantic salmon fed either Diet A or Diet B in triplicate tanks for 100 days, in substrates at pH 3 and 5.5 (mean ± SE; n = 3). One-way ANOVA mean effect of feed ($P = 0.85$) and pH ($P < 0.01$).

The apparent digestibility of the dry matter and crude protein was similar between the diets throughout the intestinal sections and there was similar total digestibility of lipids in the last section. Crude protein digestibility was 50% in the proximal gut and increased to 80% throughout the midgut section, with a total protein digestibility of 90%. The overall digestibility of dry matter in the feeds occurred in the region between the proximal and distal gut section, with an increase in digestibility from 30% to a total dry matter digestibility of 72%. Although the apparent digestibility data indicate similar stomach evacuation of dry matter between the dietary groups, analysis indicated a faster transport of crude protein to the proximal gut in fish fed Diet B compared with those fed Diet A (Table 4).

Gallbladder bile volume in continuously fed fish fed Diets A and B were measured in six fish per diet, with an average bile volume of 0.8 and 0.6 mL per kg body weight in salmon fed Diets A and B, respectively. In addition, 14 fish per dietary treatment were sampled 48 h after their last meal (fasted), among which two fish fed Diet B were not measured for bile volume. The fasted fish had a higher average bile volume of 1.7 and 2.1 mL per kg in those fed Diets A and B, respectively, although this was not significantly different (Fig. 10).

Table 4
Apparent digestibility of content from different gastrointestinal sections in pooled samples per tank from 10 Atlantic salmon fed either Diet A or Diet B in triplicate tanks for 100 days (mean ± SE, n = 3).

Apparent digestibility	Diet A	Diet B	*P*-value[a]
Stomach			
Dry matter			0.915
	4.1 ± 0.8	4.0 ± 1.3	
Crude protein			0.119
	9.5 ± 1.1	13.0 ± 1.4	
Proximal			
Dry matter			0.931
	30.2 ± 0.7	29.9 ± 4.0	
Crude protein			0.869
	53.8 ± 1.7	54.4 ± 2.8	
Midgut 1			
Dry matter		57.9 ± 0.6	0.344
	59.6 ± 1.6		
Crude protein			0.407
	78.0 ± 2.1	76.0 ± 0.5	
Midgut 2			
Dry matter			0.151
	66.2 ± 0.6	64.9 ± 0.4	
Crude protein			0.168
	84.8 ± 0.8	82.9 ± 0.9	
Hindgut 1			
Dry matter			0.683
	70.5 ± 1.6	71.3 ± 0.9	
Crude protein			0.618
	88.6 ± 1.0	87.8 ± 1.2	
Hindgut 2			
Dry matter			0.710
	71.9 ± 0.9	72.4 ± 0.8	
Crude protein			0.668
	90.4 ± 0.6	89.8 ± 0.6	
Total lipid	95.9 ± 0.3		0.537
		96.0 ± 0.1	

[a] One-way ANOVA for dietary differences at $P < 0.05$.

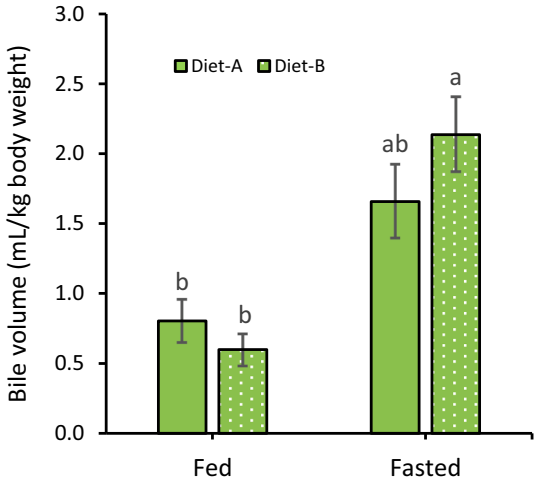

Fig. 10. Gallbladder bile volume (mL/kg fish) in individual sampled Atlantic salmon during continuous feeding of either Diet A or Diet B or fasted for 48 h (mean ± SE). Two-way ANOVA mean effect of feed ($P = 0.64$) and sampling time ($P < 0.01$) followed by Tukey's *post hoc* test. Superscripts with different letters indicate significant differences ($P < 0.05$).

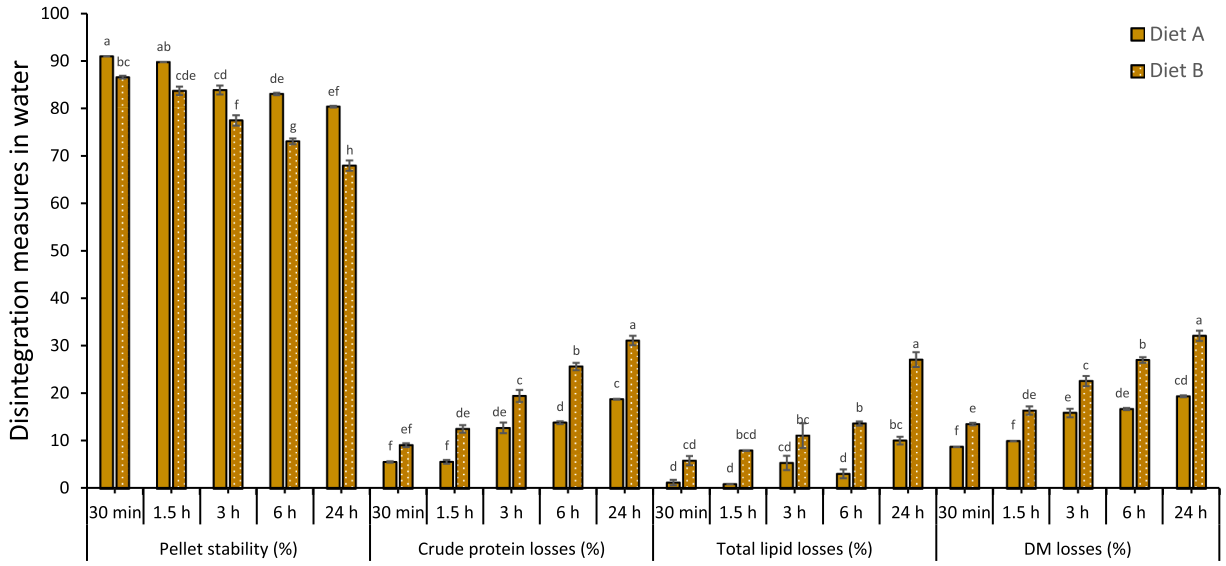

Fig. 11. Disintegration measures of pellet stability, and losses of crude protein, total lipid, and dry matter (DM) after 0.5, 1.5, 3, 6, and 24 h (mean ± SE; n = 3). Two-way ANOVA mean effect of time and feed showed values of $P < 0.001$ for all measures. Superscripts with different letters indicate significant differences ($P < 0.05$) using Tukey's *post hoc* test for each of the pellet measures.

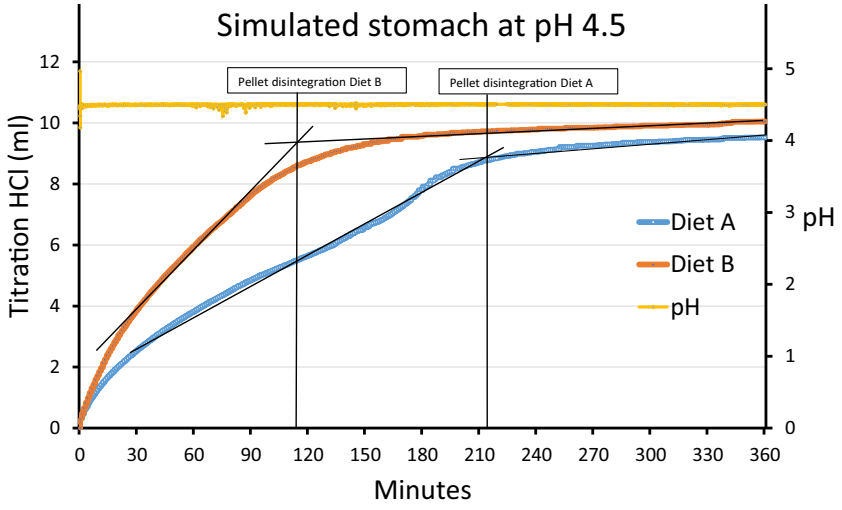

Fig. 12. *In vitro* simulated gastric method with automatic titration of HCl to maintain a constant pH of 4.5 in a seawater–pepsin solution with pellets from Diets A or B.

3.3. Pellet quality properties and simulated gastric method

Both feeds were properly cooked with a high degree of starch gelatinization (100% and 97.4% of total starch content for Diets A and B, respectively). The disintegration properties in the water bath showed overall good water stability of both feeds (Fig. 11). However, a significant higher loss in all measured disintegration properties was observed for Diet B compared with Diet A. Crude protein loss appeared to be faster than lipid loss within the first 6 h in water (Fig. 11.)

The stomach titration method with a controlled pH of 4.5 showed that stomach fluid solution with Diet B required a faster titration of HCl to maintain a stable pH compared with stomach fluid solution with Diet A. At the time of complete pellet disintegration of Diet B (approximately 2 h), the required titration volume of HCl to solution with Diet B was twofold greater than that required for Diet A (Fig. 12).

However, the solvent used in the method affected the titration volume of HCl; therefore, seawater was used instead of distilled water to avoid rapid fluctuations in pH. Titration volume of HCl required to maintain a stable pH of 4.5 was significantly higher for Diet B than that required for Diet A, both in solutions of seawater (33‰) and seawater with pepsin, and at all timepoints (Table 5). The water-soluble protein fraction increased during the measured period. There was a difference between the two diets, with a higher amount of intermediate-sized peptides (4000–6000 Da) in the hydrolyzed pellets from Diet B compared with those from Diet A. However, the main difference was the presence or absence of pepsin in the solution. A higher amount of water-soluble protein and small to intermediate-sized peptides (200–8000 Da) were observed after 3 h of disintegration in the solution with pepsin, whereas the distribution of peptides in solution with only seawater was similar to the feeds prior to HCl titration (Table 5).

In the *in vitro* gastric method, equal volumes of pepsin were added to the solution prior to starting the experiments for both feeds. The solution with greater protein hydrolysis (Diet B) appeared to use more pepsin over time and therefore showed lower pepsin activity after 1.5 h compared with Diet A. However, the pepsin activity after 3 h was similar for both feeds (Fig. 13).

Table 5
Total volume of HCl added to maintain a constant pH of 4.5 in a seawater/seawater + pepsin solution with pellets from Diets A or B after 0.5–3 h and content of water-soluble peptides after 3 h.

	Feed samples before		HCl titration in seawater solution		HCl titration in seawater + pepsin solution		*P*-value	
	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B	Method	Feed
HCl titration (mL)								
30 min			2.3 ± 0.2^{a}	$3.6 \pm <0.1^{b}$	2.3 ± 0.1^{a}	3.5 ± 0.2^{b}	0.84	<0.01
1 h			3.4 ± 0.2^{a}	5.3 ± 0.1^{b}	3.6 ± 0.1^{a}	5.5 ± 0.4^{b}	0.51	<0.01
1.5 h			4.4 ± 0.2^{a}	6.9 ± 0.3^{b}	4.7 ± 0.1^{a}	6.9 ± 0.3^{b}	0.51	<0.01
2 h			5.2 ± 0.2^{a}	8.3 ± 0.2^{b}	5.6 ± 0.1^{a}	7.8 ± 0.1^{b}	0.76	<0.01
3 h			6.6 ± 0.2^{a}	9.5 ± 0.3^{b}	7.5 ± 0.2^{a}	8.5 ± 0.3^{b}	0.92	<0.01
Water-soluble protein (g/100 g) and peptide distribution (%) after 3-h stomach hydrolysis								
WSP (g/100 g DM)	5.9	6.2	7.2 ± 0.1^{a}	7.4 ± 0.3^{a}	$14.8 \pm <0.1^{b}$	15.9 ± 0.6^{b}	<0.01	0.16
Pep >20,000 Da	6.4	6.6	2.1 ± 0.1^{a}	2.2 ± 0.1^{a}	0.9 ± 0.3^{b}	0.6 ± 0.1^{b}	<0.01	0.64
Pep 20,000–150,00 Da	6.8	6.9	5.0 ± 0.1^{a}	$5.3 \pm <0.1^{a}$	1.9 ± 0.3^{b}	$1.6 \pm <0.1^{b}$	<0.01	0.94
Pep 15,000–10,000 Da	15.0	15.3	$20.0 \pm <0.1^{a}$	$21.3 \pm <0.1^{a}$	9.0 ± 0.2^{b}	8.8 ± 0.9^{b}	<0.01	0.36
Pep 10,000–8000 Da	7.6	7.7	11.2 ± 0.1^{a}	$11.7 \pm <0.1^{a}$	7.9 ± 0.5^{b}	8.6 ± 0.6^{b}	<0.01	0.31
Pep 8000–6000 Da	8.6	8.7	$8.2 \pm <0.1^{a}$	$8.3 \pm <0.1^{a}$	10.0 ± 0.4^{ab}	11.2 ± 0.4^{b}	<0.01	0.12
Pep 6000–4000 Da	8.2	8.3	$9.0 \pm <0.1^{a}$	$9.3 \pm <0.1^{a}$	12.3 ± 0.4^{b}	13.8 ± 0.2^{c}	<0.01	0.02
Pep 4000–2000 Da	6.8	6.7	$8.0 \pm <0.1^{a}$	$7.5 \pm <0.1^{a}$	16.6 ± 0.3^{b}	16.7 ± 0.3^{b}	<0.01	0.53
Pep 2000–1000 Da	3.5	3.4	$4.3 \pm <0.1^{a}$	$4.0 \pm <0.1^{a}$	13.2 ± 0.1^{b}	12.8 ± 0.6^{b}	<0.01	0.38
Pep 1000–500 Da	2.4	2.4	$3.0 \pm <0.1^{a}$	$2.9 \pm <0.1^{a}$	8.8 ± 0.4^{b}	8.1 ± 0.7^{b}	<0.01	0.44
Pep 500–200 Da	3.8	3.7	$3.7 \pm <0.1^{a}$	3.9 ± 0.1^{a}	6.2 ± 0.4^{b}	5.7 ± 0.6^{b}	<0.01	0.77
Pep <200 Da	30.9	30.2	25.7 ± 0.1^{c}	$23.8 \pm <0.1^{b}$	13.3 ± 0.5^{a}	12.1 ± 0.2^{a}	<0.01	<0.01

Data represent mean ± SE (n = 3). Two-way ANOVA mean effect of solution in the *in vitro* gastric method and feed. Superscripts with different letters for each GI segment indicate significant differences ($P < 0.05$)
DM, dry mass; Pep, peptide; WSP, water-soluble protein.

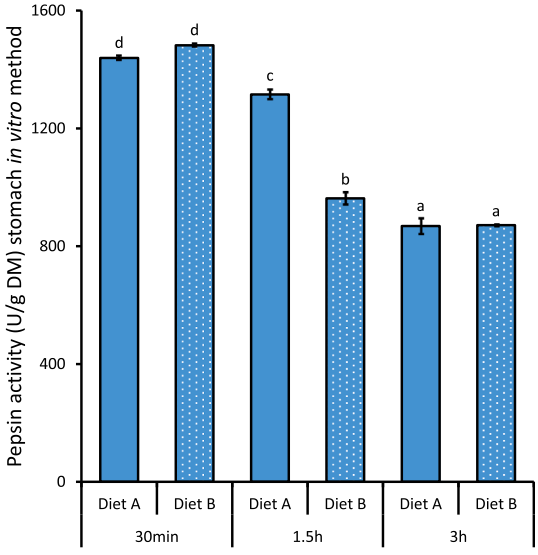

Fig. 13. Pepsin activity in the seawater–pepsin solution during disintegration of pellets from Diets A or B (0.5, 1.5, and 3 h) in the *in vitro* simulated stomach experiment with automatic titration of HCl to maintain a constant pH of 4.5 (mean ± SE, n = 2). Superscripts with different letters indicate significant differences ($P < 0.05$) using Tukey's *post hoc* test.

4. Discussion

In the present study, different disintegration stabilities of feeds were produced by impacting viscous heat dissipation in the extruder. The different disintegration stabilities affected stomach function, GI passage rate, and feed intake in Atlantic salmon. These differences in process responses and feed properties resulted from different feed mix oil inclusion levels prior to extrusion and support earlier observations reported in response to inclusion of oil-rich microalgae (Samuelsen et al., 2018). In the present study, both feeds were properly cooked and showed similar degrees of starch gelatinization and nutrient composition; therefore, the observed nutritional effects may be due to differences in disintegration stability.

The disintegration properties of a pellet can affect palatability through leakage of water-soluble components into the water during feeding (Kasumyan and Døving, 2003) and improve feed intake due to faster disintegration in the stomach. This enables the stomach to process more feed within a set period of time (Grove et al., 1978; Andersen and Beyer, 2005). In the present study, feeds extruded with a higher lipid content disintegrated faster in water and released more dry matter, protein, and fat into the water over 24 h. Despite large differences in oil inclusion at extrusion (8% and 20%), both feeds were properly cooked, good quality, and did not disintegrate completely during the 24-h test in water. This enabled both feeds to be used in a feeding experiment, and pellets not eaten by the salmon were collected to calculate feed intake.

Inclusion of high-quality fishmeal could explain the low rate of pellet disintegration in the present study, as water-soluble fishmeal components have been shown to be important binders and plasticizers in feed pellets (Oterhals and Samuelsen, 2015).

Feeds that enters the stomach begins to disintegrate by motility and peristalsis together with the presence of HCl and pepsin that start predigestion of proteins (Koelz, 1992; Einarsson et al., 1996). Studies have shown that Atlantic salmon have a relatively high gastric pH, between pH 4 and 6 (Krogdahl et al., 2015), that is greatly affected by the pH in the ingested feed (Bravo et al., 2018). In the present study, salmon showed a high stomach pH of 4.7–5.8 in Experiments 1 and 2, either 30 min to 3 h after a single meal or with continuous feeding. Our *in vitro* gastric method indicated faster disintegration of Diet B compared with Diet A, resulting in the requirement for a higher amount of HCl to maintain a stable pH (set at pH 4.5 in our study). A high *in vivo* pH indicates that the salmon stomach has limited capacity for HCl secretion, indicating that stomach pH is largely determined by the properties of the feeds. In rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), and gilthead sea bream (*Sparus aurata*), gastric pH increase at feeding and is lower prior to and after a meal (Bucking and Wood, 2009; Nikolopoulou et al., 2011). In this study, the pH decreased after a meal as the feed disintegrated and the digesta was transferred to the proximal gut, concomitant with a reduced dry matter content that indicated drinking water. However, the low gastric pH levels observed prior to a meal (pH 2–3) were not reached until 48 h after a meal in rainbow trout (Bucking and Wood, 2009) and 25 h after a meal in juvenile salmon (Bravo et al., 2018), and were not achieved in the present study. Krogdahl et al. (2015) indicated that the pH in the mucus layer of the stomach wall may be <3. The reduced pH in the stomach digesta after a meal results from a reduced buffer capacity of the feed as it disintegrates and dry matter is moved from stomach to proximal intestine.

The optimal enzyme activity of salmon pepsin occurs at pH 3.0 (Krogdahl et al., 2015), and this was also verified to be the pH that reflects the highest enzyme activity in the gastric content from continuously fed salmon in the present study. However, the measured pepsin activity was 10-fold lower at pH 5.5 compared with that seen at pH 3.0 and water-soluble peptides sized 4000–6000 Da increased 30 min after a meal and after 3 h of hydrolysis of feeds in the *in vitro* gastric method at pH 4.5. Thus, analysis of pepsin at low pH (pH 2 or 3) overestimates the true pepsin activity in the fish stomach (Yúfera et al., 2012), which has a sufficient activity to predigest feed and hydrolyze proteins. The peptide size distribution in salmon stomach digesta showed the highest levels of hydrolyzed proteins in the stomach 30 min after a meal, followed by a lower content of water-soluble proteins, likely due to the transfer of digesta to the proximal gut. In comparison, these digestive products were accumulated in the *in vitro* method. Previous studies have shown that overload of free amino acids in salmon feeds may lower overall protein digestibility (Espe, 1999; Hevrøy et al., 2005), while removal of these (*e.g.*, from fish protein hydrolysates) to increase the amount of intermediate-sized peptides may increase feed intake (Aksnes et al., 2006). Thus, we hypothesize that the increased production of intermediate-sized peptides in Diet B compared with Diet A both *in vivo* and *in vitro* may be due to an increased hydrolysis of proteins and faster pellet disintegration. Higher content of intermediate-sized peptides entering the anterior gut stimulating appetite regulation, together with faster GI passage rate, may explain the higher feed intake observed in the salmon fed Diet B (Experiment 2).

Appetite, hunger, and satiety are regulated *via* several cues, including stomach filling and distention, time taken for disintegration from feed pellets to digesta, transport of digesta to the proximal gut, and rate of passage through the GI tract (Grove et al., 1978; Soengas, 2014). This is all controlled by peptides that regulate appetite in the hypothalamus and mediated *via* signaling pathways between the gut and the brain (Hoskins and Volkoff, 2012; Rønnestad et al., 2017). Valen et al. (2011) showed that gastric emptying in Atlantic salmon was relatively rapid and 50% of the stomach content was transferred to the proximal intestine at 4.5 h, 85% at 9–12 h, and the stomach was empty by 24 h after the meal. Their experiment was performed on 44-g salmon in fresh water at 8 °C, while Experiment 1 in the present study was performed using 374-g postsmolt salmon in 13 °C seawater. In salmon fed Diet B, 90% of the stomach content had moved to the proximal and distal gut 9 h after the meal compared with only 65% of the stomach content in salmon fed Diet A. CCK, which is produced by enteroendocrine cells, is an important regulator of digestion. It delays gastric emptying, stimulates the release of bile, and affects feed intake *via* a separate mechanism when released CCK stimulates vagal afferent fibers that communicate with the appetite-controlling centers in the hypothalamus (Rehfeld, 2017). In the present study, there was a reduced CCK content in the area between the proximal and distal gut of Atlantic salmon fed diet A, with slower disintegration compared with salmon fed Diet B. This was valid at both 1.5 and 9 h after a meal, as well as compared with prior to the meal. At these time points the relative content of dry matter in the stomach to GI content and the absolute amount in the GI were lower in salmon fed Diet B, indicating faster emptying of the stomach and higher absorption of nutrients from the gut compared with salmon fed Diet A.

Furthermore, higher yttrium(III) oxide and lower crude protein content in the stomachs of salmon continuously fed on Diet B indicated a faster transit of protein into the gut as the pellets disintegrated in the stomach. Protein is mainly digested further along the GI tract at the proximal intestine (>50%) and through the midgut section (>80%). The remaining dry matter is, to a greater extent, digested through the midgut, increasing the digestibility from 30% in the proximal gut to 70% in the hindgut. There was a tendency towards different digestibility of the feeds, although this was not significantly different ($P > 0.05$). A lower digestibility and more rapid GI passage rate of the faster disintegrating pellets may explain our observation of no significant differences in final weight and growth in the present study. The significantly higher feed intake in salmon fed Diet B compared with those fed Diet A resulted in a range of values that tended to be higher for salmon fed the faster disintegrating pellets, including final weight, specific growth rate, and condition factor, but also a tendency towards a higher feed conversion ratio.

5. Conclusions

The present study demonstrates that increased feed intake does not necessarily result in a significantly higher growth rate. The lipid levels in the feed mix prior to extrusion are important for the pellet disintegration pattern in the salmon stomach and affects feed intake. This occurs through faster emptying of disintegrated chyme from the stomach and faster reduction in absolute GI content due to nutrient absorption in the gut. Our *in vitro* gastric method revealed that faster disintegration required a greater amount of secreted HCl to maintain a stable pH. Thus, feed properties, including pH and disintegration behavior in water, determine the gastric response and handling of food. However, there is a fine balance between an increased disintegration rate of the feeds that increases the feed intake and whether this has positive effect on growth or leads to inefficient utilization of the feeds due to a faster gut transit and lower digestibility.

Credit author statement

All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737006.

References

Aas, T.S., Terjesen, B.F., Sigholt, T., Hillestad, M., Holm, J., Refstie, S., Baeverfjord, G., Rørvik, K.-A., Sørensen, M., Oehme, M., Åsgård, T., 2011. Nutritional responses in rainbow trout (*Oncorhynchus mykiss*) fed diets with different physical qualities at stable or variable environmental conditions. Aquac. Nutr. 17, 657–670.

Aas, T.S., Sixten, H.J., Hillestad, M., Sveier, H., Ytrestøyl, T., Hatlen, B., Åsgård, T., 2017. Measurement of gastrointestinal passage rate in Atlantic salmon (*Salmo salar*) fed dry or soaked feed. Aquacult. Rep. 8, 49–57.

Aas, T.S., Ytrestøyl, T., Åsgård, T., 2019. Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: an update for 2016. Aquacult. Rep. 15, 100216.

Aas, T.S., Ytrestøyl, T., Sixten, H.J., Hillestad, M., Sveier, H., Åsgård, T., 2021. Physical feed properties affect gastrointestinal passage rate in Atlantic salmon, *Salmo salar*. Aquac. Nutr. 27, 386–394.

Adamidou, S., Nengas, I., Alexis, M., Foundoulaki, E., Nikolopoulou, D., Campbell, P., Karacostas, I., Rigos, G., Bell, G.J., Jauncey, K., 2009. Apparent nutrient digestibility and gastrointestinal evacuation time in European seabass (*Dicentrarchus labrax*) fed diets containing different levels of legumes. Aquaculture 289, 106–112.

Ahmad, R., Oterhals, Å., Xue, Y., Skodvin, T., Samuelsen, T.A., 2019. Impact of fish protein concentrate on apparent viscosity and physical properties of soy protein concentrate subjected to thermomechanical treatment. J. Food Eng. 259, 34–43.

Aksnes, A., Asbjørnsen, B., 2003. Method for the definition of peptide size in fish hydrolysate, ensilage and gelatine. Fiskeriforskning Rep. K-289 (in Norwegian).

Aksnes, A., Hope, B., Jonsson, E., Bjornsson, B.T., Albrektsen, S., 2006. Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. I: growth, growth regulation and feed utilization. Aquaculture 261 (1), 305–317.

Andersen, N.G., Beyer, J.E., 2005. Mechanistic modelling of gastric evacuation in predatory gadoids applying the square root model to describe surface-dependent evacuation. J. Fish Biol. 67, 1392–1412.

Baeverfjord, G., Refstie, S., Krogedal, P., Åsgård, T., 2006. Low feed pellet water stability and fluctuating water salinity cause separation and accumulation of dietary oil in the stomach of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 261, 1335–1345.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bravo, J.P., Hernández, A.J., Morales, G., Dantagnan, P., Márquez, L., 2018. Digestive coordination of the gastric function in Atlantic salmon *Salmo salar* juveniles. Lat. Am. J. Aquat. Res. 46, 1083–1090.

Bucking, C., Wood, C.M., 2009. The effect of postprandial changes in pH along the gastrointestinal tract on the distribution of ions between the solid and fluid phases of chyme in rainbow trout. Aquac. Nutr. 15, 282–296.

Dias, J., Gomes, E.F., Kaushik, S.J., 1997. Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. Aquat. Living Resour. 10, 385–389.

Einarsson, S., Davies, P.S., Talbot, C., 1996. The effect of feeding on the secretion of pepsin, trypsin and chymotrypsin in the Atlantic salmon, *Salmo salar* L. Fish Physiol. Biochem. 15, 439–446.

Einen, O., Holmefjord, I., Åsgård, T., Talbot, C., 1995. Auditing nutrient discharges from fish farms: theoretical and practical considerations. Aquac. Res. 26, 701–713.

Espe, M., 1999. Fish silage prepared from different cooked and uncooked raw materials: chemical changes during storage at different temperatures. J. Sci. Food Agric. 79 (2), 327–332.

Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579.

Glencross, B., Hawkins, W., Evans, D., Rutherford, N., McCafferty, P., Dods, K., Hauler, R., 2011. A comparison of the effect of diet extrusion or screw-press pelleting on the digestibility of grain protein products when fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture 312, 154–161.

Grove, D.J., Loizides, L.G., Nott, J., 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. J. Fish Biol. 12, 507–516.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139, 157–163.

Hevrøy, E.M., Espe, M., Waagbo, R., Sandnes, K., Ruud, M., Hemre, G.I., 2005. Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. Aquac. Nutr. 11 (4), 301–313.

Hoskins, L.J., Volkoff, H., 2012. The comparative endocrinology of feeding in fish: insights and challenges. Gen. Comp. Endocrinol. 176, 327–335.

Jacobsen, H.J., Samuelsen, T.A., Girons, A., Kousoulaki, A., 2018. Different enzyme incorporation strategies in Atlantic salmon diet containing soybean meal: effects on feed quality, fish performance, nutrient digestibility and distal intestinal morphology. Aquaculture 491, 302–309.

Kasumyan, A.O., Døving, K.B., 2003. Taste preferences in fishes. Fish Fish. 4, 289–347.

Koelz, H.R., 1992. Gastric acid in vertebrates. Scand. J. Gastroenterol. 27, 2–6.

Kousoulaki, K., Albrektsen, S., Langmyhr, E., Olsen, H.J., Campbell, P., Aksnes, A., 2009. The water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon (*Salmo salar* L.) given high plant protein diets. Aquaculture 289, 74–83.

Krogdahl, Å., Sundby, A., Holm, H., 2015. Characteristics of digestive processes in Atlantic salmon (*Salmo salar*). Enzyme pH optima, chyme pH, and enzyme activities. Aquaculture 449, 27–36.

Morais, S., 2017. The physiology of taste in fish: potential implications for feeding stimulation and gut chemical sensing. Rev. Fish. Sci. Aquacult. 25, 133–149.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, 927 Washington (376 pp).

Nikolopoulou, D., Moutou, K.A., Fountoulaki, E., Venou, B., Adamidou, S., Alexis, M.N., 2011. Patterns of gastric evacuation, digesta characteristics and pH changes along the gastrointestinal tract of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). Comp. Biochem. Physiol. 158, 406–414.

Oterhals, Å., Samuelsen, T.A., 2015. Plasticization effect of solubles in fishmeal. Food Res. Int. 69, 313–321.

Oterhals, Å., Samuelsen, T.A., Ahmad, R., 2019. A novel approach to determine optimal protein texturization conditions - a critical moisture level with increased effect of temperature on viscosity reduction in the rubbery state. J. Food Eng. 261, 66–75.

Rehfeld, J.F., 2017. Cholecystokinin-from local gut hormone to ubiquitous messenger. Front. Endocrinol. (Lausanne) 8, 8.

Rønnestad, I., Gomes, A.S., Murashita, K., Angotzi, R., Jönsson, E., Volkoff, H., 2017. Appetite-controlling endocrine systems in teleosts. Front. Endocrinol. (Lausanne) 8, 73.

Samuelsen, T.A., Oterhals, Å., 2016. Water-soluble protein level in fishmeal affects extrusion behavior, phase transitions and physical quality of feed. Aquac. Nutr. 22, 120–133.

Samuelsen, T.A., Mjøs, S.A., Oterhals, Å., 2013. Impact of variability in fishmeal physicochemical properties on the extrusion process, starch gelatinization and pellet durability and hardness. Anim. Feed Sci. Technol. 179, 77–84.

Samuelsen, T.A., Mjøs, S.A., Oterhals, Å., 2014. Influence of type of raw material on fishmeal physicochemical properties, the extrusion process, starch gelatinization and physical quality of fish feed. Aquac. Nutr. 20, 410–420.

Samuelsen, T.A., Oterhals, Å., Kousoulaki, K., 2018. High lipid microalgae (*Schizochytrium* sp.) inclusion as a sustainable source of n-3 long-chain PUFA in fish feed—effects on the extrusion process and physical pellet quality. Anim. Feed Sci. Technol. 236, 14–28.

Soengas, J.L., 2014. Contribution of glucose- and fatty acid sensing systems to the regulation of food intake in fish. A review. Gen. Comp. Endocrinol. 205, 36–48.

Valen, R., Jordal, A.E.O., Murashita, K., Rønnestad, I., 2011. Postprandial effects on appetite-related neuropeptide expression in the brain of Atlantic salmon, *Salmo salar*. Gen. Comp. Endocrinol. 171, 359–366.

Yúfera, M., Moyano, F.J., Astola, A., Pousão-Ferreira, P., Martínez-Rodríguez, G., 2012. Acidic digestion in a teleost: postprandial and circadian pattern of gastric pH, pepsin activity, and pepsinogen and proton pump mRNAs expression. PLoS One 7, e33687.