



Feed intake, nutrient digestibility and nutrient retention in Atlantic salmon (*Salmo salar* L.) fed diets with different physical pellet quality

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Abstract

Three feeds with different physical pellet quality but the same formulation were fed to Atlantic salmon for 97 days. Pellet quality was measured as bulk density, durability, fat leakage, hardness, water stability and porosity. The largest difference among the feeds was measured in hardness (201, 236 and 86 Newton for Diet 1, Diet 2 and Diet 3 respectively). The relative feed intake was highest in salmon fed Diet 2 and Diet 3 with no effects of pellet hardness. Growth and feed efficiency ratio were similar among all fish. The apparent digestibility of energy, dry matter, nitrogen and lipid was similar for all feeds, whereas the apparent digestibility of phosphorus and zinc was the lowest in Diet 3. Retention of energy, lipid and nitrogen was also similar for all feeds. Salmon fed Diet 1 and Diet 3 retained more phosphorus than those fed Diet 2. An increased retention of the digested zinc was found in salmon fed Diet 3. This study concludes that physical pellet quality affects feed intake and improve utilisation of feed in salmon if optimised properly. Moreover, pellet hardness can be optimised in commercial scale without compromising feed intake as it has no influence on feed intake.

Keywords: Apparent digestibility; Atlantic salmon; drinking rate; feed intake; mineral utilisation; physical feed quality

1 | INTRODUCTION

Feed represents the highest single cost factor in Norwegian salmon farming (Zahirovic 2012) and high utilisation and minimal loss of feed is important for a good profitability. The nutrients and energy in feeds are also valuable resources (Ytrestøyl *et al.* 2015) that should be utilised

effectively. Several tonnes of feed may be distributed to a cage daily in today's large salmon farming units. The systems for transport, storage and spreading of such amounts of feed challenge the physical quality of the feeds (Aas *et al.* 2011a; Oehme *et al.* 2012). Nutrient and energy losses occur due to pellet breakage and dust for-

mation during transport and storage (Aas *et al.* 2011a), but also indirectly when growth and feed utilisation in the fish is suboptimal. All these sources of loss can be affected by the pellet quality. The optimal pellet quality should therefore have properties appropriate for both transport and feeding systems and for the biology of the fish.

Pellet breakage can be measured relatively easily (Aas *et al.* 2011a), but indirect losses caused by suboptimal feed utilisation and growth are far more difficult to quantify. The feed utilisation in salmon is most effective at high feed intake (Einen *et al.* 1995, 1999; Grisdale-Helland *et al.* 2013). Feeding rainbow trout (*Oncorhynchus mykiss*) feeds with either high or low water stability resulted in more than 20% difference in feed intake, being the highest in trout fed the feed with low water stability (Aas *et al.* 2011b). With such effect on feed intake, and thus growth, there is potential for improving cost efficiency of feed for farmed salmonids by optimising the pellet quality.

The understanding of how the physical pellet quality affects the nutritional responses in fish is limited. There are studies demonstrating an interaction between physical and nutritional properties of the feed (Sveier *et al.* 1999; Baeverfjord *et al.* 2006; Venou *et al.* 2009; Aas *et al.* 2011b, 2017; Glencross *et al.* 2011a; Morken *et al.* 2011). Producing different pellet qualities only by varying drying time in the feed production, did not significantly affect feed intake in Atlantic salmon (Oehme *et al.* 2014). Soaking the feed increased feed intake, particularly in periods with low feed intake (Oehme *et al.* 2014). This indicates that there is a potential for improving the pellet quality of commercial salmon feeds. This may be particularly important when the feed intake is low, such as at outbreak of disease, after transfer of smolt to sea water and after farming routines that imply stressing of the fish.

The effect of physical pellet quality on feed intake in salmonids may be related to the rate at which the pellet disintegrates and passes through the gut (Aas *et al.* 2011b, 2017). Feed intake appears to increase when gastrointestinal passage rate increases (Aas *et al.* 2011b; 2017). The apparent digestibility of macronutrients seems to be less efficient as gastrointestinal passage rate increases (Aas *et al.* 2011b; Oehme *et al.* 2014). Pellet breakage also varies among different pellet qualities (Aas *et al.* 2011a). Pellet qualities that are optimal for the fish may produce some breakage in the logistic systems at the fish farm. Pellet breakage, feed intake, and apparent digestibility are all factors that must be considered when evaluating the physical quality of feeds for intensive aquaculture.

In the present study, Atlantic salmon were fed three feeds intended to have identical formulation but different physical pellet qualities. Feed intake, growth, apparent

digestibility and retention of nutrients and energy were measured.

2 | METHODOLOGY

2.1 Feeds

Three feeds, intended to have identical formulation but different physical pellet quality, were produced from the same feed mash and thereafter dried and coated with oil. The feeds were added yttrium oxide as an inert digestibility marker (Austreng 1978; Austreng *et al.* 2000; Hatlen *et al.* 2015). The feeds were formulated to represent commercial salmon feed (Tables 1 and 2). The difference in physical pellet quality among the feeds (Table 3) was achieved by using different process conditions in the extruder for each diet. In this setup, Diet 1 was the starting point. When producing Diet 2 extra moisture was added in the extruder, while Diet 3 was prepared by adding extra oil in the mix. Water or oil was added to change the level of gelatinisation of starch and interactions in the extruded mix compared to Diet 1. The settings in the preconditioner, drier, cooler and coater were the same for all three diets. The feeds were produced at pilot line by BioMar AS (Tech Centre, Brande, Denmark).

TABLE 1 Formulation of experimental feeds.

Ingredient	Inclusion level (g kg ⁻¹)
Fish meal, North Atlantic	99
Fish meal, South American	99
Soy protein concentrate	214
Corn gluten	79
Wheat gluten	79
Wheat	165
Fish oil	174
Rapeseed oil	75
Mono calcium phosphate	7.3
Lysine	2.6
Methionine	0.2
Yttrium oxide	0.5
Pigment/antioxidant	0.7
Premix (vitamins, minerals) ^a	6.1

^a BioMar commercial vitamin and mineral premix. Content of vitamins and minerals in feed is in accordance with requirements (National Research Council (NRC) 2011).

2.2 Fish trial

A fish trial was run in triplicate in a flow through system at Nofima's research facilities at Sunndalsøra for 99 days (7 May to 14 August 2014). Prior to the trial, the salmon were kept in a tank of 3 m diameter and 11 m³ volume. The last three weeks prior to the trial, the fish were fed 9 mm commercial feed (Skretting, Stavanger, Norway), and fasted the last two days before the trial. The water temperature was 6.3°C when the trial started.

TABLE 2 Chemical compositions of experimental feeds.

Composition	Diet 1	Diet 2	Diet 3
Dry matter (g kg ⁻¹)	938.6	938.8	928.4
In dry matter			
Crude lipid (g kg ⁻¹)	297	291	338
Nitrogen (g kg ⁻¹)	67	66	62
Ash (g kg ⁻¹)	56	57	53
Energy (MJ kg ⁻¹)	25.2	25.0	26.1
Yttrium (digestibility marker, g kg ⁻¹)	0.312	0.333	0.327
Minerals, in dry matter			
Phosphorus (mg kg ⁻¹)	9779	9920	9493
Zinc (mg kg ⁻¹)	186	193	178

Table 3 Physical properties of the feeds (mean ± standard deviation).

Properties	n	Diet 1	Diet 2	Diet 3
Diameter (mm)	20	9.1±0.3	8.9±0.2	9.8±0.3
Length (mm)	20	7.1±0.5	7.0±0.2	7.8±0.5
Bulk density (g/L)	2	692±2.0	664±4	682±0.4
Durability				
Ligno test (%)	2	97.6±1.1	98.5±0.1	98.8±0.1
DORIS test				
Particles > 9mm (%)	3	68.7±1.6	71.5±3.0	85.6±0.9
Particles 4-9 mm (%)	3	29.0±1.9	26.5±2.9	13.3±0.9
Particles 2-4 mm (%)	3	1.5±0.5	1.6±0.1	0.6±0.2
Particles < 2 mm (%)	3	0.9±0.1	0.4±0.1	0.5±0.1
Fat leakage (%)	3	8.8±0.2	6.1±0.4	7.5±0.2
Hardness (N)	20	201±33	236±53	87±19
Water stability (%)	2	87.0±0.8	82.3±1.0	82.3±5.4
Total porosity (%)	1	45.8	42.6	42.5

Atlantic salmon from the breeding nucleus of SalmoBreed AS (Gjerde *et al.* 2011) with mean initial body weight of 1.3 kg were allocated to nine 3.3 m³ tanks, aiming at 75 kg biomass per tank. The tanks were supplied with sea water (salinity 32 g L⁻¹) and continuous light. The temperature was gradually increased from 6.3 to 11°C during the first 12 days of the trial, and thereafter kept at this temperature.

The daily ration of feed was placed on disc feeders above each tank, and one daily meal was fed at 07:00 to 08:00 h. The daily ration at start of the trial was 500 g per tank. Throughout the trial the ration was adjusted individually for each tank based on the three last days feed intake, aiming at 20% overfeeding. The daily ration for one tank ranged from 200 g early in the trial to 1050 g at the end. Due to moderate feed intake in all treatment groups, the feed ration was delivered from two disk feeders in each tank from day 62, and from day 75 an additional daily meal was given at 19:30 to 20:00 h. The feed spill was collected daily at approximately 09:00 h, and the feed intake estimated according to Helland *et al.* (1996).

2.3 Sampling

The trial lasted for 99 days. Bulk weight was registered at

start and end of the trial. In addition, body weight was registered for all sampled individuals. Three replicates of 10 whole fish were sampled for chemical analysis at start of the trial, and ten fish from each tank were sampled on day 97. Each specimen of 10 fish was pooled and stored at -20°C until homogenisation for chemical analysis of whole body composition. The content of the small intestine and distal intestine of five fish from each tank was thoroughly examined for whole, undigested pellets on day 92. On day 97, faeces were sampled by dissecting out the gut and collecting the content of the distal intestine. The faeces from at least 10 fish per tank, or more if necessary for sufficient amount of sample material, were collected and pooled by tank. The sampled fish were weighed after emptying the gut. The remaining fish were fasted two days prior to bulk weight on day 99.

During handling and weighing, the fish were sedated with Aqui-S® (clove oil, isoeugenol 2 to 5 mg L⁻¹). Fish to be euthanized were given a lethal dose of Finquel MS-222 (tricaine methanesulfonate).

2.4 Chemical analyses

Feeds and freeze dried faeces were dried at 105°C to constant weight for dry matter estimation and analysed further for ash by combustion at 550°C to constant weight, crude protein by nitrogen × 6.25 (Kjeltec Auto Analyser) and crude lipid (SOXTEC hydrolysing and extraction systems). Gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter). Minerals and yttrium were analysed by inductively coupled plasma mass spectroscopy (ICP-MS, at Eurofins, Moss, Norway). The same analyses, except for measurement of dry matter and yttrium, were performed for homogenised whole fish samples.

2.5 Measurement of physical feed quality

Diameter and length of the pellets were measured with an electronic caliper. Bulk density was measured by loosely pouring the feed from a funnel into a 1000 ml measuring cylinder and recording the weight.

Mechanical pellet durability was measured in a Ligno tester (LT-II, Borregaard Lignotech, Sarpsborg, Norway). Samples of 100 g feed without dust or broken pellets were placed in the Ligno tester which was run for 90 seconds. Subsequently, the samples were sieved (8.0 mm sieve) and intact pellets weighed. Durability (%) was calculated as the per cent of sample that was intact after the test.

Doris Durability Index (DDI) was measured in an AkvaMarina DORIS Feed Tester (Aquasmart ASA, Bryne, Norway). Pre-sieved samples of 350 g pellets were put into the inlet of the DORIS Feed Tester, conveyed by a screw onto a rotating paddle, and collected in an accumulation box at the end. The samples were then carefully sieved on three sieves (9.0, 4.0 and 2.0 mm) to measure the amount of

whole pellet (> 9.0 mm), fracture (2.0 to 9.0 mm), and fines (< 2.0 mm). The DDI is given as the percentage of pellets in each category.

Fat leakage was measured as the loss of fat from the feed. Samples of 20 g feed were placed in a plastic box with blotting paper and incubated at 40°C for 24 h. Fat leakage was calculated as the % of sample that the leaked fat constituted.

Pellet breaking force (hardness) was measured on standing pellets by use of a texture analyzer (TA-HDi®, Stable Micro Systems Ltd, Surrey, UK). The speed of the load arm was set to 1 mm second⁻¹ and the penetration depth was set to 3 mm. The load arm was equipped with a cylindrical flat-ended aluminium probe (70 mm diameter). Pellets were broken individually between the probe and the bottom plate. The major break of the pellet (the peak force) was measured and given in Newton (N).

A modified version of the method of Baeverfjord *et al.* (2006) was used to measure water stability of the feeds. Twenty grams of feed were placed in a custom-made, cylindrical mesh wire container that was placed in a 600 ml beaker containing 300 ml distilled water. The beakers were shaken (100 shakings per minute, 2×4.9 cm swing distance) for 120 minutes at 23°C and remaining dry matter measured, giving the water stability as % remaining material.

The total porosity (%) was measured in one pellet from each feed with X-ray microtomography (micro CT; described in Draganovic *et al.* 2013; Figure 1). Micro CT analyses were carried out at Danish Technological Institute using a SkyScan 1172 Xray microtomography scanner (MicroCT, Kontich, Belgium) with a Hamamatsu C9300 (Naka-ku, Japan) 11-megapixel CCD camera. The pixel size was 8.8 µm, voltage 59 kV and current 167 µA. The image data were computed with SkyScan software CTAn v.1.13.2.1. using the Multilevel Otsu method at 4 threshold levels for optimal channel adjustment.

2.6 Calculations

Feed intake was estimated according to Helland *et al.* (1996).

$$\text{Feed intake (DM basis)} = \text{Feed fed (g, DM)} - \frac{\text{Waste feed (g, DM)}}{\text{Recovery}}$$

where Recovery = $\frac{\text{Feed spill (g, DM)}}{\text{Feed used (g, DM)}}$, estimated by following the experimental feeding routines, but with no fish in the tanks. DM = Dry matter.

$$\text{Weight gain (\%)} = 100 \times \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}}$$

Relative feed intake (% of body weight per day

$$= 100 \times \frac{\text{Feed intake (g, DM)}}{\text{Days fed} \times \left[\frac{\text{Initial weight (g)} + \text{Final weight (g)}}{2} \right]}$$

Feed efficiency ratio

$$FER = \frac{\text{Weight gain (g)}}{\text{Feed intake (g, DM)}}$$

Specific growth rate (%)

$$SGR = \frac{100 \times [\ln(\text{final weight}) - \ln(\text{Initial weight})]}{\text{Days fed}}$$

Thermal growth coefficient

$$TGC = 1000 \times \frac{\text{Final weight}^{\frac{1}{3}} - \text{Initial weight}^{\frac{1}{3}}}{\text{Sum daydegrees}}$$

Apparent digestibility (AD, as %) of nutrients and energy were calculated as

$$AD = 100 \times \frac{a - b}{a}$$

where *a* represents the nutrient to marker ratio in feed, and *b* represents the nutrient to marker ratio in faeces.

Nutrient retention (% of ingested or digested)

$$= \frac{100 \times [\text{Nutrient at end (g)} - \text{Nutrient at start (g)}]}{\text{Nutrient ingested or digested (g)}}$$

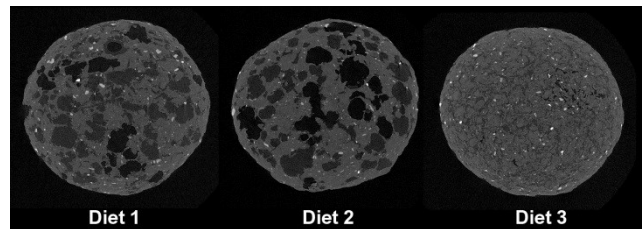


FIGURE 1 Micro CT scan of one pellet from Diet 1 to Diet 3. Black spots within the pellet represent air while white, light grey and dark grey represent bone fragments, pellet structure and oil residues respectively. The total porosity (%; Table 3) was similar in all feeds, but Diet 2 had larger pores and Diet 3 smaller pores compared to Diet 1.

2.7 Statistical analysis

Tank was used as the statistical unit. Unless otherwise specified, data are given as mean ± S.E.M. Data were analysed with ANOVA. Differences were considered significant if $P \leq 0.05$. If $0.05 < P < 0.1$, this was reported as a trend. If significant, comparisons among treatment means were analysed using Duncan's multiple range test. Statistical analyses were performed with the SAS computer software (SAS 1985, SAS Institute Inc, Cary, USA).

3 | RESULTS

3.1 Growth and feed intake

During the first month of the trial, the feed intake in all tanks was poor, but increased gradually. There were no significant differences among groups in feed intake when calculated as g feed eaten per individual (Table 4). The relative feed intake, which expresses feed intake as % of body weight per day, was significantly higher in salmon fed Diet 2 and Diet 3 than in those fed Diet 1. The overall mean of specific growth rate (SGR) in the trial was 0.47%.

There was no significant effect of physical feed properties on weight, weight gain, SGR or thermal growth coefficient (TGC). Numerically, growth corresponded with relative feed intake was the highest in salmon fed Diet 2 and Diet 3. The feed efficiency ratio (FER) was similar in all treatment groups (Table 4).

TABLE 4 Body weight, growth, feed intake and feed utilisation in Atlantic salmon fed three diets with different physical properties. The fish were fed for 96 days. Data are given as mean ± S.E.M. (*n* = 3).

Measurement	Diet 1	Diet 2	Diet 3	P-value
Initial weight (g)	1363±10	1339±4	1326±22	0.263
Final weight (g)	2 094±44	2 119±40	2 109±47	0.920
Weight gain (g)	731±41	780±38	783±33	0.578
SGR (% per day)	0.45±0.02	0.48±0.02	0.48±0.02	0.368
TGC	1.69±0.08	1.81±0.07	1.82±0.06	0.440
Individual feed intake (g DM)	684±18	747±20	732±18	0.120
Relative feed intake (DM, % of body weight day ⁻¹)	0.41±0.01 ^b	0.45±0.01 ^a	0.44±0.01 ^a	0.044
FER	1.07±0.03	1.04±0.02	1.07±0.03	0.774

DM, dry matter; SGR, specific growth rate; TGC, thermal growth coefficient; FER, feed efficiency ratio; ^{a, b}, significant differences (*P* ≤ 0.05) of means within a row are indicated by different letters

3.2 Apparent digestibility

The apparent digestibility (AD; Table 5) of dry matter, energy, lipid and nitrogen was similar for all feeds. The AD of dry matter ranged from 72.1% (Diet 3) to 73.1% (Diet 2). The overall mean AD of energy, lipid and nitrogen was 87.6%, 96.2% and 89.6% respectively. The AD of phosphorus was significantly higher in Diet 1 (47.5 ± 0.9%) and Diet 2 (45.4 ± 1.4%) than in Diet 3 (40.7 ± 1.6%). The AD of zinc was also higher in Diet 1 (41.6 ± 2.3%) and Diet 2 (43.2 ± 0.6%) compared to Diet 3 (36.3 ± 0.9%; Table 5).

The AD of ash was below zero, which is expected as fish drink sea water containing ions. There was a trend (0.05 < *P* < 0.1) to larger negative AD value of ash in salmon fed Diet 3 compared to those fed Diet 1 and Diet 2. No undigested pellets (whole or kernels of pellets) were found in the intestinal content of the fish.

3.3 Retention

There were no significant differences in retention of the ingested energy, lipid, nitrogen or zinc among salmon fed the three different diets (Table 6). The overall mean retention of ingested energy, lipid, nitrogen and zinc was 47.2%, 45.1%, 48.9% and 22.5% respectively. The retention of ingested phosphorus was significantly higher in salmon fed Diet 1 (41.9 ± 1.7%) and Diet 3 (34.2 ± 3.0%) than in those fed Diet 2 (22.8 ± 0.4%). The retention of digested energy, lipid and nitrogen was also similar among the groups (Table 6). The overall mean retention

of digested energy, lipid and nitrogen was 55.8%, 46.9% and 54.6% respectively. Salmon fed Diet 1 and Diet 3 retained more phosphorus (88.2 ± 3.8 and 84.8 ± 9.8% respectively) than salmon fed Diet 2 (51.5 ± 2.8%). The amount retained from the digested zinc was significantly higher in salmon fed Diet 3 (71.9 ± 8.9%) than in salmon fed Diet 2 (43.1 ± 5.6%) with intermediate values in those fed Diet 1 (54.4 ± 1.1%; Table 6).

TABLE 5 Apparent digestibility (%) of dry matter, energy and main nutrients in Atlantic salmon fed three diets with different physical properties. Data are given as mean ± S.E.M. (*n* = 3).

Measurement	Diet 1	Diet 2	Diet 3	P-value
Dry matter	72.9±1.3	73.1±0.5	72.1±0.3	0.716
Energy	84.6±0.8	84.6±0.2	84.8±0.1	0.936
Lipid	96.3±0.3	96.2±0.2	96.0±0.1	0.677
Nitrogen	90.1±0.8	89.9±0.2	89.0±0.1	0.296
Ash	-9.4±5.8*	-9.1±4.1*	-25.0±2.8*	0.070
Phosphorus	47.5±0.9 ^a	45.4±1.4 ^a	40.7±1.6 ^b	0.026
Zinc	41.6±2.3 ^a	43.2±0.6 ^a	36.3±0.9 ^b	0.034

^{a, b}, significant differences (*P* ≤ 0.05) of means within a row are indicated with different letters; *, a trend (0.05 < *P* ≤ 1)

TABLE 6 Retention (%) of ingested and digested energy and main nutrients in Atlantic salmon fed three diets with different physical properties. Data are given as mean ± S.E.M. (*n* = 3).

Properties	Diet 1	Diet 2	Diet 3	P-value
Retention of ingested material				
Energy	47.8±1.3	45.2±1.9	48.7±4.5	0.689
Lipid ¹	47.2±4.5	45.3±0.9	42.8±2.1	0.585
Nitrogen	48.9±2.4	47.1±1.0	50.8±0.8	0.334
Phosphorus ²	41.9±1.7 ^a	22.8±0.4 ^b	34.2±3.0 ^a	0.007
Zinc	22.6±1.0	18.6±2.2	26.2±3.8	0.197
Retention of digested material				
Energy	56.5±1.0	53.5±2.3	57.5±5.3	0.698
Lipid ¹	49.0±4.5	47.1±1.0	44.5±2.2	0.590
Nitrogen	54.3±2.5	52.4±1.1	57.1±0.9	0.219
Phosphorus ²	88.2±3.8 ^a	51.5±2.8 ^b	84.8±9.8 ^a	0.038
Zinc	54.4±1.1 ^{ab}	43.1±5.6 ^b	71.9±8.9 ^a	0.042

¹, lipid retention includes lipid from non-lipid precursors; ², *n* = 2 in Diet 2. One of the replicates of phosphorus concentration in whole fish fed Diet 2 was excluded because the analysed value was considered too high to be reliable (4560 mg kg⁻¹ as opposed to mean value 3325 mg kg⁻¹ in fish from the other tanks). This was assumed to be an analytical error for this particular sample; ^{a, b}, significant differences (*P* < 0.05) of means within a row are indicated by different letters

4 | DISCUSSION

There was no mortality in the trial and fish appeared to be at good health. The feed intake was poor during the first month but increased gradually throughout the trial.

In the first part of this period fish were acclimatised to increasing temperature from 6.3 to 11°C. Due to the initial low feed intake, the total feed intake of the salmon was lower than expected. Correspondingly, the overall growth of the salmon was 0.47% per day, which is below expected values (Austreng *et al.* 1987; Skretting 2011). According to Austreng *et al.* (1987) and feeding tables given by Skretting (2011), salmon of 1300 g is expected to grow 0.90% per day at 11°C. The fish was weighed at start and end of the trial and growth during separate periods could not be measured. But the daily feed intake could be used to estimate growth at different periods. Assuming the FER was constant during the trial, mean SGR would be 0.23% for the first 30 days of the trial and 0.58% for the remaining 66 days. However, since feed utilisation is expected to be the highest at high feed intake (Einen *et al.* 1995, 1999; Grisdale-Helland *et al.* 2013), the FER was probably not constant during the trial. This implies that the true SGR would be lower than 0.23% during the initial period with low feed intake and higher than 0.58% during the last part of the trial. The real SGR for the last period can thus be assumed to be closer to the expected values.

The physical properties of feeds depend both on the feed ingredients and the processing conditions (Sørensen *et al.* 2009; Glencross *et al.* 2010; Draganovic *et al.* 2011; Kraugerud *et al.* 2011; Kraugerud and Svihus 2011; Morken *et al.* 2012; Samuelsen *et al.* 2013, 2014; Oterhals and Samuelsen 2015). An infinite number of pellet qualities can be achieved and producing a feed with predetermined physical properties is challenging even for the most experienced operators. In commercial feed production, different process conditions and levels of macronutrients in the different parts of the process lines are actively used to control physical pellet quality. The physical properties of the feed are also influenced by other factors including addition of steam or moisture, addition of slurry with moisture and/or macronutrient like starches, oils or other components to the dry mix, preconditioner, extruder and coater. Several methods are used to measure and describe the physical pellet quality (Winowisky 1995; Thomas and van der Poel 1996; Kaliyan and Vance Morey 2009; Sørensen 2012). The various methods measure the feed's ability to withstand different forces, but none of the commonly used methods can predict very well how the feed will withstand the forces in a pneumatic feeding system at the fish farm (Aas *et al.* 2011a). Durability measurements are assumed to be the best methods currently available to predict the pellets' durability in feeding systems. Consequently, high durability is used as a desirable property of commercial salmonid feeds.

In the present trial, different expansion of the pellets resulted in higher lipid absorption in Diet 3 during coating, and thus higher lipid content in this feed than in Diet 1 and Diet 2. Correspondingly, the energy content was also higher and the nitrogen content somewhat lower in Diet

3 than other diets. Except for this, the chemical composition was similar among all feeds. Ideally, all feeds should be identical in composition. Due to the complexity of the extrusion process the differences in physical pellet quality that can be achieved while at the same time have identical composition of the feeds, are limited. To be able to test different pellet qualities, some variation in composition has to be accepted.

The largest effect of feed processing on pellet quality was found in measured hardness, ranging from 87 N in Diet 3 to 236 N in diet 2. The measured water stability was lower in Diet 2 and 3 (82.3% remaining material for both) than in Diet 1 (87.0% remaining). The difference in water stability was not very large, but a difference in visual appearance of the feeds was evident. Visually, Diet 3 had larger pellets and lighter brown colour than the two other diets. After two hours shaking in water bath in the water stability test, the pellets of Diet 1 appeared intact, the pellets of Diet 2 had become smaller with rounded edges and signs of attrition, whereas pellets of Diet 3 were swollen and greyish. Interestingly, Diet 3, which had the lowest hardness and the lowest water stability (together with Diet 2), was most durable according to the DORIS test. Diet 3 had slightly larger pellets than the two other diets, which may affect the DORIS measurements, particularly the largest size fraction (particles > 9 mm). The total breakage in the DORIS test was 31.3, 28.5 and 14.4% for Diet 1, Diet 2 and Diet 3 respectively, confirming that the highest DORIS durability was found in Diet 3. Measured with the Ligno test, Diet 2 and Diet 3 were most durable. The fat leakage was the highest in Diet 1 and the lowest in Diet 2. Regarding feeding systems, where high pellet durability and low fat leakage is required, Diet 2 (with lowest fat leakage) or Diet 3 (with the highest durability) seemed to be the most desirable. Diet 1 had the least desirable physical pellet quality among these three diets.

Micro CT scans showed different pore size in the three feeds produced with different process parameters. Diet 2, produced with extra moisture in the extruder, had large pore size compared to Diet 1. Diet 3, produced with increased oil in the mix, had smaller pores in the pellet. Measurement of pore size and total porosity may be a valuable addition to the standard methods for characterisation of feeds and may contribute to better prediction of the feed's properties in the logistic systems and how the fish will utilise it.

There is limited knowledge available about how the pellet quality affects the feed utilisation in fish, and the existing data are somewhat conflicting (reviewed by Sørensen 2012). It has been shown that the water stability of the feed did not affect feed intake in rainbow trout significantly (Baeverfjord *et al.* 2006). While another study the feed intake was more than 20% higher in rainbow trout fed a diet with low water stability compared to a diet with

high water stability (Aas *et al.* 2011b). In accordance with the latter study, low water stability was found to affect feed intake positively in study with Atlantic salmon (Oehme *et al.* 2014). This may be related to increased passage rate of stomach content when feed disintegrates easily (Aas *et al.* 2017). In the present study, all feed intake and growth estimates were numerically higher for salmon fed Diet 2 and Diet 3 than those fed Diet 1, although the difference was only significant when expressed as relative feed intake. The higher relative feed intake in Diet 2 and Diet 3 corresponded with the lower water stability in these diets. The initial poor feed intake and hence an overall low feed intake, resulted in a total average weight increase of 57% in the trial. This is somewhat low to achieve the power necessary to demonstrate existing differences in growth and feed efficiency among groups. In a longer trial, the differences in feed intake and growth might have become clearer. In accordance with previous studies (*e.g.* Aas *et al.* 2011b; Oehme *et al.* 2014), the feed intake in the present study was highest in salmon fed the diets with lowest water stability (Diet 2 and Diet 3). Interestingly, these diets also had higher durability than Diet 1, showing that increasing pellet durability does not necessarily reduce feed intake. The hardness of Diet 2 and Diet 3 were measured to 236.15 and 86.84 N respectively, which is a considerable difference in hardness. It should be noted that feeds can be produced with an infinite range of physical pellet qualities, which can be measured with several different methods. In the present study one can only compare the feed qualities tested, and it is difficult to make general conclusions based on one study. However, the data clearly show that pellet hardness does not necessarily affect feed intake.

The apparent digestibility (AD) of phosphorus and zinc was different across diets. Previous data have also showed that mineral digestibility in rainbow trout can be affected by pellet quality (Aas *et al.* 2011b). In accordance with the present study, the digestibility of minerals, but not that of main nutrients, was affected by pellet quality in rainbow trout. In that study, the difference in feed intake was large and the effect of pellet quality and feed intake could not be separated (Aas *et al.* 2011b). Oehme *et al.* (2014) showed that apparent nutrient digestibility was negatively affected by increased feed intake. In the present study, the AD of phosphorus and zinc was significantly different between Diet 2 and Diet 3. Feed intake was similar for these two diets. The AD of these minerals was the highest in Diet 1 and Diet 2, which had the hardest pellets. To measure the real AD of minerals from feed, the minerals must be fed at sub-optimal levels as fish. This study was not designed to measure AD of minerals and these data should therefore be used with care. The data do however indicate that mineral absorption is affected by the physical properties of the feed. Due to fish welfare as well as optimising use of limited resources

such as phosphorus, this needs to be further elucidated.

The AD for certain minerals and for ash cannot be estimated when fish is kept in sea water as fish absorb high concentration of ions from water. The high negative AD values of ash in salmon fed Diet 3 indicate a high drinking rate in these fish compared to those fed Diet 1 and Diet 2. Increased intake of sea water increases the sodium load in the fish, which in certain situations may affect fish health negatively. For example, during transfer to sea water, the physical quality of the feed may have a considerable impact on fish health.

Retention of energy, lipid and nitrogen was not significantly affected by physical pellet quality. The mineral retention was different among the diets. This can be ascribed to differences in feed intake and AD, with increased efficiency of mineral retention at low feed intake and/or low AD. The lowest AD of zinc was found in salmon fed Diet 3, which had the most efficient retention of digested zinc. The highest retention of phosphorus was found in salmon fed Diet 1, with lowest feed intake, and in those fed Diet 3, with the lowest AD of phosphorus. As for growth data, a longer trial might have been advantageous to develop clearer differences in body composition and retention data.

5 | CONCLUSIONS

Physical pellet quality can have significant effect on feed intake in Atlantic salmon. Among the three feed qualities tested, the highest feed intake was found in salmon fed the two diets with highest durability, lowest fat leakage and lowest water stability, whereas pellet hardness did not affect feed intake. The pellet quality did not affect the apparent digestibility of energy, dry matter, nitrogen and lipid, whereas the apparent digestibility of phosphorus and zinc was significantly affected by pellet quality. The mineral retention varied among the diets, probably due to differences in feed intake and mineral digestibility.

Reproducing the exact same feed qualities is not possible and the results from this trial are only valid for the specific feeds tested. But as a general conclusion, the study showed that physical pellet quality affects feed intake. To improve the utilisation of commercial salmon feeds, physical feed properties that promote high feed intake need to be defined. Pellet hardness is an important feature for the utility of feed in today's logistic systems. In this trial, pellet hardness did not affect feed intake, indicating that feeds can be produced with certain hardness level without compromising feed intake in salmon.

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CONFLICT OF INTEREST

Three of the authors are employed by the industry, one feed company and one salmon farming company. Their respective companies are given in the authors' affiliations. We consider their participation not to have biased the results or interpretation of data. The remaining three authors are employed by a research institute and have no economic or other conflicts of interests.

DATA AVAILABILITY STATEMENT

The data supporting the study are available on request from Turid Synnøve Aas or Hanne Jorun Sixten.

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