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Feed intake and nutrient digestibility and retention in Atlantic salmon fed diets with different physical pellet quality

A CREATE project

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Summary/recommendation:

Three extruded salmon diets (9 mm) were produced with the same formulation but different physical pellet qualities. Pellet quality was measured in terms of bulk density, durability (Ligno and DORIS test), fat leakage, hardness and water stability. The largest difference among the feeds was found in hardness, which was measured to 201 N, 236 N and 86 N, respectively, for Diet 1, Diet 2 and Diet 3. The water stability, which in previous trials has seemed to affect feed intake, was 87.0 % for Diet 1 and 82.3 % for both Diet 2 and Diet 3.

The three feeds were fed to Atlantic salmon with start weight 1.3 kg for three months. The relative feed intake (% of body weight per day) was significantly higher in salmon fed Diet 2 and Diet 3 than of those fed Diet 1. There were no significant differences in growth or feed conversion ratio. The apparent digestibility of energy, dry matter, nitrogen and fat was similar for all feeds, whereas the apparent digestibility of phosphorus and zinc was highest in Diet 1 and 2.

No significant differences in retention of ingested nutrients were found, whereas salmon fed Diet 3 retained significantly more Zn than those fed Diet 2, with those fed Diet 1 in an intermediate position.

In conclusion, feed intake in Atlantic salmon was different among diets with different pellet quality. The highest feed intake was found in salmon fed the diets with highest durabilities (Ligno and DORIS test), lowest water stabilities and lowest fat leakages, whereas pellet hardness did not affect feed intake. The digestibility of P and Zn, and the retention of digested Zn, was affected by the pellet quality. The measured parameters of physical pellet quality did not explain the significant differences that were found in feed intake and mineral utilisation in the salmon.

Summary/recommendation in Norwegian:

Atlantisk laks med startvekt 1.3 kg ble fôret med tre fôr med lik formulering, men ulik pelletkvalitet, i tre måneder. Det var signifikant forskjell i relativt fôrinntak (% av kroppsvekt per dag), og det høyeste fôrinntaket ble funnet hos fisk som fikk fôrene med lavest vannstabilitet. Det var stor forskjell i hardhet mellom fôrene, men dette påvirket ikke fôrinntaket i dette forsøket. Det var ingen signifikante forskjeller i vekst eller fôrfaktor.

I forsøket ble det funnet at pelletkvaliteten påvirker den tilsynelatende fordøyeligheten av fosfor og sink. Det var også signifikant effekt av pelletkvalitet på retensjon av fordøyd sink.

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1 Introduction

Feed represents the highest single cost factor in Norwegian salmon farming. Consequently, high utilization and minimal losses of feed is important for a good profitability. Nutrient and energy loss occurs both due to pellet breakage and dust formation during transport and storage (Aas et al., 2011a), and due to suboptimal growth and feed utilization. All these loss factors can be affected by the pellet quality. The optimal pellet quality must therefore have properties appropriate for both transport and feeding systems and for the fish.

Pellet breakage may be discovered visually and can be measured relatively easily, whereas loss due to suboptimal growth is far more difficult to measure during production. The strong effect of difference in feed intake on nutrient and energy retention was clearly demonstrated by Grisdale-Helland et al. (2013). In another study, feeding rainbow trout with feeds with either high or low water stability resulted in more than 20 % difference in feed intake, being 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 improving the pellet quality.

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). However, 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, and that this may be particularly important when the feed intake is low, such as at outbreak of disease and at transfer to sea water.

Studies have shown that the effect of pellet quality on feed intake may be related to the rate at which the pellet disintegrates and passes through the gut (Aas et al., 2011b; Aas et al., 2014; Aas et al., 2013). Although feed intake appears to increase when gut evacuation rate increases, the apparent digestibility of macronutrients seems to be less efficient as gut evacuation rate increases (Aas et al., 2011b; Oehme et al., 2014). Furthermore, pellet breakage varies between different pellet qualities (Aas et al., 2011a), and the pellet quality that is optimal for the fish may produce some breakage in the logistic systems at the fish farm. Thus, pellet breakage, feed intake, and apparent digestibility are factors that all must be considered when evaluating the physical feed quality.

In the present study, Atlantic salmon was fed three feeds with different physical feed quality. Feed intake, growth and apparent digestibility and retention of nutrients were measured, and the morphology of the gut was evaluated.

2 Materials and methods

2.1 Feeds

Three feeds with different physical pellet quality were produced from the same feed mash and thereafter coated with oil (BioMar Tech Centre, Brande, Denmark). The difference in physical pellet quality was achieved by changing process conditions in the extruder for each diet. The settings in the pre-conditioner, drier, cooler and coater were the same for all three diets. The formulation of the feeds, the chemical composition and the physical properties of the feeds are shown in Table 1, 2 and 3.

Table 1 Formulation of experimental feeds. The same recipe was used for Diet 1, Diet 2 and Diet 3.

Ingredient	**Inclusion level (%)**
Fish meal, North Atlantic	9.9
Fish meal, South American	9.9
Soy protein concentrate	21.4
Corn gluten	7.9
Wheat gluten	7.9
Wheat	16.5
Fish oil	17.4
Rapeseed oil	7.5
Mono calcium phosphate	0.73
Premix 1 (vitamins, minerals)	0.42
Premix 2 (amino acids, pigment, yttrium oxide)	0.59

Table 2 Chemical composition of experimental feeds.

	Diet 1	**Diet 2**	**Diet 3**
Dry matter,%	93.9	93.9	92.8
In dry matter, % or MJ/kg:			
Fat	29.7	29.1	33.8
Nitrogen	6.7	6.6	6.2
Crude protein (Nx6.25)	41.5	41.2	39.0
Ash	5.6	5.7	5.3
Energy	25.2	25.0	26.1
Yttrium (digestibility marker)	0.0312	0.0333	0.0327
Minerals, in dry matter, ppm:			
P	9779	9920	9493
Ca	9918	9807	9330
Mg	1607	1657	1584
Na	4345	3921	4291
Fe	150	148	150
Mn	33.0	35.7	30.2
Zn	186	193	178
Cu	6.3	6.5	6.4

Table 3 Physical properties of the feeds.

	n	Diet 1	Diet 2	Diet 3
Measured at Nofima's laboratory:				
Diameter (mm)	20	9.05 SD=0.32	8.94 SD=0.21	9.76 SD=0.28
Length (mm)	20	7.05 SD=0.48	6.99 SD=0.20	7.75 SD=0.53
Bulk density (g/L)	2	691.5 SD=1.8	663.7 SD=3.7	682.4 SD=0.4
Fat leakage (%)	3	8.8 SD=0.2	6.1 SD=0.4	7.5 SD=0.2
Water stability (%)	2	87.0 SD=0.8	82.3 SD=1.0	82.3 SD=5.4
Durability				
Ligno test (%)	2	97.6 SD=1.1	98.5 SD=0.1	98.8 SD=0.05
DORIS test				
Particles >9mm (%)	3	68.7 SD=1.6	71.5 SD=3.0	85.6 SD=0.9
Particles 4-9 mm (%)	3	29.0 SD=1.9	26.5 SD=3.0	13.3 SD=0.9
Particles 2-4 mm (%)	3	1.5 SD=0.5	1.6 SD=0.1	0.6 SD=0.2
Particles <2 mm (%)	3	0.8 SD=0.1	0.4 SD=0.1	0.5 SD=0.1
Hardness (N)		201.4 SD=32.9	236.1 SD=52.6	86.8 SD=18.9
Measured at BioMar's laboratory:				
DORIS test				
Particles >8mm (%)	1	92	93	98
Hardness (N)	20	71.6 SD=10.8	98.7 SD=18.8	62.8 SD=8.3

2.2 Fish trial

Prior to the trial, the fish was kept in a tank with diameter 3 m and 11 m^3 water volume, and the water temperature was 6.3 °C when the trial started. The last three weeks prior to the trial, the fish was fed 9 mm commercial feed (Skretting, Stavanger, Norway), and then fasted the last two days before the start of the trial.

Atlantic salmon from the breeding nucleus of SalmoBreed AS (Gjerde et al., 2011) with mean initial body weight of 1.3 kg was placed in the tanks on May 7th 2014, with approximately 75 kg biomass per tank. The fish trial was run in triplicate in 3.3 m^3 tanks supplied with sea water (salinity 32 ‰) in a flow through system at Nofima's research facilities at Sunndalsøra for 98 days. 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 a disc feeder placed above the tank, and one meal was fed daily, from 07:00-08:00 h. Due to moderate feed intake in all treatment groups, the feed ration was delivered from two disk feeders in each tank from July 7th, and from July 20th, an additional daily meal was given at 19:30-20:00 h. The feed spill was collected daily at approximately 09:00, and the feed intake estimated according to Helland et al. (1996). The uneaten feed was collected and weighed, and dry matter measured. The recovery (%) of uneaten feed was estimated by following the same routine as in the trial, but with no fish in the tanks. The recovery for each feed was measured both for the feeding regime with one daily meal, and with two daily meals. The recovery values were used to correct the amount of uneaten feed, and daily feed intake was calculated as feed given minus corrected uneaten feed.

2.3 Sampling

To avoid the risk of reducing the feed intake during the trial, weighing and sampling was performed only at start and end of the trial. On August 6th, 5 fish from each tank was sampled and the weight of whole fish, liver, stomach, and intestine was weighted, and the content of the gut evaluated using BioMar gut evaluation system. The sampling of faeces was performed by dissecting out the gut on August 11th. The faeces from at least 10 fish per tank, or more fish if necessary to obtain 40 g faeces, were collected from the hind gut and pooled by tank. The sampled fish was weight after emptying the gut. The remaining fish was weighed on August 13th, after two days of starvation.

At start of the trial, three replicates of 10 whole fish were sampled, and on August 11th, 10 fish from each tank was sampled. Each sample of 10 fish was pooled and homogenized for chemical analysis of whole body composition.

At handling and weighing, the fish was sedated with Aqui-S® (clove oil, isoeugenol 2-5 mg/L). Fish to be euthanized, was 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 calculation, and analyzed further for ash by combustion at 550 °C to constant weight, crude protein by nitrogen x 6.25 (Kjeltec Auto Analyzer) and crude lipid (SOXTEC hydrolyzing and extraction systems). Gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter). Minerals and marker (yttrium) were analyzed 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 homogenized 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 1 000 ml measuring cylinder. The top was gently flattened before the weight was registered.

Fat leakage was measured as the loss of fat from the feed. Samples of 75 g feed were placed in plastic box with blotting paper and incubated at 40 °C for 24 h. Fat leakage (%) was calculated as the per cent of sample that the leaked fat constituted.

A modified version of the method of Baeverfjord et al. (2006) was used to measure water stability of the feeds. 20 g feed was placed in a custom-made, cylindrical mesh wire container that each was placed in a 600 ml beaker containing 300 ml distilled water (see Fig. 1). The beakers were shaken (100 shakings per minute, 2x4.9 cm swing distance) for 120 minutes at 23 °C and remaining dry matter (%) measured.

The 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 sec. Subsequently, the sample was sieved and intact pellets weighed. The 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) at Nofima's laboratory. A pre-sieved sample 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 sample was then carefully sieved on three sieves (9.0, 4.0 and 2.0mm) to measure the amount of whole pellet (>9.0mm), fracture (2.0 - 9.0mm), and fines (<2.0 mm). The DDI is given as the percentage of pellets in each category. The DORIS test was also performed at BioMar's laboratory using the same procedure, but with different sieves (10, 8 and 2.36 mm).

At Nofima's laboratory, pellet breaking force (hardness) was measured on standing pellets by use of a texture analyser (TA-HDi®, Stable Micro Systems Ltd, Surrey, UK). The speed of the load arm was set to 1 mm/sec and the penetration depth was set to 3 mm. The load arm was equipped with a cylindrical flat-ended aluminum 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 presented in Newton (N). Pellet breaking force (hardness) was also measured at BioMar's laboratory on laying pellets by use of a KAHL Pellet Hardness Tester.

Figure 1 Feed sample in a mesh wire container placed in a beaker for water stability test.

2.6 Calculations

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

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

estimated by following the experimental feeding routines, but with no fish in the tanks.

Apparent digestibility and nutrients and energy were calculated as

$$\text{Apparent digestibility (ADC, \%)} = 100 \cdot \frac{a - b}{a}$$, where a represents the nutrient to marker ratio in feed, and b represents the nutrient to marker ratio in faeces.

$$\text{Weight increase (\%)} = 100 \cdot \frac{\text{Final weight (g)} - \text{Start weight (g)}}{\text{Start weight (g)}}$$

$$\text{Relative feed intake (\% of body weight per day)} = 100 \cdot \frac{\text{Feed intake (g, DM)}}{\text{Days fed} \cdot \left[\frac{\text{Start weight (g)} + \text{Final weight (g)}}{2}\right]}$$

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

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

$$\text{Specific growth rate (SGR, \%)} = \frac{100 \cdot [\ln(\text{Final weight}) - \ln(\text{Start weight})]}{\text{Days fed}}$$

$$\text{Thermal growth coefficient (TGC)} = 1000 \cdot \frac{\text{Final weight}^{\frac{1}{3}} - \text{Start weight}^{\frac{1}{3}}}{\text{Sum day degrees}}$$

$$\text{Specific feeding rate (SFR)} = \text{SGR} \cdot \text{FCR}$$

Nutrient retention (% of ingested or digested)

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

$$\text{Dress out percentage (D\%)} = \frac{100 \cdot \text{Gutted weight (g)}}{\text{Body weight (g)}}$$

$$\text{Condition factor (CF)} = \frac{100 \cdot \text{Body weight (g)}}{\text{Body length (cm)}^3}$$

$$\text{Hepatosomatic index (HI)} = \frac{100 \cdot \text{Liver weight (g)}}{\text{Body weight (g)}}$$

Somatic indices of stomach (SSI), pyloric intestine (PISI), mid intestine (MISI) and distal intestine (DISI) were calculated the same way as HSI.

2.7 Statistical analysis

Tank data were analyzed with one-way ANOVA. Tank was used as the statistical unit for feed intake and growth data (n=3). For individual data (morphometrics, somatic indices and gut scores), all

individuals from one treatment (three tanks, and five fish per tank) were grouped and tank effect was not considered (n=15).

Unless otherwise specified, data are given as mean±S.E.M.

Differences were considered significant if $P \leq 0.05$, and if $0.05 < P < 0.1$, this was reported as a trend. If significant, comparisons among treatment means were analyzed using Duncan's multiple range test.

Statistical analyses were performed with the SAS computer software (SAS 1985, SAS Institute Inc, Cary, USA) and SPSS v.19.

3 Results

3.1 Growth and feed intake

There was no significant effect of physical feed properties on weight, weight gain, SGR or TGC (Table 4). The overall mean of SGR in the trial was 0.47 %.

During the first month of the trial, the feed intake in all tanks was poor, but increased gradually (Fig. 2). There was no significant differences among groups in feed intake when calculated as g feed eaten per individual (Table 4). The relative feed intake however, where feed intake is expressed as % of body weight per day, was significantly higher in salmon fed Diet 2 and Diet 3 than in those fed Diet 1 (Fig. 3). Similarly, the specific feeding rate (SFR) was significantly higher in salmon fed Diet 2 and 3 than in salmon fed Diet 1 (Table 4). The feed utilisation (FER or FCR) was similar in all treatment groups (Table 4).

Table 4 Weight, growth, feed intake and feed utilisation in Atlantic salmon fed three diets with different physical properties. The fish was fed for 96 days. Data are given as mean ± SEM (n=3).

	Diet 1	**Diet 2**	**Diet 3**
Start weight (g)	1 363±10	1 339±4	1 326±22
Final weight (g)	2 094±44	2 119±40	2 109±47
Weight gain (g)	731±41	780±38	783±33
SGR (% per day)	0.45±0.02	0.48±0.02	0.48±0.02
TGC	1.69±0.08	1.81±0.07	1.82±0.06
Total individual feed intake (g dry matter)	684±18	747±20	732±18
FER	1.07±0.03	1.04±0.02	1.07±0.03
FCR	0.94±0.03	0.96±0.02	0.94±0.03
SFR	0.42±0.01[b]	0.46±0.01[a]	0.45±0.01[a]

[a, b] – Significant differences (P≤0.05) of means within a row are indicated with different letters

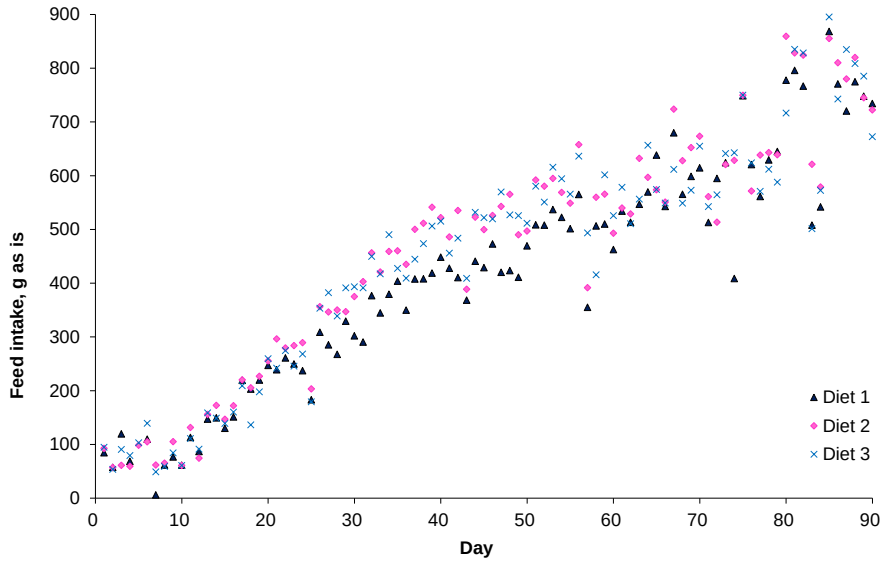

Figure 2 *Feed intake during the trial, given as g feed 'as is' eaten per tank per day (mean, n=3).*

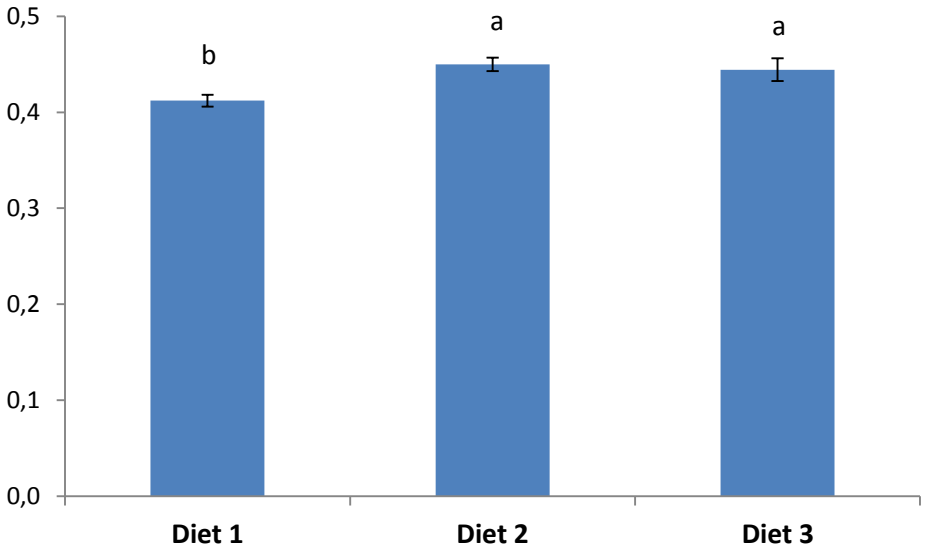

Figure 3 *Relative feed intake (% of body weight per day) in Atlantic salmon fed three diets with different physical properties. The fish was fed for 96 days. Data are given as mean ± SEM (n=3). Significant differences (P≤0.05) of means are indicated with different letters.*

3.2 Apparent digestibility (AD)

The measured AD is shown in Table 5. The AD of dry matter was similar in all feeds, and ranged from 72.1 % (Diet 3) to 73.1 % (Diet 2). The AD of energy was approximately 85 %, the AD of fat approximately 96 % and of nitrogen approximately 90 % for all feeds. The AD of P however, was significantly higher in Diet 1 and Diet 2 (47.5±0.9 and 45.4±1.4, respectively) than in Diet 3 (40.7±1.6).

Likewise, the AD of Zn was higher in Diet 1 and 2 (41.6±2.3 and 43.2±0.6, respectively) compared to Diet 3 (36.3±0.9; Table 5).

The AD of ash was below zero, which is expected as the fish drinks sea water containing ions. There was a trend (0.05<P<0.1) to higher negative AD value of ash in salmon fed Diet 3 compared to those fed Diet 1 and 2.

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 ± SEM (n=3).

	Diet 1	**Diet 2**	**Diet 3**
Dry matter	72.9±1.3	73.1±0.5	72.1±0.3
Energy	84.6±0.8	84.6±0.2	84.8±0.1
Fat	96.3±0.3	96.2±0.2	96.0±0.1
Nitrogen	90.1±0.8	89.9±0.2	89.0±0.1
Ash	-9.4±5.8*	-9.1±4.1*	-25.0±2.8*
Phosphorus	47.5±0.9[a]	45.4±1.4[a]	40.7±1.6[b]
Zinc	41.6±2.3[a]	43.2±0.6[a]	36.3±0.9[b]

[a, b] – Significant differences (P≤0.05) of means within a row are indicated with different letters

* – A trend (0.05<P<0.1)

3.3 Retention

There were no significant differences in retention of ingested nutrients among salmon fed the three different diets (Table 6). The overall mean retention of energy, fat, N, P and Zn was 47.2 %, 45.1 %, 48.9 %, 38.1 % and 22.5 %, respectively.

The retention of digested energy, fat, nitrogen and phosphorus was also similar among the groups (Table 6). The overall mean retention of energy, fat, N and P was 55.8 %, 46.9 %, 54.6 % and 85.3 %, respectively. The amount retained from the digested zinc however, was significantly higher in salmon fed Diet 3 (71.9±8.9 %) than in salmon fed Diet 2 (43.1±5.6 %) with those fed Diet 1 in an intermediate position (54.4±1.1 %; Table 6).

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 ± SEM (n=3).

	Diet 1	Diet 2	Diet 3
Retention of ingested material:			
Energy	47.8±1.3	45.2±1.9	48.7±4.5
Fat	47.2±4.5	45.3±0.9	42.8±2.1
Nitrogen	48.9±2.4	47.1±1.0	50.8±0.8
Phosphorus	41.9±1.7	38.2±15.4	34.2±3.0
Zinc	22.6±1.0	18.6±2.2	26.2±3.8
Retention of digested material:			
Energy	56.5±1.0	53.5±2.3	57.5±5.3
Fat	49.0±4.5	47.1±1.0	44.5±2.2
Nitrogen	54.3±2.5	52.4±1.1	57.1±0.9
Phosphorus	88.2±3.8	82.9±31.4	84.8±9.8
Zinc	54.4±1.1^{ab}	43.1±5.6^{b}	71.9±8.9^{a}

$^{a, b}$ – Significant differences (P<0.05) of means within a row are indicated with different letters

3.4 Morphometrics, gut scores and somatic indices

Based on 5 fish per cage (15 fish per diet), the measured condition factor (CF), dressout percentage (D %), hepatosomatic index (HSI) and somatic indices for stomach, pyloric intestine, mid intestine and distal intestine (SSI, PISI, MISI and DISI) is shown in Table 7. In addition, body length (cm), body weight (g) and gutted weight (g) for these fish are listed in table.

The condition factor ranged from 1.46 (Diet 1) to 1.53 (Diet 2) and salmon fed Diet 1 had a significantly lower condition factor than those fed Diet 2. There was a trend (0.05<P<0.1) to higher dressout percentage for salmon fed Diet 2 compared to those fed Diet 3. The hepatosomatic index followed a similar pattern as the conditions, but there were no significant differences between the diets. There were no significant differences in gut somatic indices. No significant differences were revealed in liver and gut tissue scores between salmon fed the three different diets (data not shown).

Table 7 Morphometrics and somatic indices of Atlantic salmon fed three diets with different physical properties. Data are given as mean ± SEM (n=15).

	Diet 1	**Diet 2**	**Diet 3**
Final body weight (g)	2321±104	2258±113	2198±118
Gutted weight (g)	2036±100	1988±103	1899±110
Length (cm)	54.0±0.7	52.6±0.8	52.4±0.8
CF	1.46±0.02[b]	1.53±0.02[a]	1.51±0.02[ab]
Liver weight (g)	30.7±1.7	33.7±1.8	29.9±2.9
D%	87.5±0.6*	87.9±0.3*	86.2±0.7*
HSI	1.33±0.06	1.50±0.05	1.34±0.08
SSI	0.52±0.02	0.51±0.02	0.50±0.02
PISI	2.66±0.15	2.66±0.12	2.74±0.15
MISI	0.22±0.01	0.23±0.01	0.21±0.01
DISI	0.46±0.03	0.47±0.02	0.41±0.03

a, b – Significant differences (P≤0.05) of means within a row are indicated with different letters

* – A trend (0.05<P<0.1)

CF – Condition factor

D% – Dressout percentage

HSI, SSI, PISI, MISI, DISI – Somatic indices of liver, stomach, pyloric intestine, mid intestine and distal intestine, respectively.

4 Discussion

There was no mortality in the trial, and the fish appeared to be at good health. The feed intake was poor during the first month but increased gradually throughout the trial. 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, 2012). According to Skretting (2012), salmon of 1300 g is expected to grow 0.90 % per day at 11 °C. Based on the measured daily feed intake and the assumption that the FCR was constant during the trial, the mean SGR of the first 30 days of the trial was 0.23 %, and 0.58 % for the remaining 66 days. However, as feed utilisation is highest at high feed intake (Einen et al., 1995; Einen et al., 1999; Grisdale-Helland et al., 2013), the FCR was not constant during the trial and thus 0.23 % is an overestimate of SGR during the initial period with low feed intake. Correspondingly, 0.58 % is an underestimate of SGR during the last part of the trial, and the real SGR for this period was closer to the expected values.

Except for low feed intake during the adaption period, no health or welfare problems were observed during the trial. The results from the trial are therefore considered to be representative for the feeds qualities tested.

The three experimental feeds were produced from the same feed mix and coated with fat after extrusion. Different expansion of the pellets resulted in higher fat uptake in Diet 3 during coating, and thus higher fat content in this feed than in Diet 1 and Diet 2. Correspondingly, the energy content was also higher, the moisture slightly higher and the nitrogen content lower in Diet 3 than in the other diets. Except for this, the chemical composition of the feeds was similar among all feeds.

The pellets of Diet 3 were slightly larger than the two other diets. The largest effect of feed processing on pellet quality however, was found in hardness. The hardness (measured with Texture Analyzer) of Diet 3 was measured to 86.8 N, whereas the hardness of Diet 1 and 2 was 201.4 and 236.1 N, respectively. The measured water stability was lower in Diet 3 and 2 (82.3 % remaining material for both) than in Diet 1 (87.0 % remaining). Interestingly, Diet 3, which had the lowest hardness and low water stability, was most durable according to the DORIS test. The pellets in Diet 3 were slightly larger than in the two other diets, which may affect the DORIS measurements, particularly the largest size fraction (particles > 9 mm). However, the total breakage in the DORIS test was 30.5, 28.1 and 14.0 % for Diet 1, Diet 2 and Diet 3, respectively, confirming that the highest DORIS durability was found in Diet 3. Accordingly, using a sieve of 0.8 mm, the DORIS values were 92, 93 and 98 % (breakage 8, 7 and 2 %) for Diet 1, Diet 2 and Diet 3, respectively. Measured with the Ligno test, Diet 2 and Diet 3 was most durable. The fat leakage was highest in Diet 1 and lowest in Diet 2.

The water stability test was performed within few days after the feeds were produced. Although the difference in water stability was not very large (87.0, 82.3 and 82.3 % for Diet 1, 2 and 3, respectively), 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 shaking in water bath in the water stability test, the pellets in Diet 1 appeared intact after two hours shaking in water bath, the pellets of diet 2 were smaller and had some rounded edges and signs of attrition, whereas pellets of Diet 3 were swollen and greyish.

The measurement of water stability of the three experimental diets was repeated eight months after feed production. At this time, the remaining dry matter in the water stability test was 93.5 (SD=0.5), 94.8 (SD=0.4) and 93.8 (SD=0.8) for Diet 1, Diet 2 and Diet 3, respectively. Thus, after eight months storage at 4 °C, the water stability of the feeds was increased, and was similar for all feeds. Besides, the difference in visual disappearance of the feeds after shaking on water bath was no longer so evident (Fig. 4). This shows that the physical properties of feeds change during storage, which is in accordance with previous findings (Aas et al., 2011a). Thus, the physical feed properties measured by the feed manufacturer may not be valid if the feed is stored for prolonged time.

Figure 4 *Experimental feeds eight months after production. Upper panel, from left: Diet 1, 2 and 3. The visual appearance resembled that right after production. Lower panel, from left: Diet 1, 2 and 3 after two hours shaking in water bath in the water stability test. Diet 1 had intact pellets, pellets of Diet 2 had some rounded edges, and Diet 3 had swollen pellets. The difference in visual appearance among the feeds after the water stability test was larger shortly after production.*

Previous data indicate that water stability may be negatively correlated with feed intake in salmonids (Aas et al., 2011b; Oehme et al., 2014). Thus, according to these studies pellets with low water stability may be advantageous to obtain a high feed intake. On the other hand, durable pellets which produce minimal dust and small particles during storage and transport are needed (Aas et al., 2011a). These may seem as contradictory properties. Among the three feeds produced in the present trial though, Diet 3 had both low water stability and hardness, and the highest durability. Based on data from previous studies, Diet 3 therefore seems to have the most desirable physical properties among the three feeds tested in the present trial.

Numerically, all feed intake and growth estimates was higher for salmon fed Diet 2 and 3 than those fed Diet 1, although3333 the difference was only significant when expressed as relative feed intake and specific feeding rate. The condition factor at termination of the trial was also significantly higher in salmon fed Diet 2 than in salmon fed Diet 1. The condition factor of those fed Diet 3 was numerically, but not significantly higher than salmon fed Diet 1. 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 among groups. Thus, in a longer trial, the differences in feed intake and growth might have become clearer. In accordance with previous studies (Aas et al., 2011b; Oehme et al., 2014) however, 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 3 were measured to 236.15 and 86.84 N which is a very large difference in hardness. Thus, for the feeds used in this trial, hardness did not affect feed intake. This is contradictory to the hypothesis that hard pellets cause reduction in feed intake because pellets disintegrate slowly in the stomach, which again reduces feed intake due to a slow passage rate through the gut (Aas et al., 2014; Aas et al., 2013). According to the present data, low water stability of the pellets seems to enhance feed intake, which is in accordance with earlier findings (Aas et al., 2011b; Oehme et al., 2014). This is possibly related to rapid disintegration of pellets in the stomach when the water stability is low, and a higher transit time through the gut, compared to pellets with higher water stability (Aas et al., 2014; Aas et al., 2013). It should be noted that feeds can be produced with an infinite number of pellet qualities, which can be measured with several different methods. These results show that the response in fish also depends on which process parameters are used to achieve the pellet quality, not only the quality analysis itself. One can therefore only compare the feed qualities tested, whereas it is difficult to make general conclusions based on one study. However, the data clearly show that pellet hardness does not necessary affect feed intake negatively.

The AD (apparent digestibility) of P and Zn was different among the different diets. Previous data have also shown that mineral digestibility in rainbow trout is affected by pellet quality (Aas et al., 2011b). As in the present study, the digestibility of minerals, but not main nutrients, was affected by pellet quality in rainbow trout. In that study however, 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 is affected by feed intake. In the present study however, the AD of P and Zn was significantly different in Diet 2 and Diet 3, whereas feed intake was similar for these two diets. It can therefore be concluded that in the present study, the AD of P and Zn was affected by pellet quality. The AD of these minerals was highest in Diet 1 and 2, which had the hardest pellets.

The salmon retained similar amounts (%) of ingested nutrients and energy for all diets. The amount retained from the digested energy and most nutrients measured was also similar among the diets. For Zn however, the retention was highest in salmon fed Diet 3, which had lowest AD of Zn, indicating an effective retention compensating for a low AD. A similar significant compensation was not found for P. As for growth data, a longer trial might have been advantageous with regard to develop differences in body composition and retention data.

Conclusion

Pellet quality was found to have a significant effect on feed intake. Among the three feed quailies 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 hepatosomatic index or gut indices. Neither did the pellet quality affect the digestibility of energy, dry matter, nitrogen and fat, whereas the apparent digestibility of P and Zn was significantly affected by pellet quality. The retention of ingested nutrients was similar for all feeds, whereas the retention of digested Zn was significantly affected by pellet quality.

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