Gut evacuation rate in Atlantic salmon (*Salmo salar*) fed diets with different physical properties
A CREATE project

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Report

Title:	*Report No.:*
Gut evacuation rate in Atlantic salmon (Salmo salar) fed diets with different physical properties. A CREATE project	33/2014
	Accessibility:
	Open
Author(s)/Project manager:	*Date:*
Turid Synnøve Aas, Trine Ytrestøyl, Bjarne Hatlen, Hanne Jorun Sixten, Marie Hillestad, Harald Sveier, Roger Selset and Torbjørn Åsgård	June 16th 2014
Department:	*Number of pages and appendixes:*
Nutrition and feed technology	14
Client:	*Client's ref.:*
SINTEF Fiskeri og havbruk AS	Arne Fredheim
Keywords:	*Project No.:*
Atlantic salmon, pellet quality, gut evacuation rate	2424

Summary/recommendation:

To measure the effect of physical feed quality on gut evacuation, Atlantic salmon with mean weight 1047 g (measured after finishing the sampling) was fed two extruded feeds with different physical quality to satiation. Each feed quality was produced in three batches to contain three different markers (La, Yb and Y). The measured hardness for Diet 1 was 128.5, 140.5 and 152.1 N for the batches added La, Yb and Y, respectively. For Diet 2, the corresponding figures were 148.2, 172.0 and 153.3 N, respectively. The mean water stability, given as % of dry matter remaining after 2 hours shaking in water bath, was 78.6, 76.9 and 77.9 % for Diet 1 added La, Yb and Y, respectively. For Diet 2, the corresponding figures were 84.9, 85.2 and 81.8, respectively. The durability, given as remaining intact pellets in the DORIS test, was 64.6, 65.9 and 81.6 for Diet 1, and 80.1, 81.4 and 78.3 for Diet 2, respectively, in the batches added La, Yb and Y. The pellet size was 10 mm. The salmon was fed one meal daily. The feeds added La was fed for a period of 26 days. On day 27, feeds containing Yb was given, and on day 28 and thereafter, the salmon was fed feeds with Y added. Faeces were collected from the outlet water during 30 minutes intervals at 8, 16, 24, 32, 40 and 48 hours after the feeding on day 27, and analyzed for La, Yb and Y. The ratio of the markers was calculated as concentration of each marker divided by concentration of sum of markers.

For both feed groups, some Yb appeared in faeces 8 h after feeding diets containing this marker, and amount of Yb peaked around 24 h and almost no Yb was left after 48 h. Sixteen hours after feeding, at the time when the change in marker concentrations had happened fastest, the relative concentration was lowest for La, and highest for Yb, in faeces from fish fed Diet 1, indicating a higher gastrointestinal transit rate for this feed in this period. The apparent digestibility of fat was approximately 2 % higher in Diet 1 than in Diet 2.

The data indicate that even small differences in the physical quality of feeds may have an impact on the gut evacuation rate in Atlantic salmon. This may affect how well the genetically inherent growth capacity of the fish is expressed (and utilized).

Summary/recommendation in Norwegian:

For å måle effekten av fysisk pelletkvalitet på passasjehastigheten gjennom mage og tarm ble laks med snittvekt 1047 g ble fôret med to fôr med lik formulering men ulik pelletkvalitet. Hvert fôr ble produsert i tre batcher og tilsatt ulike markører (La, Yb og Y). Målt hardhet for Fôr 1 var henholdsvis 128.5, 140.5 and 152.1 N for batchene tilsatt La, Yb og Y. Hardheten til Fôr 2 var henholdsvis 148.2, 172.0 og 153.3 N. Vannstabilitet, målt som % gjenværende tørrstoff etter 2 t i ristevannbad, var 78.6, 76.9 og 77.9 % for Fôr 1 tilsatt hhv La, Yb og Y. De tilsvarende verider for Fôr 2 var hhv 84.9, 85.2 og 81.8. Slitestyrke, gitt som mengde intakt pellets i DORIS-testen, var 64.6, 65.9 og 81.6 for Fôr 1, og 80.1, 81.4 og 78.3 for Fôr 2, henholdsvis, i batchene tilsatt La, Yb og Y. Pelletstørrelsen var 10 mm.

Fisken ble fôret en gang per dag. De første 26 dagene ble det brukt fôr tilsatt La. Dag 27 ble det brukt fôr tilsatt Yb, og dag 28 og deretter ble det brukt fôr tilsatt Y. Faeces fra fisken ble samlet over 30 minutt på rist fra utløpsvannet 8, 16, 24, 32, 40 og 48 timer etter måltidet på dag 27, og analysert for markører. Den raskeste endringen i markørratioer i gjødsel ble funnet i tidsrommet 8-16 timer etter fôring. Etter 16 timer var de relative konsentrasjonene av La lavest, og av Yb høyest, i faeces fra fisk som fikk fôret med lavest hardhet. Dette tyder på en raskere passasje gjennom fiskens mage-tarm-system for dette fôret enn for det hardeste fôret. Fordøyeligheten av fett var ca. 2 % høyere i fôret med lavest hardhet enn i det hardeste fôret.

Disse dataene indikerer at selv små ulikheter i fysisk kvalitet kan ha innflytelse på passasjehastigheten i atlantisk laks. Dette kan ha betydning for hvordan fiskens genetiske vekstkapasitet blir utnyttet.

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1   Introduction

Due to mechanical stress during storing, transport and handling in pneumatic feeding devices in today's salmon farming, feeds with high pellet quality are demanded (Aarseth 2004; Aarseth et al., 2006). However, pellet quality has been shown to affect feed intake in salmonids (Aas et al. 2011, Aas et al, 2013). Feed represents more than 50 % of the cost in Norwegian salmon farming, and sustainability in food production requires effective utilization of resources. The utilization of feed is highest at high feed intake and high growth rate (Einen et al. 1995, Einen et al. 1999). Studies have shown that there is a potential for increasing feed intake and growth in commercial salmon farming (Hatlen, unpublished data).

Oehme et al. (2013) reported that soaking the feed prior to feeding increased feed intake in salmon, particularly at low feed intake. The effect of soaking on feed intake may have several causes, such as release of feed components to the water that stimulate feed intake, a feed consistency that the salmon prefer or a consistency that causes feed to be rapidly disintegrated in the stomach and released into the small intestine. The latter will allow the soaked feed to have an increased gastrointestinal passage rate compared to dry feed. At high passage rate of the feed, the fish may be capable to eat more, and have a higher flow of feed through the gastrointestinal system. This hypothesis was tested by Aas et al. (2013), which used the same feeds as tested by Oehme et al (2013). Aas et al. (2013) found an increased gastric evacuation rate in salmon fed soaked feed compared to salmon fed dry feed, which thus may explain the increased feed intake in salmon fed the soaked diet.

Aas et al. (2011) reported a higher feed intake when rainbow trout were fed a diet with low water stability compared to a diet with high water stability, whereas Baeverfjord et al. (2006) found no significant difference in feed intake and growth in rainbow trout fed diets with high or low water stability. The apparent digestibility coefficient (ADC) of nitrogen and lipid (Baeverfjord et al., 2006) and amino acids, starch, energy and dry matter (Aas et al., 2011) was highest in feed with high water stability and hardness. Adamidou et al. (2009) showed that inclusion of faba bean and chickpea in diets for sea bass increased the hardness of the extruded pellet, giving prolonged gut evacuation rate These studies suggest that higher water stability and hardness of pellets result in longer gastric retention time and prolonged evacuation time of chime through the gastrointestinal tract.

This leads to the hypothesis that feed intake in salmon can be increased by optimizing the pellet quality. Furthermore, the rate at which the pellet dissolves in the stomach and the corresponding gastrointestinal passage rate may be factors that affect feed intake. In the present study therefore, two feeds with identical formulations were produced to have different water stability and hardness and fed to Atlantic salmon. By adding inert markers in the feeds and analyzing these in the faeces sampled 8, 16, 24, 32, 40 and 48 h after one of the meals, the gut evacuation rate between the two feeds with different water stability were compared. Also, the apparent digestibility of dry matter, nitrogen, lipid, ash and energy in the two feeds were estimated.

2 Materials and methods

An overview of the fish trial is shown in Table 1. Briefly, the gut evacuation rate (GER) of two feeds with different physical qualities was measured by adding different inert markers to the feeds, and the content of these markers were analyzed in collected faeces. The fish was fed the two diets added lanthanum (La) day 1-26, the feeds used on day 27 were added ytterbium (Yb) and from day 28 and thereafter, feeds with yttrium (Y) were fed. All feeds were given as one meal daily, lasting one hour (from 07:00 to 08:00). During day 27-29, faeces were collected from the outlet water, and analyzed for La, Yb and Y to estimate GER of the two feeds.

Table 1   Overview of the fish trial

Day	Feeding time (h)	Marker in feed	Sampling	Time for sampling (h)
1	07:00-08:00	La	-	-
2	07:00-08:00	La	-	-
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
24	07:00-08:00	La	-	-
25	07:00-08:00	La	Faeces for ADC estimation	09:00-11:00
26	07:00-08:00	La	Faeces for ADC estimation	09:00-11:00
27	07:00-08:00	Yb	Faeces for GER estimation	16:00-16:30, 24:00-00:30
28	07:00-08:00	Y	Faeces for GER estimation	08:00-08:30, 16:00-16:30, 24:00-00:30
29	07:00-08:00	Y	Faeces for GER estimation	08:00-08:30
30	-	-		-
31	-	-		-
32	-	-		-
33	-	-	Weighing	

2.1 Feed production

Two feeds with identical formulation but different physical quality were produced by BioMar AS (Tech Centre, Brande, Denmark). To add three different markers, each feed was produced in three batches (in total 6 feeds). The feeds were denoted Diet 1La, Diet 1Yb, Diet 1Y, Diet 2La, Diet 2Yb and Diet 2Y, reflecting which marker that is added to Diet 1 or 2. The difference in physical feed quality between Diet 1 and Diet 2 was achieved by using different process conditions for the diets in the extruder. The formulation of the feeds is shown in Table 2, the chemical composition in Table 3 and measured physical quality in Table 4.

Table 2   Formulation of feed (g kg^{-1} diet)

	Diet 1 and Diet 2
North Atlantic fishmeal	100
South American fishmeal	100
Soy protein concentrate	160
Sunflower expeller	160
Wheat gluten	30
Micronized Pea starch	71
Wheat	71
Standard fish oil	210
Rapeseed oil	90
Mono calcium phosphate	9.7
Amino acid mix	6.1
Vitamin and Mineral premix	3.2

Table 3   Chemical composition of experimental feeds

	Diet 1La	Diet 1Yb	Diet 1Y	Diet 2La	Diet 2Yb	Diet 2Y
Dry matter (%)	94.1	94.4	93.2	94.9	94.9	95.6
In dry matter (% or MJ/Kg):						
Lipid	36.5	35.3	35.9	35.4	34.7	33.5
Nitrogen	5.49	5.59	5.48	5.58	5.52	5.63
Ash	5.98	6.16	6.10	6.22	6.30	6.35
Energy	26.78	26.22	26.44	26.12	26.13	25.84
Starch	11.0	11.5	11.5	11.7	11.4	11.4
Fibre	3.6	3.5	3.6	3.7	3.6	3.7
P	1.15	1.17	1.17	1.18	1.19	1.20
Zn	0.016	0.015	0.016	0.016	0.019	0.017
Digestibility markers (g/kg in dry matter):						
La	0.35	0.07	0.01	0.39	0.03	-
Yb	-	0.37	0.02	-	0.37	-
Y	0.02	0.01	0.39	0.02	0.02	0.41

Table 4   Physical quality of experimental feeds. Data are given as mean ± 1 SD

	Diet 1La	Diet 1Yb	Diet 1Y	Diet 2La	Diet 2Yb	Diet 2Y
Diameter (mm)	9.4 ± 0.4	9.4 ± 0.4	9.3 ± 0.2	9.4 ± 0.3	9.6 ± 0.2	9.4 ± 0.3
Length (mm)	9.2 ± 0.6	9.0 ± 0.4	9.0 ± 0.4	9.9 ± 0.5	9.6 ± 0.5	10.3 ± 0.6
Bulk density (g/L)	624.7 ± 2.0	625.3 ± 6.4	651.6 ± 4.5	660.7 ± 3.0	670.7 ± 2.2	670.5 ± 3.7
Hardness (N):						
Texture analyzer	128.5 ± 18.9	140.5 ± 17.5	152.1 ± 16.4	148.2 ± 11.8	172.0 ± 14.2	153.3 ± 14.0
Kahl	49.0	50.4	57.4	58.9	63.0	66.1
Water stability test (Remaining dry matter, %)	78.6	76.9	77.9	84.9	85.2	81.8
Durability:						
Ligno test (%)	98.2 ± 0.6	98.2 ± 0.1	99.2 ± 0.04	98.2 ± 0.1	98.3 ± 0.04	98.1 ± 0.1
DORIS test:						
Whole pellets (%)	64.6 ± 4.0	65.9 ± 2.7	81.6 ± 2.1	80.1 ± 0.6	81.4 ± 0.7	78.3 ± 0.4
Fracture (%)	30.0 ± 3.9	29.0 ± 2.1	15.9 ± 1.6	16.6 ± 1.2	15.3 ± 0.9	18.6 ± 0.4
Fines (%)	5.4 ± 0.4	5.1 ± 0.7	2.5 ± 0.5	3.3 ± 0.6	3.2 ± 0.3	3.1 ± 0.3
Fat leakage (%)	6.2 ± 0.5	6.4 ± 0.2	6.6 ± 0.4	3.6 ± 0.5	3.5 ± 0.6	3.5 ± 0.3

2.2   Fish trial

The fish trial was run in 3.3 m^3 tanks supplied with sea water in a flow through system at the Nofima Centre for Recirculation in Aquaculture, Sunndalsøra. The temperature on day 27, 28 and 29 was 11.5 °C, 12.0 °C and 12.3 °C, respectively. Atlantic salmon (approximately 150 fish per tank) was placed in the experimental tanks May 22nd and 23rd 2013 for acclimation to the experimental conditions. During the first two weeks, the fish was gradually accustomed to one daily meal lasting 1 hour.

The fish was fed Diet 1La and Diet 2La from August 2nd (Day 1 of trial). August 28th (Day 27 of trial), Diet 1Yb and Diet 2Yb were fed, and thereafter (day 28 and 29 of trial) Diet 1Y and Diet 2Y. During the whole trial, the salmon was fed one meal daily, lasting 1 h (from 7.00 AM to 8.00 AM). The fish was weighed and counted on day 33 after three days fasting after finishing the trial. The mean weight was 1047 g.

The feed intake was measured for 25 days prior to sampling, following the procedures of Helland et al. (1996). Briefly, the waste feed was collected and weighed daily. The recovery (%) was determined by following the experimental procedure, but with no fish in the tanks. The recovery value was used to correct the amount of waste feed, and daily feed intake was calculated as feed given minus corrected waste feed.

Twenty per cent overfeeding was aimed at based on the last three days' estimated feed intake during the first 25 days. On day 26 and thereafter, 50% overfeeding was aimed at, to assure large amounts of available feed at each meal during the period samples for GER was collected.

2.3   Sampling

Faeces were collected from the outlet water by placing a container made of wire mesh with openings < 1 mm under the outlet of each tank. Every 5 minutes during collection, the containers were emptied and cleaned, and placed back at the water outlet. On day 25 and 26, faeces for digestibility estimation were collected, and the sampled material was pooled by tank. Samples for estimation of gut evacuation rate were collected at 8 h intervals, at 6 points in time, after the feeding on day 27. Each sampling lasted for 30 min (emptying the containers every 5 min). The sampled faeces were stored at -20 °C until freeze drying.

2.4   Chemical analysis

All faecal samples were freeze dried. Feeds and faecal samples used for digestibility estimation 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 x 6.25 (Kjeltec Auto Analyser) and crude lipid (SOXTEC hydrolyzing and extraction systems). Gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter). Minerals and markers (La, Yb and Y) in feeds and all faecal samples were analysed with ICP-OES (Perkin Elmer Optima 5300 DV, Perkin Elmer, Inc 2004 Shelton, USA) or ICP-MS (Agilent 8800 Triple Quad) after decomposition in concentrated HNO_3 at 260 °C (UltraClave, Milestone mikrowave Ultraclave III) and thereafter dilution to 10% HNO_3.

2.5   Measurement of physical feed quality

Diameter and length of the pellets were measured with an electronic caliper. The measurements were conducted on 20 pellets from each diet.

Bulk density was measured by loosely pouring the dried uncoated feed from a funnel into a 1 000 ml measuring cylinder. The top was gently flattened before measuring the weight. The measurement was repeated three times per diet.

Pellet breaking force (hardness) was measured with two different methods. One was with 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). Measurements were conducted for 20 pellets from each of the feed samples. Pellets were abraded with sandpaper P120 before measurements in order to make the pellet stand. Pellet breaking force (hardness) was also measured on laying pellets on a Kahl Pellet Hardness Tester (Amandus Kahl GmbH & Co. KG., Hamburg, Germany) and given as the mean of 10 measurements.

A modified version of the method of Baeverfjord et al. (2006) was used to measure water stability of the feeds. Four replicates of 20 g of each feed was placed in custom-made, cylindrical mesh wire containers that each were placed in a 600 ml beaker containing 300 ml distilled water. 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. The test was run in triplicate.

Doris Durability Index (DDI) was measured on oil coated pellets in an AkvaMarina DORIS Feed Tester (Aquasmart ASA, Bryne, Norway). 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 (8.00, 5.60 and 2.36mm) to measure the amount of whole pellet (> 8.00mm), fracture (2.36 - 8.00mm), and fines (<2.36mm). The DDI is given as the percentage of pellets in each category. Each diet was analyzed in triplicate.

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, and was recorded three times per diet.

2.6 Calculations

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

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

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

Apparent digestibility and nutrients and energy were calculated as

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

and b represents the nutrient to marker ratio in faeces.

The ratio of marker in each faecal sample was calculated for each marker as $\frac{[X]}{[La]+[Yb]+[Y]}$, where X

represents one marker (La, Yb or Y).

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 by comparing the two feed groups with an ANOVA (t-test) at each sampling time. Differences were considered significant if P≤0.05, and if 0.05<P<0.1, this was reported as a trend.

All statistical analyses were performed with the SAS computer software (SAS 1985, SAS Institute Inc, Cary, USA).

3 Results

3.1 Feed intake

No significant differences in feed intake between fish fed Diet 1 and Diet 2 were found in the present trial (Table 5).

Table 5 Individual feed intake (g per individual, DM) in Atlantic salmon fed two diets with different physical properties. The feed intake is given as cumulative feed intake over 26 days with feeds added La, and for day 27 when the fish was fed diets added Yb (Mean±S.E.M., n=4)

	Diet 1La	Diet 2La	P-value (ANOVA)
Cumulative feed intake day 1-26	104 ± 4	108 ± 6	0.5680

	Diet 1Yb	Diet 2Yb	
Feed intake day 27	8 ± 0.2	8 ± 0.5	0.5490

3.2 Apparent digestibility

Faeces for digestibility estimation were sampled on day 25 and 26 of the trial. The apparent digestibility of lipid was significantly higher in Diet 1La (94.1+0.5%) than in Diet 2La (92.3+0.3%). No other significant differences were found in apparent digestibility of nutrients and energy between the two feeds, but Kalium showed a trend for higher digestibility in Diet 1 (Table 6).

Table 6 Apparent digestibility (%) of nutrients and energy in Diet 1La and Diet 2La fed to Atlantic salmon (Mean±S.E.M., n=4)

	Diet 1La			Diet 2La			P-value (ANOVA)
Dry matter	66.7	+	1.5	66.8	+	0.8	0.9648
Lipids	94.1	+	0.5	92.3	+	0.3	0.0175
Nitrogen	91.4	+	0.5	90.7	+	0.5	0.3067
Ash	-59.0	+	3.9	-58.3	+	1.9	0.8819
Energy	85.2	+	0.9	84.2	+	0.5	0.3491
Na	-818.3	+	38.8	-854.8	+	18.5	0.4288
P	39.2	+	2.8	39.7	+	2.4	0.9030
K	89.7	+	0.3	88.7	+	0.3	0.0513
Ca	-23.9	+	5.9	-21.5	+	4.7	0.7653
Fe	13.4	+	3.2	5.7	+	4.8	0.2330
Cu	63.3	+	2.9	60.5	+	1.5	0.4348
Zn	21.0	+	4.8	22.8	+	3.4	0.7678

3.3 Gut evacuation rate

The ratios of the concentrations of the three markers in faeces sampled during 30 min periods starting 8, 16, 24, 32, 40 and 48 hours after feeding diets added Yb (time 0) are shown in Fig. 1. Eight hours after Diet 1Yb and Diet 2Yb were administered, Yb was found in faeces from fish from both fed groups. A large drop in relative La-concentration (red graphs in Fig. 1), and correspondingly, a large

increase in relative Yb-concentration (green graphs in Fig. 1) occurred in faeces from both feed groups from 8 to 16 hours after feeding the diets containing Yb. At time 8 h, there were no significant differences in the ratios [La]/[Sum of markers] or [Yb]/[Sum of markers], whereas at time 16 h the ratio [La]/[Sum of markers] tended (P<0.1) to be lower in faeces from salmon fed Diet 1La than from those fed Diet 2La. Correspondingly, the ratio [Yb]/[Sum of markers] was significantly higher in faeces from salmon fed Diet 1Yb than from those fed Diet 2Yb (Fig. 1). In other words, the graphs for La-ratio and Yb-ratio in faeces were shifted to the right in fish fed Diet 2, which had the highest water stability and hardness, compared to fish fed Diet 1, which had lowest water stability and hardness. This indicates that during this time interval, when the concentrations in marker ratios changes fastest, Diet 1 passed faster through the gastrointestinal system than Diet 2.

This was also confirmed by the calculated slopes for the marker ratios for the time interval 8-16 h (Table 7); there was a trend (P<0.1) to steeper slopes for the declining La-concentration and increasing Yb-concentration in faeces of salmon fed Diet 1 compared to those fed Diet 2.
During the interval at time 32-40 h, a similar difference in gastrointestinal passage rate between the two diets was not revealed. However, also at this time interval, there was a trend (P<0.1) to faster increase in the ratio [Y]/[Sum of markers] in faeces from salmon fed Diet 1 than from salmon fed Diet 2.

For both feed groups, the peaks in Yb-concentrations were found at time 24 h after the meal, and Yb was almost completely evacuated from the intestine at time 48 h.
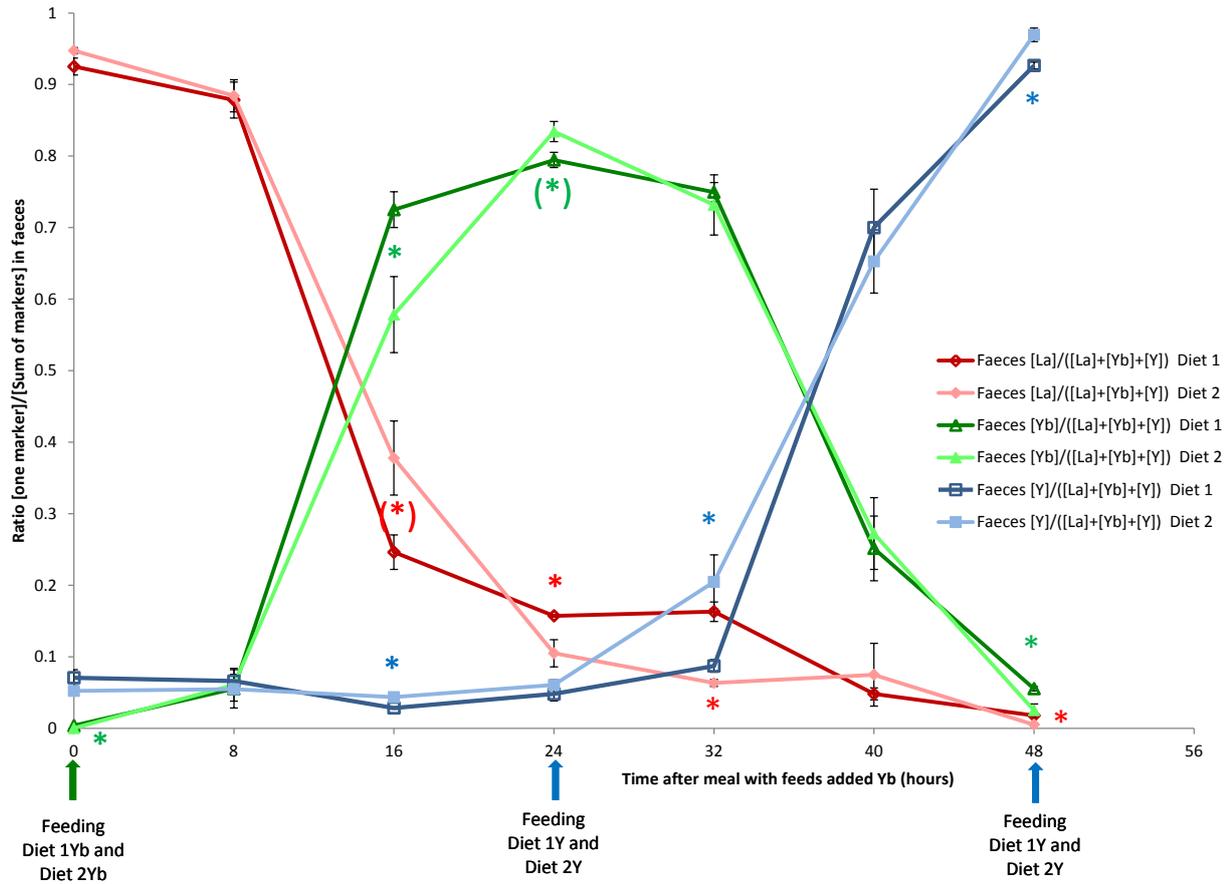
Figure 1   *Ratio of markers in faeces from Atlantic salmon sampled 8, 16, 24, 32, 40 and 48 hours after a meal with feeds added Yb (time 0). The salmon was fed diets added Y at 24 and 48 h. Prior to these samplings, the salmon was fed diets added La, and samples collected the last two days before time 0 are used for time 0 in the figure. (Mean±S.E.M., n=4)*

* Significantly different (P≤0.05)
(*)Trend (0.05<P<0.1)

Table 7   *Slope of the graphs (shown in Fig. 1) for marker ratios in faeces in the time interval 8-16 h after the meal with added Yb. (Mean±S.E.M., n=4)*

	Diet 1			Diet 2			p-value
8-16 h							
[La]/[Sum of markers]	-0.079	±	0.004	-0.063	±	0.007	0.0846
[Yb]/[Sum of markers]	0.084	±	0.004	0.065	±	0.007	0.0569

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4 Discussion

The method used in this trial has the large advantage that the GER was studied in undisturbed fish. Since digestive processes are affected by stress (Peters, 1982, Bolasina et al. 2007, Chen and Fernald 2008, Oxley et al. 2010), sampling and handling the fish, and thus causing stress, will affect the results when examining GER. The data obtained in the present trial however, are representative for salmon kept at the conditions used in this trial. Also, the fish was fed both before and after the sampling, so the data reflect the normal nutritional state in salmon. The major limitation of the method is that only total GER could be studied, and digestive processes in stomach and different segments of the intestine could not be examined.

Physical feed quality

To obtain feeds with different markers, each feed quality must either be produced in several batches with one marker mixed with the ingredients in each batch, or the markers can be coated on the feeds after extrusion. When coating, it is a risk that the coated marker is inhomogeneously distributed in each pellet with the highest concentration in the outer layer. In the stomach, the feed is broken down by disintegrating the outermost part of the feed particles (Aas et al. 2011). Thus, adding the markers to the feed by coating may create a source of error when studying GER. In the present trial therefore, adding the marker to the feed mix and producing each feed in three batches was chosen. The disadvantage of this method is that the different batches of a feed may vary in physical quality. In the present trial, the water stability was similar in the three batches of each feed, and lower in Diet 1 than in Diet 2. However, with respect to hardness and DORIS durability, Diet 1Y was similar to the three batches of Diet 2.

The diets used in the trial were of commercial-like quality with regard to formulation and physical quality. The difference in the measured physical properties between the diets classified as Diet 1 and those of Diet 2 were not very large, and thus large differences in GER could not be expected.

Feed intake

In previous studies, it has been shown that the physical properties of the feed can affect feed intake in salmonids, and that this may be related to the GER (Sveier et al. 1999, Aas et al. 2011, Aas et al. 2013, Oehme et al. 2013). In the present study, there was not found any effect of physical feed quality on feed intake. In a previous study with rainbow trout, feed intake was approximately 20% higher when fed a diet with low water stability compared to a diet with high water stability (Aas et al. 2011). In that trial however, the difference in water stability between the two feeds was considerably larger than in the present study. Baeverfjord et al. (2006) found no significant difference in feed intake and growth in rainbow trout fed diets with high or low water stability. These results are in contrast to studies by Glencross et al. (2011) finding positive correlation between hardness and feed intake. Differences in raw material and process conditions might explain the different results as specific knowledge of the right process parameters for the different raw materials is required in order to achieve the desired pellet quality (Morken et al. 2012; Kraugerud et al. 2011; Sørensen et al. 2011). Although no relation between the feeds' water stability and feed intake was found in the present trial, it cannot be ruled out that with larger differences in water stability between feeds, this can affect feed intake in Atlantic salmon too.

Apparent digestibility

The apparent digestibility of lipid was approximately 2% higher in Diet 1La than in Diet 2La in the present study. It has earlier been shown that high feed intake may reduce the apparent digestibility of nutrients Atlantic salmon (Oehme et al. 2013). Since there were no differences in feed intake in the present study however, the data indicate that for the feed qualities used, the lowest water stability and hardness of the feed (Diet 1) increased lipid digestibility compared to the diet with higher water stability and hardness (Diet 2). Studying rainbow trout, Aas et al. (2011) showed that a feed a with low water stability resulted in higher feed intake and lower digestibility of amino acids, starch, energy and dry matter than a feed with high water stability. In that study, the effect of feed quality could not be separated from the effect of feed intake. Beaverfjord et al (2006) found that the digestibility of nitrogen and lipid was affected by the water stability of the feed in rainbow trout. Oehme et al. (2013) showed that in Atlantic salmon, nutrient digestibility is generally lower at high feed intake than at low feed intake. The data from the present study is a further indication that the differences in nutrient digestibility found in rainbow trout fed diets with high or low water stability (Aas et al. 2011), may be an effect of feed intake rather than the feeds for most nutrients, except for lipid.

Gastrointestinal passage rate

There was a certain amount of all three markers in all feeds and thus there was a background level for all markers. However, these background levels were low and did not seem to interrupt the course of the marker ratios in faeces.

Marker ratios estimated in faeces sampled the two days prior to the meal at time 0 h was used as data for time 0. The salmon was fed Diet 1La and Diet 2La for 26 days prior to time 0 h and therefore, the marker ratios in faeces were assumed to be the constant until the diets were changed at time 0 h.

The gastrointestinal passage rate varies between species, and is dependent on temperature (Fänge and Grove 1979). Furthermore, several studies suggest that higher water stability and hardness of pellets result in longer GER of chime through the gastrointestinal tract (Aas et al., 2011; Aas et al., 2013; Baeverfjord et al., 2006). Adamidou et al. (2009) showed that inclusion of faba bean and chickpea in diets for sea bass increased the hardness and gave prolonged GER of the extruded pellet. The GER found in the present study correspond with data from a similar study (Sveier et al. 1999). In that study, Atlantic salmon was fed diets containing coarse, standard or micro ground fish meal. Twelve hours after feeding, marker concentrations indicated that gastric emptying was fastest for feed with standard fish meal, and slowest for feed with coarse fish meal. Correspondingly, marker concentration in hind gut was highest in fish fed feeds with standard ground fish meal, and lowest in fish fed feed with coarsely ground fish meal 12 hours after feeding (Sveier et al. 1999). In the present trial, the marker ratios in faeces changed rapidly between 8 and 16 hours after feeding. The slope in this interval, and the significantly different marker ratios at 16 hours indicate that in this time interval, when the change in marker ratios were large, the transit through the gastrointestinal tract was fastest for Diet 1 (lowest water stability and hardness).

Earlier studies have shown that the gastric evacuation in Atlantic salmon is affected by the physical properties of the feed (Sveier et al. 1999, Aas et al. 2013). This may explain the findings from the present study, where the marker ratios in faeces indicated that the total GER was affected by pellet quality in the first phase of digestion of a meal (8- 16 hours post feeding).

Corresponding to the interval at 8-16 hours after feeding, the marker ratios also changed rapidly between 32 and 40 hours after feeding the diets labelled with Yb, which was 8-16 hours after feeding diets labelled with Y. During this interval, a clear indication of differences in passage rate between the two feeds was not found. However, the two diets added Y, fed at 24 h, were very similar in hardness, and also more similar in water stability than the diets added La and Yb. Therefore, an effect on GER cannot be expected from the diets added Y and the measurements from time 24 hours and onwards only give information about the GER in general.

In conclusion, although the difference in physical quality of the tested feeds was not very large, a significantly higher gut evacuation rate and apparent digestibility of lipid was found in salmon fed the diet with lowest water stability than in those fed the diet with highest water stability 8-16 hours after feeding. No significant effect of physical feed quality on feed intake was found.

5   Acknowledgements

6 References

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