Effects of dietary moisture content of extruded diets on physical feed quality and nutritional response in Atlantic salmon (*Salmo salar*)

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Abstract

Feed pellets in modern aquaculture must be of high physical quality to withstand mechanical impacts. However, physical quality of feed has been shown to affect nutritional responses. In the present study, different moisture contents were used to modify physical feed qualities. Effects of dietary moisture content on physical feed quality, feed intake, nutrient digestibility and growth performance in Atlantic salmon were investigated.

Different drying times of four commercial-like extruded diets resulted in in dry matter contents of 95.9%, 94.1%, 92.3%, 90.8%. A fifth diet containing 70% dry matter was made by soaking pellets in sea water. Triplicate groups of Atlantic salmon (961 ± 12.1 g ind^{-1}, mean ± SEM) were fed *ad libitum*, and then with restricted feeding.

The results showed that *ad libitum* feed intake was affected by soaking but not by drying time. The diets differed significantly in dry matter, bulk density, DORIS durability and hardness. None of these measurements correlated with feed intake or growth. Long term water stability of the diets was not affected by drying time and soaking, and had a positive linear effect on feed intake and growth. Digestibility of most nutrients was highest in fish fed restricted compared to *ad libitum* feeding, but was not affected by drying time.

Keywords

Pellet durability, Pellet hardness, Water stability, Feed intake, Digestibility, Dietary moisture content, Atlantic salmon (*Salmo salar*)

Introduction

The grow-out phase of Atlantic salmon takes place in large sea cages. With cage volumes of up to 80,000 m^3 and stocking density up to 25 kg m^3 (Oppedal *et al.*, 2011) one cage may hold a fish biomass of 2,000 tons. At modern farm sites, several cages are supplied by one feed barge, that daily deliver up to 20 tons feed to every cage. To deal with these large volumes, feed often is transported in bulk. At the farm, feed is often kept in large silos and conveyed with use of pneumatic feeding systems to the cages (Aas *et al.*, 2011a, Oehme *et al.*, 2012). Developments in feed transportation have increased the mechanical impacts on feed pellets. Feed producers thus need to produce feeds with high pellet quality to avoid pellet breakage. Pellet breakage represents loss of valuable feed resources and has consequences for the cost-efficiency of salmon production (Aas *et al.*, 2011a).

Salmon feeds are produced by extrusion process, a combination of high pressure, high temperature and shear forces, as reviewed by Sørensen (2012). Moisture also plays an important role (Sørensen *et al.*, 2002). The final quality of the pellets is affected by production conditions during grinding, conditioning, extruding and drying, as well as physiochemical properties of ingredients. The gelatinization and expansion of starch in combination with denaturing of proteins result in water stable, durable pellets with bulk densities that can be adjusted according to the demand for oil absorption post extrusion and sinking velocity.

Different methods and equipment can be used to describe the characteristics of extruded pellets such as durability tests, hardness test and water stability tests (Sørensen, 2012). Physical feed quality is most often not reported in feeding experiments and there exist no standards for reporting of physical feed quality, although studies have shown an interaction between physical feed quality and nutritional responses in fish (Aas *et al.*, 2011b, Bæverfjord *et al.*, 2006, Glencross *et al.*, 2011a, Morken *et al.*, 2011, Sveier *et al.*, 1999, Venou *et al.*,

2009). It remains unclear how different physical quality methods relate to feed intake and feed utilization in fish. More research is needed to understand how different pellet quality parameters interfere with feed intake, growth and feed utilization in order to utilize the full growth potential of the fish. It is important to optimize the physical feed quality in order to minimize the losses as pellet breakage during transportation and handling at the same time as feed intake is maximized.

Morken *et al*. (2011) reported higher pellet durability and nutrient digestibility in rainbow trout fed pellets produced at high extrusion temperatures (141 °C) compared to low temperature (110 °C). A correlation was found between extruded and screw-pressed diets for nutrient digestibility in diets based on different ingredients (Glencross *et al.*, 2011a). Use of fish meal gave the slowest degradation of feed in the stomach followed by lupin kernel meal and soybean meal. These latter authors suggested that lupin meal gave a harder and more durable pellet than soybean meal during the extrusion process. However, they did not find any correlation between digestibility and the pellet integrity in the stomach. Venou *et al.* (2009) reported a slight reduction in feed intake, but an increase in growth, feed utilization, and digestibility of starch and energy in sea bream fed an extruded diet compared to steam pelleted feed. The decreased feed intake of the extruded diet was attributed to a nearly doubled gastric retention time measured in fish fed this diet. A feed with low water stability gave 20% higher feed intake compared to a feed with high water stability in rainbow trout (Aas *et al.*, 2011b). The more water stable feed also gave higher nutrient digestibility compared to the less water stable feed (Aas *et al.*, 2011b). Still it remains unclear whether the increased nutrient digestibility observed in the water stable diet was caused by physical feed quality or by lower feed intake. A high feed intake is related to a faster gastrointestinal evacuation rate and is negatively associated with nutrient digestibility (Adamidou *et al.*, 2009, Venou *et al.*, 2009). Gastrointestinal evacuation rate in Atlantic salmon is also affected by

coarseness of the ingredients. Sveier *et al*. (1999) showed increased retention time in the gastrointestinal tract when feeds are produced with coarse ground fish meal compared to fine ground fish meal.

High feed intake and feed utilization is an assumption to fully realize the growth potential of the salmon, and for an efficient utilization of the feed (Bergheim & Åsgård, 1996, Einen *et al.*, 1995, Einen *et al.*, 1999, Gjedrem *et al.*, 2012). In recirculating systems, total suspended solids and particle size distribution are important variables for water quality (Summerfelt & Vinci, 2007). Removing particles smaller than 40 µm by sedimentation or filtration is not efficient with the existing technology (Chen *et al.*, 1993, Cripps & Bergheim, 2000). The pellet quality may thus have a significant impact on water quality.

A study carried out with rats suggested that hardness of feed plays an important role in selection and ingestion of food (Ishizuka *et al.*, 2010). Texture of the feed interfered with feed intake in pigs (Sola-Oriol *et al.*, 2009) and chickens (Picard *et al.*, 2000). Interestingly, dietary moisture content has an impact on feed intake and nutrient utilization of poultry (Scott, 2002) and weaning piglets (Schellingerhout, 2002) and is also important for physical quality of pellets (Gilpin *et al.*, 2002, Lundblad *et al.*, 2011, Moritz *et al.*, 2001). It can therefore be hypothesized that feed moisture content can be used as a mean to adjust pellet quality of feed for Atlantic salmon. Different pellet qualities can be obtained by changing post extrusion drying time or by soaking feed pellets. Producing pellets with different physical qualities using means such as changing feed formulations or extrusion parameters may result in confounded effects and difficulties interpreting the results.

The aim of this study was to investigate the effect of moisture content of extruded feed on physical pellet quality, feed intake, growth and nutrient digestibility in Atlantic salmon. Nutrient digestibility was analyzed at both *ad libitum* feeding and constant feeding ration to

separate between effects of dietary treatment and feed intake. Also, the amount of total suspended solids and particle size distribution of released fines from pellets was analyzed.

Materials and methods

The study was carried out in two experiments; a pre-study and a main study. The pre-study tested the effect of soaked and unsoaked feed on feed intake. In the main study, five diets differing in moisture contents were tested for the effect on feed intake, growth rate and nutrient digestibility (Table 1).

1. Diets

All feeds (feeds for pre-study and main study) were produced by Biomar AS (Tech Centre, Brande, Denmark).

Diets for pre-study

One experimental feed with identical formulation and similar extrusion conditions as diets in the main study, was produced with dry matter content (DM) of 91.2%. This was fed either as is or the pellets were moistened by immersing the daily feed ration for each tank in 500 ml sea water (4 °C) for 120 min prior to feeding. The pellets were kept in a 4 °C cooler during the soaking. The soaking resulted in pellets with a DM of 73.2%, a soft surface and a core that remained hard. The diets were labeled according to their DM content (D91 and S73). The chemical compositions are given in Table 2.

Diets for main study

Four experimental diets were produced with identical ingredient composition and extrusion conditions. The different feed qualities of dietary treatment 1-4 were achieved by adjusting the drying conditions post extrusion in order to provide diets of 95.9%, 94.1%, 92.3%, 90.8% DM. The diets were labeled according to their DM content D96, D94, D92, D91, respectively. The fifth dietary treatment, a soaked feed (diet S70) was produced by soaking feed D92 in sea water to a DM of 69.5%. Soaking was performed as described for the pre-study. Chemical composition of the feeds are shown in Table 2. The feeds were formulated on a 95% dry

matter basis (20% fishmeal, 15.5% soy protein concentrate, 3% wheat gluten, 15.5% sunflower expeller, 15.2% dehulled bean, 21.1% fish oil, 9% rape oil, 1.26% monocalciumphosphate 0.36% amino acids, 0.23% mineral and vitamin mix, 0.05% yttriumoxid). A feed batch including all ingredients was mixed and preconditioned in an atmospheric conditioner (Clextral, Firminy, France) prior to extrusion (BC 45, Clextral, Firminy, France). After extrusion the diets were dried in a six layer column dryer (Geelen type), aiming at different DMs by varying the drying time. The times of drying were 350 seconds (s), 230 s, 115 s and 50 s, respectively for the four diets. The temperature of inlet air to the top layer was kept constant at 117-118 °C, and at 80-81°C to the fifth layer. After drying and cooling, the feeds were packed in 25 kg plastic bags and were stored at 4 °C.

2. Fish trial

The two studies were carried out at Nofima's research station at Sunndalsøra, Norway from August 2011 until March 2012.

Pre-study

Daily feed intake of the two diets was measured for 41 days in triplicate tanks. In total, 120 Atlantic salmon, with an average initial weight of 809 ± 90.2 g (mean ± SD, N=120) were distributed to 6 tanks (1 m^2). The two feeds were randomly assigned to the tanks. Automatic belt feeders were used to feed the fish once per day, in a meal lasting for two hours.

Main trial

A total of 240 Atlantic salmon were distributed to 15 tanks (1 m^2). All fish were PIT tagged and distributed to the tanks based on randomization of the PIT tag identity. The first 35 days was an adaptation period, and the fish were fed a mixture of a commercial 7 mm diet (Skretting) and a marine ingredient-based 9 mm diet produced at Nofima.

At start of the feeding trial the body weight was 961 ± 187 g (mean ± SD, N=240). The five experimental feeds were randomly assigned to three replicate tanks. The fish were fed one meal a day lasting for approximately 30 min using automatic belt feeders. Waste feed collection and calculation of feed intake was done according to Helland *et al.* (1996). The fish were kept in sea water (salinity 32 ppt) with average temperature 9.4 °C (range 7.8 °C - 11.2 °C), and at continous light. During the first period (91 days) fish were fed *ad libitum*. The feed amount was adjusted every day based on the estimated feed intake for the last three days aiming at 20% overfeeding. During period two (19 days), fish were first fed *ad libitum* (12 days) until stable feed intake was obtained. For the last 7 days of period two, the fish in all tanks was fed a fixed DM ration of 0.4% of biomass. Fish were weighed individually at the start and end of each period. After period one and two, faeces was stripped from all fish and pooled by tank for analyses of digestibility (Austreng, 1978). The last meal was fed 22 to 27 hours prior to sampling. Sampling was carried out in three blocks, with one replicate feed in each block to avoid effects due to sampling at different times after feeding.

3. Analysis of physical feed quality

Hardness was analyzed by diametrical compression using a texture analyzer (TA-XT2, Model 1000 R, SMS Stable Micro Systems, Blackdown Rural Industries, Surrey, UK) as described in Aas *et al*. (2011b). For each diet, 35 pellets were analyzed, and strength at rapture (N) was recorded. The texture analyzer also recorded the diameter on the pellets used for the hardness measurements. Bulk density was analyzed in three replicates by pouring pellets in a 1000 ml cylinder with subsequent weighing (described in Aas *et al.,* 2011b and Sørensen, 2012). Pellet durability was analyzed by the DORIS tester (AKVAsmart, Bryne, Norway) in three replicates. Pellets (350 g) were loaded in the DORIS tester and sieved afterwards for 30 s at amplitute of 1.5 using a Retsch AS 200 Control sieving machine (Retsch GmbH, Haan, Germany). The screens used for collection had a mesh width of 8.0 mm, 5.6 mm and 2.4 mm

and the fractions were recorded as intact pellets, large fracture, small fracture and dust, respectively (described in Aas *et al*., 2011b and Sørensen, 2012). Bulk density and DORIS durability could not be analyzed on the soaked feed. Water stability was measured in three replicate samples for 60 min, 120 min and 240 min by filling 20 g of pellets in a custom made steel-mesh placed inside a glass beakers containing 300 ml distilled water. The beakers were shaken at 100 shakings per minute in a water bath at 25 °C. After incubation the retained DM was measured (described in Sørensen, 2012).

4. Chemical analyses

Faeces was freeze dried prior to analysis. Feed and faeces was analysed for dry matter by drying at 105 °C to constant weight, ash was analyzed by combustion at 550°C to constant weight, crude protein by nitrogen x 6.25 (Kjeltec Auto Analyser), crude lipid by SOXTEC hydrolyzing and extraction systems, while gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter). Starch was determined as glucose after enzymatic hydrolysis, by a commercial kit based on the GODPOD method (Megazyme, Bray, Ireland). Amino acids were analysed by using a Biochrom 30 amino acid analyser (Biochrom, Cambridge, UK). Tryptophan was analysed after basic hydrolysis (Hugli & Moore, 1972), and the remaining amino acids according to Davies (2002). During sample preparation, glutamine and asparagine are converted to glutamice acid and aspartic acid, respectively. Therefore, in the following, the given value for glutamic acid and aspartic acid represents the sum of glutamic acid and glutamine, and aspartic acid and asparagine, respectively. Yttrium oxide (inert digestibility marker) and minerals were analysed by inductively coupled plasma mass spectroscopy (ICP-MS, at Eurofins, Moss, Norway).

5. Particle size distribution and particles formed in-vitro

A water stability test was carried out on the 5 feeds in three replicates with the same equipment as described above. Feed pellets (20 g, as is) were shaken in 300 ml distilled water

at 18 °C in 500 ml glass beakers. Pellets were shaken for 15 min at 50 shakings per minute to mimic a normal situation, and for 120 min at 90 rpm to mimic a situation where pellets are trapped in the system or exposed to stronger mechanical impacts over longer period of time. Remaining pellets in the mesh were discarded and the water containing released feed particles was analyzed for total suspended solids and particle size distribution, immediately after the water stability test. Analysis of total suspended solids was carried out according to APHA (2005). Briefly, homogeneous samples with volumes ranging between 49 ml and 100 ml were filtered using dried and preweighted glass fibre filters (GF/C, diameter 55 mm, particle retention 1.2 µm, Whatman). The DM of the filters was determined and amount of total suspended solids (g DM per kg DM feed) calculated. Particle size distribution was analyzed with SALD Series Laser Diffraction Particle Size Analyser (Shimadzu, Kyoto, Japan) and results were analyzed using the software WingSald II, V 2.1. (Shimadzu, Kyoto, Japan).

6. Gastrointestinal content and tissue

After period two in the main trial, stomach, pyloric-, mid- and distal intestine and its content were evaluated visually and scores were given using a 0-3 point scale according to the degree of disintegration of feed pellets and abnormal appearance of gastrointestinal tissue, score 0 being normal and scores 1-3 being more og less abnormal conditions.

7. Calculations

Feed intake (DM) = Feed fed[a] (g, DM) – Waste feed (g, DM) / Recovery

[a] Soaked feed was weighed before soaking

Recovery = Feed spill (g, DM) / Feed used[b] (g, DM)

[b] Feed used for soaking was weighed before soaking and DM of that feed was corrected for DM loss (1.1 % DM) during soaking.

Feed conversion ratio (FCR) = Feed intake (g, DM) / Weight gain (g)

Only non-mature fish were included in the calculation.

Specific growth rate (SGR) = ((ln (Final weight(g) – ln (Initial weight (g)) / Days fed) x100

Only non-mature fish were included in the calculation.

Specific feeding rate (SFR) = SGR x FCR

Thermal growth coefficient (TGC) = 1000 x ((Final weight$^{1/3}$ (g) – Initial weight$^{1/3}$ (g)) / Sum daydegrees)

Only non-mature were fish included in the calculation.

Apparent digestibility (AD, %) = 100 (a-b) / a

where a is nutrient to marker ratio in feed and b is nutrient to marker ratio in feces.

Total suspended solids (g DM per kg DM feed) = ((DM of filter after filtration (g) – DM of empty filter (g)) / sample volume (ml) x 1000) / (weighted in feed (g) x DM feed (%)) x 1000

Released feed particles (DM, g per kg DM feed) = Total suspended solids (g DM per kg DM feed) x 0.3

8. Statistical analysis

All statistical analyses were carried out with the computer software SAS 9.2. (SAS Institute Inc. Cary, NC, USA). Tank mean was the statistical unit in data of the fish trial (n=3) and data are presented as mean ± SEM for each dietary treatment. The experiment was carried out using triplicate tanks in a complete randomized design. Analyses of physical quality of feed was carried out with the individual measurement as the statistical unit (DORIS, bulk density and water stability n=3; hardness and diameter n=35) and results are presented as means ± SEM for each feed.

Physical feed quality

Pearson correlation analysis among the physical feed quality data on the means of the feeds and was carried out using the corr procedure in SAS (DORIS, bulk density, DM, hardness, diameter and water stability n=1).

Effect of drying

The effect of drying time on chemical feed composition, physical feed quality, feed intake, growth, digestibility and released particles of in-vitro water quality was carried out using regression analysis. Only diets from the main trial were evaluated (D96, D94, D92, D91) using DM as the independent variable.

Effect of soaking

The effect of soaking on chemical feed composition was assessed numerically by calculating the difference between S70 and D92 from the main trial. The results are presented if the

difference was greater than 5%. The difference was calculated as: (nutrient S70 / nutrient D92) *100. Differences in physical feed quality, feed intake and growth, digestibility and released particles of in-vitro water quality between the soaked diet and the corresponding dry diet in the main trial were assessed by ANOVA procedure in SAS. A two-way ANOVA was used to analyze the effect of soaking on feed intake with feed and scenario as factors. The three scenarios that were used were the first period of the main trial, the first 12 days of period 2 (adaption period before restricted feeding was introduced) and the pre-study.

Effect of fixed feeding

Nutrient digestibility from the two faecal collection periods were analyzed by one way ANOVA, using repeated ANOVA statement.

Fish trial and physical feed quality

Regression analysis was carried out to analyze linear effects of physical feed qualities on growth and feed intake parameters using all five dietary treatments for DM, hardness, diameter, water stability measurements and using dietary treatment 1-4 for DORIS, bulk density measurements.

Results

1. Chemical feed composition

The DM content in the feeds showed a significant linear decrease with increasing drying time ($p=0.001$, $R^2=1.00$). The content of most nutrients was similar among diets based on the DM content (Table 2). Only taurine tended to have a linear reduction with increasing drying time ($p=0.087$, $R^2=0.83$). For the soaked diet (S70), higher content of Mg, Na, Fe and Tyr was measured compared to the corresponding unsoaked diet (D92). Nutrients that were present in lower levels in the soaked feed, compared to the corresponding dry diet were Ala, Gly, Hyl, Pro, Tau, His, Met, Phe (all more than 5% reduced).

2. Physical feed quality

The DM of the diets had a significant negative linear effect on DORIS intact, and bulk density ($R^2=0.92$ and $R^2=0.91$, respectively) (Table 3). Dietary DM had a significant negative linear effect on hardness, water stability at 60 min and at 120 min, but less of the variation was explained ($R^2=0.15$, $R^2=0.62$, $R^2=0.66$, respectively). For DORIS fractures and DORIS dust, dietary DM had a significantly positive linear effect and R^2 was 0.89 and 0.95, respectively. The DM content of the diets had no significant linear effect on diameter and water stability at 240 min. Soaking of the feed did not affect pellet hardness or water stability at any of the three incubation times of the feeds. Correlation of the physical quality measurements indicated that DM, DORIS durability and bulk density were strongly correlated (Table 4). Water stability after 60 min was correlated to DORIS durability and bulk density. Water stability after 120 and 240 min and hardness did not show any significant correlations to any other tests of physical feed quality. The two driest feeds had highest frequencey of broken pellets at low force ($\leq$ 10 N) (Fig. 1).

3. Particle size distribution and particles formed in-vitro

After 15 min of gentle shaking in water bath, dietary DM content had a significant positive linear effect on the amount of particles that were released from the feed pellets and measured in the water. Most particles were measured for diet D96 (7.1 ± 0.5 g per kg dry feed), followed by 5.6 ± 0.3, 3.9 ± 0.7, 2.1 ± 0.3 g DM per kg DM feed for diet D94, D92 and D91, respectively. The soaked diet released higher amount of particles than the corresponding dry diet (6.6 ± 0.8 g per kg dry feed, p=0.07). The mean particle size distribution ranged between 167.1 ± 31.2 µm and 121.9 ± 1.7 µm, for D96 and S70, respectively and no differences were observed between the diets after 15 min gently shaking (Fig. 2). The amount of particles formed during the course of 120 min intense shaking was higher than at 15 min gentle shaking, but was not significantly affected by the diet, and amounts ranged between 66.8 ± 2.4 and 113.4 ± 15.7 g DM per kg DM feed, for the diets D91 and S70, respectively. After 120 min intense shaking, the particle size was smaller compared to short and gentle shaking and mean particle size was largest for the soaked diet (mean particle size: 0.5 ± 0.3 µm), followed by diet D96 (mean particle size: 0.2 ± 0.1 µm). Mean particles size for diets D94, D92 and D91 was 0.1 ± 0.0 µm.

4. Fish experiment

One fish died during the main trial after jumping out of the tank. This fish had reduced its body weight since the start of the experiment (-32 g). Most likely it did not consume any feed during the course of the experiment and was therefore excluded from all calculations. Sexual maturation was noted on 2 to 6 male fish per tank. Weight gain recorded on individual fish, showed that mature fish did not grow (Fig. 3). Therefore, feeding and growth results presented are corrected for the mature fish. The non-mature fish grew as expected according to Austreng et al (1987).

Effect of drying time in feed production

Regression analysis showed that weight gain (Fig. 3), final body weight, feed intake, SGR, SFR, TGC and FCR (Table 5) were not significantly affected by drying time of the diets ($p>0.05$). Numerically, feed intake, weight gain, SFR and SGR were slightly higher for fish fed diet D91, second highest for D96, followed by D94 and D92. Fish fed diet D92 had lowest weight gain, feed intake, SFR, SGR and TGC. FCR was slightly higher for D92 compared to the other diets.

Effect of soaking

Soaking did not significantly affect weight gain (Fig. 3), final body weight, feed intake, SGR, SFR, TGC and FCR (Table 5) in the main trial. Numerically however, all parameters were slightly improved for the soaked diet, compared to the corresponding unsoaked diet (D92).

Effect of soaking on feed intake in three situations

Figure 4 shows feed intake (DM) for the soaked diet compared with the corresponding dry diet in three different situations, normal, intermediate and low feed intake. The specific situations were 1) the first period of the main trial 2) the first 12 days of period 2 (adaption period before restricted feeding was introduced), when feed intake was low because of handling stress during sampling and 3) the pre-study which was characterized by very low feed intake. The main effect of soaking was significant ($p=0.005$) indicating that feed intake was significantly higher for fish fed the soaked diet compared to the corresponding dry diet for these three situations. Feed intake tended to be higher for the soaked diet compared to the dry diet for the pre-study ($p=0.051$) and during the adaption period of the second feeding period ($p=0.056$).

5. Relationship between physical feed quality and feed intake

Water stability at 240 min had a significant positive linear effect on total feed intake and SGR ($R^2=0.82$ and $R^2=0.90$, respectively) (Fig. 5). Hardness, DM, DORIS durability, bulk density,

water stability after 60 min and water stability after 120 min did not have significant linear effects on feed intake and SGR (not shown).

6. Digestibility

Apparent digestibility of fish fed *ad libitum* or restricted was not affected by drying time (Table 6). Starch digestibility was slightly lower in the soaked diet compared to the corresponding dry diet when fish was fed restricted and *ad libitum*. Moreover, soaking influenced Na digestibility in fish fed restricted and *ad libitum*, while Fe digestibility was affected only when fish was fed restricted. The soaked diet gave less accumulation of minerals in feces compared to the dry diet. Differences in Na digestibility between the soaked and corresponding dry diets were 169.3% and 107.8% for fish fed *ad libitum* and restricted, respectively. Most nutrients showed higher AD when the fish was fed restricted compared to *ad libitum*. The average ADs of all five diets in fish fed *ad libitum* and restricted respectively, were 85.2% and 85.8% for nitrogen, 89.1% and 90.1% for sum of amino acids, 70.5% and 72.5% for starch, 29.7% and 34.9% for phosphorous and 60.6% and 66.9% for cysteine.

7. Gastrointestinal content and tissue

Neither amount and pellet integrity of gastrointestinal content, nor intestinal tissue were affected by drying time or by soaking of the feeds. At sampling (22-27 hours after feeding), the stomach was empty in most fish.

Discussion

All experimental feeds were formulated identically, and the differences in feed quality were achieved by either adjusting drying time after the extrusion process, or by soaking one of the feeds in water before feeding or chemical and physical analyses. Processing involving heat may result in undesirable chemical reactions reducing bioavailability of heat sensitive nutrients (Camire *et al.*, 1990, Opstvedt *et al.*, 1984). High moisture content (25-30%) is however, protecting protein and amino acids during extrusion processing ensuring high digestibility of these nutrients (Sørensen *et al.*, 2002). Heat processing at low moisture content may result in degradation or formation of undesirable cross-linkages in amino acids. The amino acids arginine, cystein, lysine, serine and threonine are most succesptible to degration (Papadopoulos, 1989, Pickford, 1992). Cysteine may form disulfide-bonds (Opstvedt *et al.*, 1984), and lysine is susceptible to form Maillard reactions at high temperature in combination with low moisture content (Singh *et al.*, 2007). Such reactions may reduce the avalibility of amino acids (Ljøkjel *et al.*, 2000). Moisture content is reduced in a dryer, from 30% after extrusion to below 8%, to increase the shelf life during storage (Sørensen, 2003). Excessive drying can be a significant factor for formation of disufild bridges and heat induced oxidation of cysteine and methionine (Opstvedt *et al.*, 1984). Opstvedt *et al.* (1984) reported that fish meals dried at temperatures ranging from 40 to 115 °C for 40 min, gave decreased protein and amino acid digestibilities at temperatures above 95 °C. In the present trial, drying temperatures measured at the top of the dryer did not exceed 118 °C, which is lower than temperatures during extrusion, however, the protecting effect of moisture disappears during drying. Nutrient content of the experimental diets was not affected by drying time, except for taurine, which was marginally reduced with drying time. Loss of taurine during processing has been described previously and was suggested to be caused by either leaching or Maillard reaction upon heating (Larsen *et al.*, 2007, Spitze *et al.*, 2003).

Digestibility of nitrogen and amino acids was not affected by drying time, indicating that there was no severe damages because of the treatments. Digestibility of cysteine was in general low for all diets, and may have resulted in low availability for the fish. Cysteine is susceptible to form disulphide bonds at treatments involving high temperature and low moisture content (Opstvedt *et al.*, 1984). Since no differences were observed in cysteine digestibility among treatments in the present experiment, the low digestibility was probably not caused by the drying of the feeds. Another explanations suggested is that the heat damage already had occurred during processing of the ingredients before feed production. Ljøkjel *et al.* (2000) showed that heat treatment at 130 °C for 30 min of soybean meal significantly reduced the cysteine concentration, probably due to Maillard reaction, and caused a reduced cysteine digestibility in soybean meal and fish meal based diets in mink. It was further suggested that reduced cysteine digestibility was due to formation of disulfid bridges. However, analytical methods used to analyze cysteine in the study by Ljøkjel *et al.* (2000) and the present study, do not discriminate between cysteine and cysteine bound in disulphide bridges (cystine). Cysteine levels analyzed in the diets were together with methionine according to the requirement of sulfur-containing amino acids of salmon (Rollin *et al.*, 2003), however, more cysteine was excreted in the feces.

When soaking feed in sea water, ions from the water will migrate into the pellets, and some nutrients may leach from the pellets. Thus, a change in chemical composition is expected. When soaking diet D92 in sea water, the concentration of ions from the sea water (Mg, Na, Fe) increased, while the concentration of some amino acids (Ala, Gly, Hyl, Pro, Tau, His, Met, Phe) was reduced. These results strongly suggest that amino acid concentration should be monitored if extruded feed is soaked in water prior to feeding.

The fish appeared to be at good health, and there was no mortality in the trial, except from one individual fish, that jumped out of the tank. Sexual maturation among male fish appeared

during the trial, resulting in anorexia and negative weight development for these fish. Therefore, mature fish were excluded from calculations. Calculations based on only non mature fish followed the same pattern compared to use of all fish. Consequently, the interpretation of results was not affected by the mature males.

Feed intake, growth and feed utilisation

The diets varied in DM, bulk density, hardness and DORIS durability and differences were achieved by varying drying time after extrusion in feed production. Among pellet qualities used in this trial, no significant effects were noted on feed intake, digestibility and growth. However, large numerical differences were noted between the best and lowest performing groups. Salmon fed D91, with the highest durability, hardness and bulk density, had 28% greater feed intake and 36% higher weight gain, compared to those fed D92. Soaking the pellets in water before feeding significantly increased feed intake but did not affect hardness and water stability of the feeds. This is in contrast to the findings of Aas *et al.* (2011b) who reported that feed intake in rainbow trout was affected by physical feed quality. In that study, feed intake was approximately 20% higher in trout fed a diet with low water stability (27.9% retained DM) than in trout fed a diet with high water stability (80.6% retained DM). In the present study, all feeds were more stable (92.6% on average) and no differences were noted in water stability among diets. Also, the diets used in the present trial were considerably harder, ranging from 33.2 to 54.3 N, compared to those used by Aas *et al.* (2011b), ranging from 20.1 to 27.3 N. Contradictive findings were previously reported about the relationship between hardness and feed intake (reviewed by Sørensen, 2012). In line with Glencross *et al.* (2011b) and Bæverfjord *et al.* (2006), the present findings indicate no evident relationship between feed intake and hardness, as well as a positive relationship between feed intake and long term water stability. Contradictive findings among experiments about the interaction of physical

quality and feed intake may be related to species differences as well as feed intake levels, and physical differences not covered by the methods used.

Soaking had a positive effect on feed intake, particularly in the adaption periods with low or increasing feed intake. The reason for the low feed intake during the pre-study is not known. The low feed intake during the adaption period before restricted feeding is explained by handling stress during sampling. The positive effect of soaked pellets on feed intake may be because of stimulated appetite due to dispersion of feed odor in the tanks. In addition to olfactory mechanisms, the increased water content of soaked pellets may positively affect pellet disintegration and gastrointestinal retention time. The digestion of pellets start with absorption of water. Kristiansen & Rankin (2001) showed that extruded pellets in juvenile rainbow trout started to disintegrate at a moisture content of 65%. Thus, soaking of pellets prior to feeding may reduce the gastric moisturing time resulting in shorter retention time in the fish stomach. However, water stability test, which is suggested to mimic intestinal disintegration of pellets (reviewed by Sørensen, 2012), was not different for the soaked and corresponding dry diet. Furthermore, visual analysis of pellet integrity throughout the gut did not reveal any differences in intestinal pellet disintegration for the soaked and corresponding dry diet. The present data showed that soaking of feed pellets affected feed intake, in particular in periods of low feed intake, indicating that moisturizing of extruded feed may be a tool to improve feed intake and growth, especially in periods when feed intake is relatively low.

The lower starch digestibility in the feeding period with *ad libitum* feeding compared to the feeding period with fixed ration may be due to a faster gastrointestinal evacuation rate. This may also explain the difference in starch digestibility for the unsoaked and the soaked diets. Faster gastrointestinal evacuation rate has previously resulted in reduced nutrient digestibility (Adamidou *et al.*, 2009, Venou *et al.*, 2009). Differences in digestibility between soaked and

unsoaked diet were only observed for starch, but not for other nutrients most likely because Atlantic salmon has low capacity to digest starch (Krogdahl *et al.*, 2005).

Digestibility of Na was negative (accumulation in feces) for both periods (restricted and *ad libitum* feeding) because the fish was kept in sea water. Fish fed the soaked diet had less accumulation of Na. This may be related to the higher level of Na in the soaked diet originating from seawater, compared to the corresponding dry diet. Different drinking rate of sea water between fish fed soaked and dry diet may influence accumulation of Na and other minerals in feces (Shehadeh & Gordon, 1969). Ruohonen *et al.* (1997) suggested that feeding dry pellets to rainbow trout increased the demand for water that was met by drinking. Fish fed the soaked diet may thus have a lower need to drink seawater than fish fed the extruded diet. A calculation taking into account the different Na-digestibility values of the dry and soaked diets and different dietary Na concentration, showed that the total amount of Na accumulating in the feces was similar between the two dietary treatments at *ad libitum* and restricted feeding. In contrast to Ruohonen *et al.* (1997) the present results suggest that drinking rate was not affected by the DM of the pellets. Thus, the differences in Na-digestibilities between the soaked and dry diet in the present experiment seems to be a result of soaking in seawater.

The digestibility of nitrogen, starch, most amino acids, P and Ca was higher in fish fed restricted compared to *ad libitum*, and was not affected by drying time of the feeds. These results are in line with Aas *et al.* (2011b), who reported that higher feed intake gave lower apparent nutrient digestibility. In the study of Aas *et al.* (2011b), the effect of physical feed quality on nutrient digestibility was confounded with effect of feed intake. The present data, however, showed that nutrient digestibility increased at low feed intake, when retention time in the gastrointestinal was high, and was not directly affected by drying time or differences in physical feed quality.

Generally, differences in average AD between *ad libitum* and restricted feeding were larger for nutrients that were digested less efficiently, such as cysteine and phosphorous. In comparison, nutrients with high ADs were not affected by feeding regime, such as lysine, lipid and energy. The results showed that in situations with high feed intake the ratio of digested nutients can be reduced. This may affect the diet's ability to fulfill requirements of essential nutrients, in particular the nutrients that are not easlisy available, such as cysteine and phosphorous.

Measurement of physical feed quality

In the present experiment, significant effects on bulk density, DORIS durability, hardness and short term water stability by drying time were found, however, these measurements did not correlate with feed intake, gastrointestinal pellet integrity or digestibility. Hardness and long term water stability measurments did not correlate with the other methods of physical feed quality indicating that these methods may describe pellet characteristics that are not captured by DM, bulk density, DORIS durability and short term water stability analyses. Non-significant, but numerical differences, were observed in feed intake and growth among treatment groups. However, the variation in physical quality induced by manipulating drying time may not have been large enough to interfere with feed intake. Hardness was significantly affected by drying. Large variation within the feeds was found for the hardness measurements for diet D96 and D94. In measurement of particle size distribution, large variation was also noted for D96 and was due to one sample with very large particles. However, the variation among the feeds were modest, ranging between 33.2 N to 52.3 N. Besides, the hardness values of the feeds used in the present experiment were high compared to other experiments (Aas *et al.*, 2011b, Morken *et al.*, 2011, Morken *et al.*, 2012). Hardness of pellets is positively associated with pellet diameter (Abdollahi *et al.*, 2012, Kaliyan & Vance Morey, 2009). The higher hardness of pellets used in the present experiment may thus be explained with the

larger diameter of the pellets compared to other experiments (Aas *et al.*, 2011b, Morken *et al.*, 2011, Morken *et al.*, 2012). The high hardness of the feeds used in the present experiment may have interfered with feed intake, restricting the realization of growth potential. Feed intake and growth was positively correlated with long term water stability, though it was not affected by drying time and no significant differences in feed and growth were noted among the feeds. Long term water stability is suggested to mimic disintegration of pellets in the gastrointestinal tract and influence gastric evacuation rate (Sørensen, 2012). This is in line with finding of Aas *et al.* (2011b) suggesting that pellets with high water stability and hardness slowed down gastric evacuation rate. Visual inspection of the feed indicates that the soaked diet differed from the dry diet, but no significant differences were observed in long term water stability and hardness. There exist no methods that objectivley describe water absorbtion and distribution in feed pellets, and disintegration of pellets in water. These pellet characteristics may be central because dietary moisture may improve feed intake and growth and be related to disintegration of feed pellets. The decreasing durability and increasing dust formation at DM above 92% indicate that low water content could potentially create pellet breakage leading to feed spill (Aas *et al.*, 2011a). Such feed spill caused by small particles and fines may reduce feed intake and feed utilization at commercial fish farms. The controlled feeding routines used in the present experiment were not expected to create fines compared to large scale facilities. However, available methods used to analyze physical feed quality did not correlate with pellet degradation in pneumatic feeding system (Aas *et al.*, 2011a). To reduce feed spill and increase feed intake and feed utilization it is important to produce pellets with a compromised physical quality to balance losses related to feed transport and conveying against potential negative effects on fish performance. Overall, the present experiment showed that most existing methods used to analyze physical feed quality did not correspond well with nutritional responses. Only long term water stability was associated with feed

intake. Nutrient digestibility depended rather on feed intake than on pellet quality. Variation in physical feed quality of commercial feeds suggest that it is important to understand the impact of physical quality on feed intake and growth, in order to realize the full growth potential and predict production of Atlantic salmon more precicly.

Physical feed quality and water quality

The driest feed (D96) released about 3.4 times more particles than the dry feed with highest water content (D91) after 15 min. in shaking water bath. These findings correspond well with the results of the water stability test. Moreover, the particle size distribution was different among the experimental diets when pellets were shaken intensely for 120 min. The present results showed that physical feed quality can affect the prevalence of small particles in water used for fish culture. Removing fine particles are challenging and require advanced solid separation techniques (Chen *et al.*, 1993). This may be of particular importance of management of recirculating systems in aquaculture.

Conclusion

Overall, no significant effects of drying time of feed were observed on feed intake in fish. Soaking the feed before feeding had a positive effect on feed intake. Resticted feeding compared to *ad libitum* feeding gave higher nutrient digestibility. Digestibility was not affected by physical quality produced by varying drying time among diets, however, soaking resulted in slightly reduced digestibility of starch. Most methods used to analyze physical feed quality did not correlate to feed intake and growth, only long term water stability was associated with feed intake but has to be further investigated. Considering the variation in physical feed quality in commercial feeds, it is important to understand potential impacts of physical feed quality on feed intake, growth and feed utilization in order to improve production planning and utilization of the full growth potential of Atlantic salmon.

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Figure legends

Figure 1.

Frequency distribution (%) of hardness measurement of feed pellets with different physical quality of diets used in the main trial. Differences were produced by different drying times (D96, D94, D92, D91) or by soaking in sea water for two hours (S70). The class interval width was 10 N, and the endpoints were 0 and 100 N.

Figure 2.

Particle size distribution of released particles from feed pellets used in the main trial at 120 min and 90 shakings per minute (black lines) and at 15 min and 50 rpm shakings per minute (grey lines) in water bath at 18 °C. Feed pellets differed in physical feed quality produced by varying drying time (D96, D94, D92, D91) and by soaking in sea water (S70).

Figure 3.

Inidividual weight gain of Atlantic salmon (g per fish) of all fish (black bars), non-mature fish (grey bars) and mature fish (white bars) fed diets of different physical feed quality *ad libitum* (period 1 of the main trial). Differences were produced by varying drying times (D96, D94, D92, D91) and by soaking in sea water (S70). Data is given as mean ± SEM. No significant differences were found.

Figure 4.

Average feed intake (g DM per fish per day) of Atlantic salmon fed a soaked and corresponding unsoaked diet for three situations: 1) period 1 of the main trial (*ad libitum*

feeding) 2) first 12 days of period 2 before feed ration was restricted 3) the pre-study. Grey bars are soaked feed and black bars the corresponding dry feed. Data is given as mean ± SEM.

Figure 5.

Linear effect of water stability at 240 min (%) of experimental diets used in the main trial on total feed intake (g DM per non-mature fish, upper panel) and specific growth rate (SGR, lower panel) at *ad libitum* feeding (period 1 of the main trial).

Tables

Table 1. Overview of the trials.

	Pre-study	Main trial			
		Adaptation	Period 1	Period 2, refeeding	Period 2, restricted
Diets	D91, S73	Commercial and Nofima diet	D96, D94, D92, D91, S70	D96, D94, D92, D91, S70	D96, D94, D92, D91, S70
Feeding	*ad libitum*	*ad libitum*	*ad libitum*	*ad libitum*	restricted
Duration	41 days	35 days	91 days	12 days	7 days

Table 2.

Chemical composition of experimental diets used in the main trial and in the pre-study. Differences were produced by different drying times (D96, D94, D92, D91) or by soaking in sea water for 2 hours (S70, S73).

	Main trial					Pre-study	
	D96	D94	D92	D91	S70	D91	S73
Dry matter (g kg^{-1})	959	941	923	908	695	912	732
In dry matter							
Crude protein[1] (g kg^{-1})	375	367	371	369	372	363	363
Sum of amino acids (g kg^{-1})	301	296	306	302	294		
Crude lipid (g kg^{-1})	347	350	351	349	355	339	356
Starch (g kg^{-1})	57.7	54.9	57.0	57.0	55.8	62.1	61.7
Energy (MJ kg^{-1})	26.3	26.1	26.0	26.3	26.2	25.8	26.0
Minerals							
Phosphorous (g kg^{-1})	10.7	10.7	10.7	10.7	10.4	NA	NA
Magnesium (g kg^{-1})	2.1	2.1	2.1	2.1	2.5	NA	NA
Sodium (g kg^{-1})	3.1	3.0	3.2	3.1	5.8	NA	NA
Iron (mg kg^{-1})	210	212	212	205	259	NA	NA
Zinc (mg kg^{-1})	178	169	169	168	165	NA	NA
Yttriumoxid (g kg^{-1})	0.47	0.46	0.45	0.47	0.45	NA	NA
Dispensible amino acids (g kg^{-1})[2]							
Alanine	13.5	13.2	13.7	13.4	12.8	NA	NA
Aspartic acid[3]	29.8	29.5	30.3	29.9	29.5	NA	NA
Cysteine	3.8	3.7	3.9	3.9	3.8	NA	NA
Glutamic acid[4]	58.6	58.2	60.3	59.7	58.3	NA	NA
Glycine	13.4	13.1	13.5	13.5	12.8	NA	NA
Proline	15.5	15.4	15.7	15.1	14.9	NA	NA
Serine	13.5	13.2	13.8	13.6	13.2	NA	NA
Taurin	1.3	1.3	1.3	1.4	1.1	NA	NA
Tyrosine	11.2	10.9	11.3	11.1	11.8	NA	NA
Indispensible amino acids (g kg^{-1})[2]							
Arginine	22.2	21.7	22.4	22.3	21.7	NA	NA
Histidine	8.4	8.1	8.5	8.2	7.9	NA	NA
Isoleucine	13.7	13.5	14.0	13.9	13.5	NA	NA
Leucine	22.7	22.4	23.1	22.8	22.2	NA	NA
Lysine	22.2	22.0	22.6	22.5	21.5	NA	NA
Methionine	8.4	8.3	8.5	8.3	7.7	NA	NA
Phenylalanine	12.9	12.5	13.0	12.7	12.3	NA	NA
Threonine	11.8	11.5	11.9	11.7	11.4	NA	NA
Tryptophan	4.0	3.8	4.2	4.1	4.2	NA	NA
Valine	15.1	14.8	15.3	15.2	14.8	NA	NA

[1] N x 6.25

[2] Amino acids are given as dehydrated residuals.

[3] Aspargine is converted to aspartic acid during analysis, thus aspartic acid represents the sum of aparagine and aspartic acid.

[4] Glutamine is converted to glutamic acid during analysis, thus glutamic acid represents the sum of glutamine and glutamic acid.

NA, not analyzed

Table 3. Measurements of physical feed quality of the experimental diets used in the main trial. Differences were produced by different drying times (D96, D94, D92, D91) or by soaking in sea water for two hours (S70). Data is given as mean ± SEM.

	D96	D94	D92	D91	S70	p-value	R^2	b
Diameter, mm	10.4 ± 0.1	10.8 ± 0.1	10.7 ± 0.1	10.8 ± 0.2	10.6 ± 0.1	0.07	0.02	-0.06
Bulk density, g/l	618.1 ± 0.5	655.3 ± 0.5	673.3 ± 0.3	678.4 ± 0.1	NA	<.0001	0.91	-11.78
Hardness, N	37.7 ± 4.8	33.2 ± 3.6	52.3 ± 2.7	50.4 ± 2.8	54.3 ± 1.8	<.0001	0.15	-4.03
DORIS intact[1], %	37.9 ± 0.9	73.6 ± 0.3	93.9 ± 0.4	98.8 ± 0.1	NA	<.0001	0.92	-12.00
DORIS large fracture[1], %	30.4 ± 0.4	12.9 ± 0.5	2.6 ± 0.2	0.4 ± 0.1	NA	<.0001	0.92	5.94
DORIS small fracture[1], %	19.0 ± 0.9	7.1 ± 0.3	1.9 ± 0.1	0.5 ± 0.0	NA	<.0001	0.89	3.60
DORIS dust[1], %	11.9 ± 0.1	6.2 ± 0.3	1.6 ± 0.1	0.4 ± 0.0	NA	<.0001	0.95	2.31
Water stability 60 min[2], %	95.4 ± 0.3	95.8 ± 0.1	96.0 ± 0.2	96.3 ± 0.0	95.8 ± 0.1	0.002	0.62	-0.17
Water stability 120 min[2], %	93.6 ± 0.2	94.0 ± 0.2	94.2 ± 0.4	95.1 ± 0.1	94.7 ± 0.4	0.001	0.66	-0.27
Water stability 240min[2], %	92.5 ± 0.2	92.3 ± 0.2	92.1 ± 0.5	93.1 ± 0.2	93.0 ± 0.3	0.362	0.08	-0.09

[1] The given DORIS values are the percentage of each size fracture present in the sample after the DORIS tester. The different size fractions of DORIS values were: DORIS intact > 8.0 mm; DORIS large fracture > 5.6 mm and < 8.0 mm; DORIS small fracture > 2.4 mm and < 5.6 mm; DORIS dust < 2.4 mm.

[2] Water stability is given as remaining DM of DM at start of test (%) at 60, 120 and 240 min.

NA, not analyzed

Effect of drying time was analyzed by regression on dry diets D96, D94, D92 and D91.

Table 4. Results of Pearson's correlation analysis of measurements of physical feed quality of the experimenetal diets used in the main trial. Differences were produced by varying drying times (D96, D94, D92, D91) or by soaking in sea water for two hours (S70).

	DORIS intact	DORIS large fracture	DORIS small fracture	DORIS dust	Bulk density	Hardness	Diameter	Water stability 60min	Water stability 120min	Water stability 240min
DM	-0.959	0.958	0.944	0.977	-0.952	-0.627	0.124	-0.075	-0.511	-0.572
	0.041	0.042	0.056	0.023	0.048	0.258	0.842	0.905	0.379	0.314
DORIS intact		-1.000	-0.998	-0.996	0.999	0.719	0.845	0.964	0.833	0.195
		<.0001	0.002	0.004	0.001	0.281	0.155	0.036	0.167	0.805
DORIS large fracture			0.998	0.996	-0.999	-0.724	-0.841	-0.962	-0.830	-0.191
			0.002	0.004	0.001	0.277	0.159	0.038	0.170	0.809
DORIS small fracture				0.988	-1.000	-0.676	-0.873	-0.956	-0.817	-0.172
				0.012	0.000	0.324	0.127	0.044	0.183	0.828
DORIS dust					-0.992	-0.775	-0.799	-0.972	-0.858	-0.242
					0.008	0.225	0.201	0.028	0.142	0.758
Bulk density						0.694	0.863	0.961	0.827	0.188
						0.306	0.137	0.039	0.173	0.812
Hardness							0.365	0.739	0.806	0.371
							0.546	0.154	0.099	0.539
Diameter								0.817	0.541	0.021
								0.091	0.346	0.973
Water stability 60min									0.845	0.332
									0.072	0.586
Water stability 120min										0.750
										0.145

DM, DORIS intact, DORIS large fracture, DORIS small fracture, DORIS dust, bulk density, hardness, diameter, water stability at 60 min, water stability at 120 min and water stability at 240 min were tested pairwise. DORIS durability and bulk density were not measrued for diet S70. DORIS intact > 8.0 mm; DORIS large fracture > 5.6 mm and < 8.0 mm; DORIS small fracture > 2.4 mm and < 5.6 mm; DORIS dust < 2.4 mm

Table 5. Weight, feed intake, growth and feed conversion in Atlantic salmon fed diets of different physical feed quality *ad libitum* (period 1 of the main trial). Differences were produced by different drying times (D96, D94, D92, D91) or by soaking in sea water for two hours (S70). Data is given as mean ± SEM.

	D96	D94	D92	D91	S70	p-value (drying time)	p-value (soaking)
Initial body weight, g (all fish)	979 ± 40	939 ± 2	934 ± 12	964 ± 39	988 ± 30	0.667	0.163
Final body weight, g (all fish)	1670 ± 111	1600 ± 65	1490 ± 47	1823 ± 182	1756 ± 121	0.599	0.110
Final body weight, g (non-mature)	1964 ± 183	1728 ± 41	1687 ± 143	1964 ± 166	2018 ± 181	0.898	0.225
Feed intake per fish, g DM (all fish)	572 ± 69	546 ± 60	478 ± 44	695 ± 122	639 ± 71	0.470	0.124
Feed intake per fish, g DM (non-mature)	796 ± 122	653 ± 43	650 ± 95	828 ± 127	832 ± 103	0.807	0.395
SGR, % (non-mature)	0.73 ± 0.06	0.67 ± 0.02	0.64 ± 0.07	0.79 ± 0.06	0.75 ± 0.06	0.634	0.299
SFR (non-mature)	0.60 ± 0.05	0.55 ± 0.03	0.56 ± 0.05	0.65 ± 0.06	0.62 ± 0.04	0.678	0.357
TGC (non-mature)	2.86 ± 0.29	2.55 ± 0.10	2.42 ± 0.31	3.09 ± 0.28	2.95 ± 0.29	0.738	0.278
FCR[1]	0.83 ± 0.02	0.82 ± 0.03	0.88 ± 0.06	0.81 ± 0.02	0.83 ± 0.02	0.976	0.577

[1] Calculated as: total feed intake (DM) per tank / biomass increase of non-mature fish.

All-fish: individual body weights of both mature and non-mature were included in the calculation; non-mature fish: individual body weights of non-mature fish only were included in the calculation.

SGR, specific growth rate; SFR, specific feeding rate; TGC, thermal growth coefficient; FCR, feed conversion ratio.

Effect of drying time was analyzed by regression on dry diets D96, D94, D92 and D91 and effect of soaking was tested by ANOVA on diet D92 and S70.

Table 6. Apparent nutrient digestibility coefficients (AD, %) in Atlantic salmon fed diets of different physical feed quality *ad libitum* (period 1 of the main trial) and restricted at 0.4 % of body weight (period 2). Differences were produced by different drying times (D96, D94, D92, D91) or by soaking in sea water for two hours (S70, S73). Data is given as mean ± SEM.

	Period 1					Period 2					
	D96	D94	D92	D91	S70	D96	D94	D92	D91	S70	Period
Crude lipid	93.5 ± 0.7	95.8 ± 0.5	95.2 ± 0.7	95.4 ± 0.0	95.3 ± 0.4	93.8 ± 0.2	95.7 ± 0.3	94.6 ± 0.8	94.8 ± 0.5	94.0 ± 0.9	NS
Nitrogen	84.1 ± 0.9	86.0 ± 0.7	85.3 ± 0.2	84.5 ± 0.5	85.9 ± 0.6	85.1 ± 0.1	86.9 ± 0.3	85.7 ± 0.6	84.9 ± 0.2	86.5 ± 0.6	*
sum of amino acids	88.5 ± 0.6	89.6 ± 0.5	89.4 ± 0.2	88.8 ± 0.3	89.2 ± 0.4	89.9 ± 0.1	90.6 ± 0.1	90.2 ± 0.6	89.8 ± 0.3	90.2 ± 0.4	*
DM	62.5 ± 1.5	66.1 ± 1.0	64.8 ± 0.8	63.8 ± 0.8	65.6 ± 0.5	63.3 ± 0.5	66.4 ± 0.7	64.1 ± 0.2	63.3 ± 0.4	64.7 ± 1.1	NS
Starch	70.8 ± 0.8	71.9 ± 0.8	73.4 ± 1.5[a]	68.9 ± 0.8	67.6 ± 1.1[b]	73.1 ± 1.0	74.0 ± 0.5	74.2 ± 0.8[a]	70.8 ± 0.3	70.2 ± 0.4[b]	*
Energy	78.3 ± 1.1	80.7 ± 0.6	79.6 ± 0.7	79.2 ± 0.4	80.5 ± 0.6	79.2 ± 0.2	81.2 ± 0.4	80.0 ± 0.6	79.2 ± 0.0	80.3 ± 0.7	NS
Minerals:											
Phosphorous	25.9 ± 3.1	33.8 ± 2.6	30.0 ± 1.6	31.7 ± 3.3	27.1 ± 0.9	32.3 ± 3.5	37.9 ± 2.3	36.5 ± 2.1	35.2 ± 2.4	32.6 ± 3.1	*
Magnesium	-364.5 ± 25.9	-312.4 ± 38.2	-303.0 ± 65.5	-318.1 ± 12.6	-237.4 ± 34.0	-411.6 ± 14.8	-386.6 ± 27.6	-436.3 ± 33.1	-390.9 ± 26.0	-326.0 ± 48.1	*
Sodium	-197.6 ± 25.8	-202.6 ± 22.0	-186.9 ± 20.7[b]	-213.0 ± 3.4	-46.1 ± 11.7[a]	-127.6 ± 12.1	-130.0 ± 9.9	-120.2 ± 14.3[b]	-135.2 ± 10.8	-26.0 ± 11.3[a]	*
Iron	-15.3 ± 4.2	6.3 ± 5.1	8.9 ± 8.8	-9.3 ± 3.9	23.7 ± 7.1	-6.0 ± 12.0	-2.1 ± 1.4	-6.5 ± 4.2[b]	-12.3 ± 2.0	14.8 ± 2.6[a]	NS
Zinc	12.2 ± 4.1	19.1 ± 7.3	21.7 ± 10.3	13.4 ± 3.5	16.3 ± 6.3	10.7 ± 2.7	12.6 ± 3.8	9.6 ± 1.4	12.8 ± 2.5	12.2 ± 2.3	NS
Dispendible amino acids:											
Alanine	88.7 ± 0.4	90.0 ± 0.3	89.9 ± 0.2	89.2 ± 0.3	89.2 ± 0.3	90.1 ± 0.0	90.8 ± 0.2	90.5 ± 0.6	90.1 ± 0.1	90.2 ± 0.3	*
Aspartic acid	81.3 ± 1.1	83.5 ± 0.8	82.7 ± 0.3	81.9 ± 0.6	82.9 ± 0.8	83.3 ± 0.1	84.5 ± 0.2	83.5 ± 1.1	83.1 ± 0.6	84.1 ± 0.9	*
Cysteine	58.9 ± 2.9	62.3 ± 3.3	60.9 ± 0.8	60.6 ± 1.2	60.1 ± 2.2	67.4 ± 0.8	67.9 ± 0.5	65.1 ± 3.5	67.0 ± 1.7	66.8 ± 1.2	*
Glutamic Acid	91.9 ± 0.4	92.7 ± 0.5	92.5 ± 0.1	92.2 ± 0.2	92.4 ± 0.3	93.3 ± 0.1	93.8 ± 0.1	93.6 ± 0.5	93.2 ± 0.3	93.5 ± 0.3	*
Glycine	80.2 ± 1.2	82.6 ± 0.8	81.7 ± 0.2	81.1 ± 0.5	81.3 ± 0.8	82.6 ± 0.3	83.8 ± 0.2	83.1 ± 1.2	82.8 ± 0.6	82.9 ± 0.7	*
Proline	87.5 ± 0.9	89.1 ± 0.7	88.6 ± 0.2	87.8 ± 0.5	88.7 ± 0.5	89.4 ± 0.2	89.7 ± 0.4	89.8 ± 0.6	88.9 ± 0.3	89.4 ± 0.3	*
Serine	86.6 ± 0.6	87.9 ± 0.5	87.9 ± 0.2	87.2 ± 0.3	87.4 ± 0.5	88.4 ± 0.1	89.2 ± 0.2	88.9 ± 0.8	88.2 ± 0.2	88.7 ± 0.4	*
Taurin	-97.2 ± 7.9	-127.2 ± 10.9	-49.1 ± 19.7	-79.1 ± 18.7	-83.4 ± 16.0	-18.8 ± 6.1	-7.3 ± 3.9	19.4 ± 2.5	-25.4 ± 4.1	-20.5 ± 20.7	*
Tyrosine	90.3 ± 0.3	91.1 ± 0.2	91.2 ± 0.2	90.5 ± 0.2	91.6 ± 0.2	91.5 ± 0.2	92.3 ± 0.2	92.1 ± 0.4	91.3 ± 0.2	92.4 ± 0.2	*
Indispensible amino acids:											
Arginine	93.9 ± 0.2	94.5 ± 0.1	94.4 ± 0.1	94.1 ± 0.1	94.3 ± 0.1	94.3 ± 0.0	94.7 ± 0.2	94.5 ± 0.2	94.2 ± 0.1	94.4 ± 0.2	*
Histidine	88.5 ± 0.6	89.3 ± 0.4	89.3 ± 0.2	88.3 ± 0.4	88.7 ± 0.5	89.8 ± 0.1	90.5 ± 0.1	90.0 ± 0.7	89.5 ± 0.3	89.8 ± 0.5	*
Isoleucine	89.5 ± 0.4	90.5 ± 0.4	90.5 ± 0.2	90.1 ± 0.2	90.3 ± 0.3	90.8 ± 0.0	91.5 ± 0.2	91.2 ± 0.5	90.8 ± 0.2	91.3 ± 0.3	*
Leucine	90.4 ± 0.3	91.3 ± 0.3	91.3 ± 0.2	90.8 ± 0.2	90.9 ± 0.2	91.3 ± 0.1	92.1 ± 0.1	91.8 ± 0.4	91.3 ± 0.1	91.7 ± 0.2	*
Lysine	91.7 ± 0.4	92.4 ± 0.5	92.4 ± 0.1	92.0 ± 0.3	92.2 ± 0.3	92.1 ± 0.0	92.9 ± 0.2	92.4 ± 0.3	92.2 ± 0.2	92.4 ± 0.3	NS
Methionine	91.8 ± 0.3	92.7 ± 0.2	92.4 ± 0.2	91.7 ± 0.3	91.6 ± 0.4	92.7 ± 0.1	93.1 ± 0.1	92.8 ± 0.5	92.0 ± 0.1	92.3 ± 0.4	*
Phenylalanine	90.3 ± 0.3	91.0 ± 0.3	91.1 ± 0.3	90.4 ± 0.2	90.5 ± 0.3	91.0 ± 0.0	91.7 ± 0.1	91.5 ± 0.4	91.0 ± 0.1	91.0 ± 0.2	*
Threonine	84.2 ± 0.9	85.7 ± 0.7	85.4 ± 0.2	84.6 ± 0.4	85.1 ± 0.5	86.2 ± 0.2	87.2 ± 0.3	86.4 ± 0.8	85.5 ± 0.3	86.5 ± 0.4	*
Tryptophan	86.4 ± 0.4	86.4 ± 0.6	87.7 ± 0.5	87.4 ± 0.7	87.7 ± 0.5	87.4 ± 0.6	88.3 ± 0.5	88.9 ± 0.8	87.8 ± 0.4	88.2 ± 0.3	*
Valine	88.8 ± 0.4	89.9 ± 0.4	89.8 ± 0.2	89.3 ± 0.2	89.5 ± 0.3	90.4 ± 0.0	91.1 ± 0.2	90.7 ± 0.6	90.3 ± 0.2	90.8 ± 0.3	*

Regression analysis gave no significant effects on digestibility for the dry diets within each period.

[ab] Different letters indicate that means of D92 and S70 in the same row for each period are significantly different ($P<0.05$), testes with ANOVA for feed D92 and S70.

* indicates significant effect of period ($p<0.05$) tested by repeated ANOVA; NS, not significant.

Figures

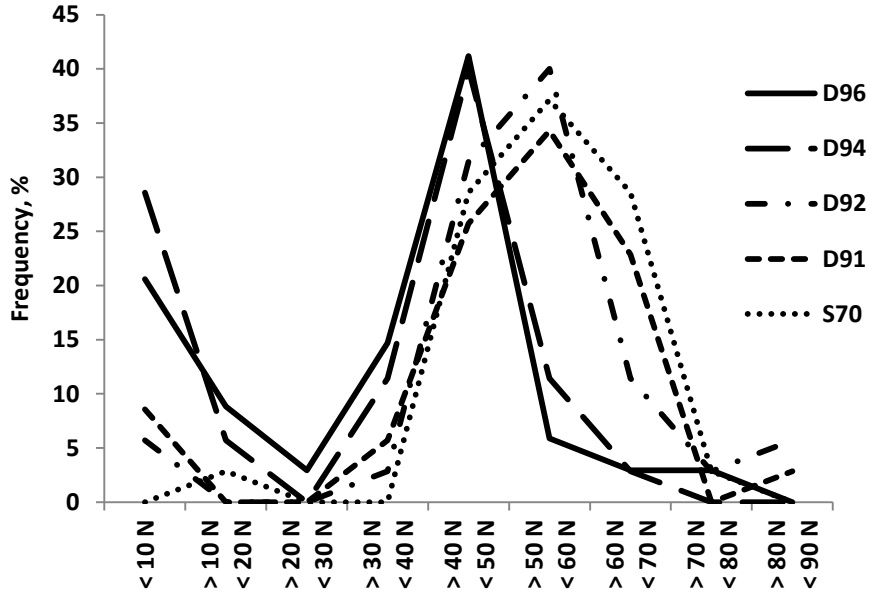


Figure 1.

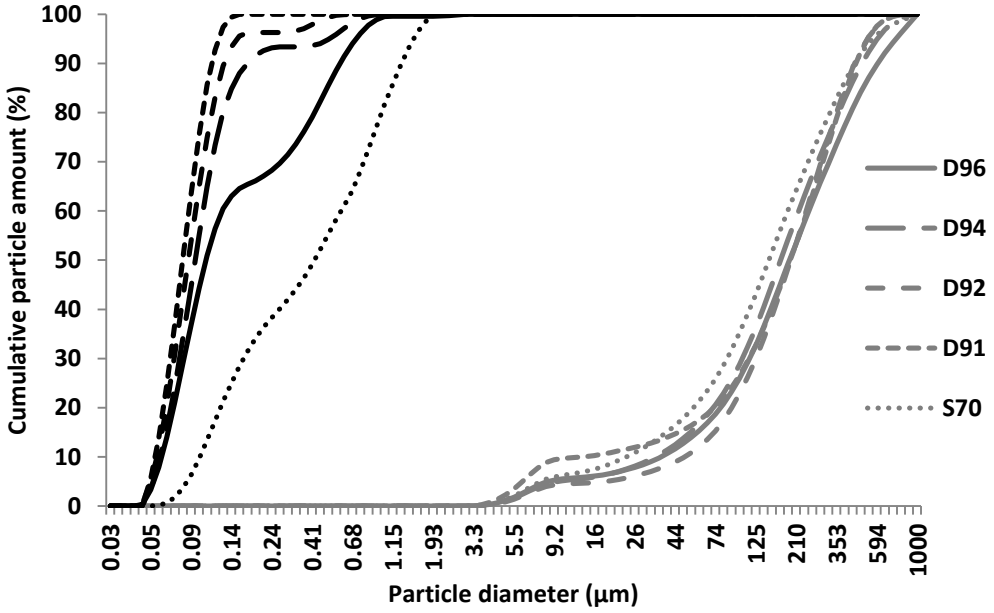

Cumulative particle amount (%)
100
90
80
70
60
50
40
30
20
10
0
0.03
0.05
0.09
0.14
0.24
0.41
0.68
1.15
1.93
3.3
5.5
9.2
16
26
44
74
125
210
353
594
1000
Particle diameter (µm)
D96
D94
D92
D91
S70

Figure 2.

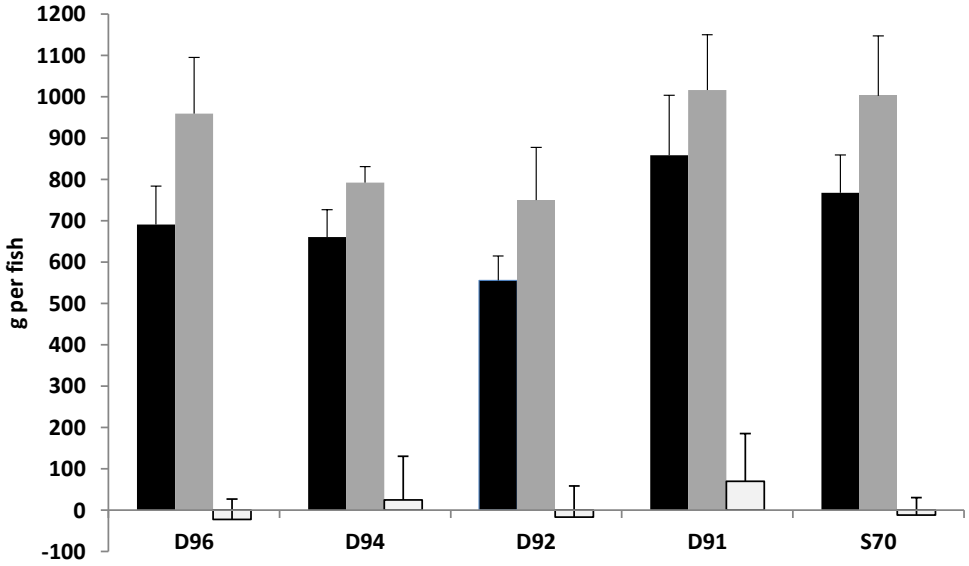

1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
g per fish
D96
D94
D92
D91
S70

Figure 3.

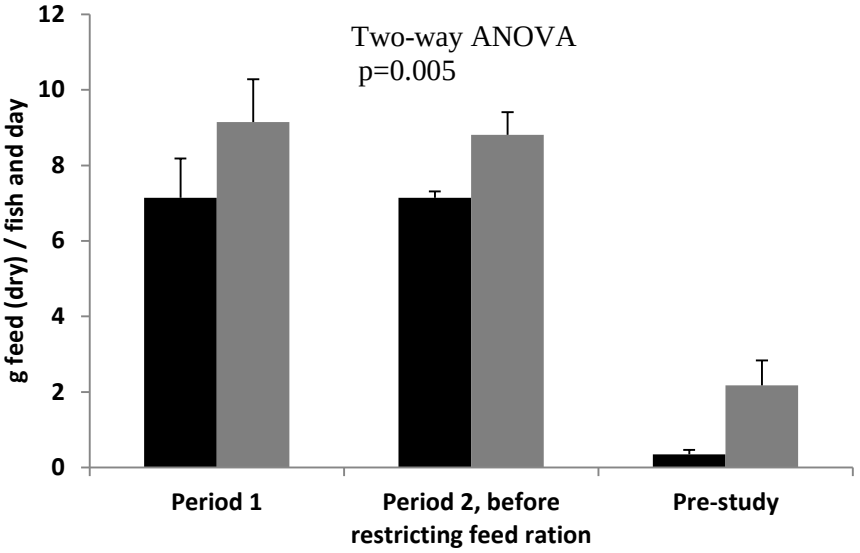
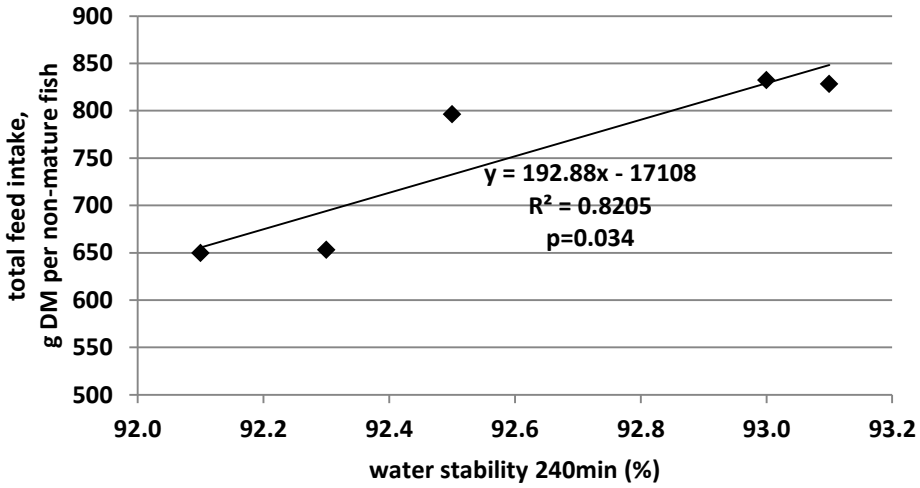

Two-way ANOVA
p=0.005
g feed (dry) / fish and day
12
10
8
6
4
2
0
Period 1
Period 2, before restricting feed ration
Pre-study

Figure 4.


900
850
800
750
700
650
600
550
500
total feed intake, g DM per non-mature fish
y = 192.88x - 17108
R² = 0.8205
p=0.034
92.0
92.2
92.4
92.6
92.8
93.0
93.2
water stability 240min (%)

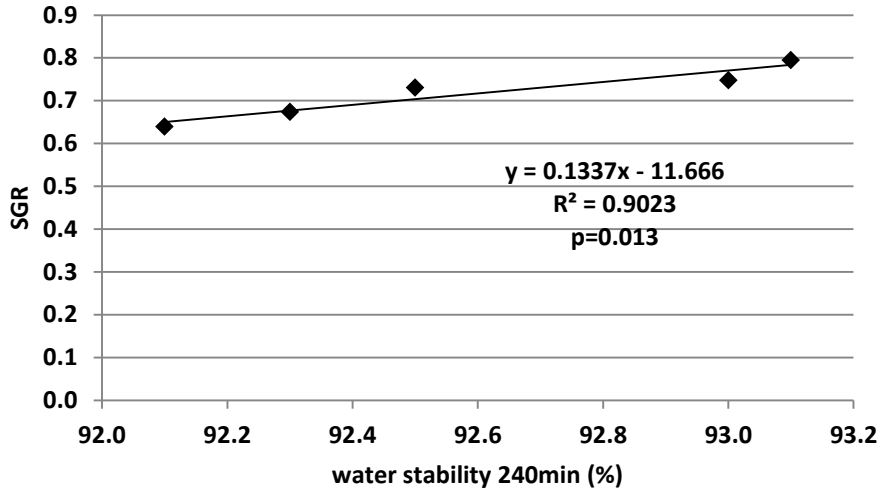

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
SGR
y = 0.1337x - 11.666
R² = 0.9023
p=0.013
92.0
92.2
92.4
92.6
92.8
93.0
93.2
water stability 240min (%)

Figure 5.

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