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Physical feed properties affect gastrointestinal passage rate in Atlantic salmon, *Salmo salar*

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Abstract

Physical feed properties affect feed utilization in fish. To study how physical feed properties affects feed intake, digestibility and gastrointestinal passage, two feeds with identical formulation, but different physical properties, were produced. Diet 1 was produced with lower water stability than Diet 2. Each feed was produced in three batches, added lanthanum (La), ytterbium (Yb) or yttrium (Y) as indigestible markers. The feeds were fed to Atlantic salmon (1,047 g), one daily meal, for 29 days. Feeds labelled with La were used the first 26 days, feeds with Yb on day 27 and feeds with Y on days 28 and 29. Faeces were collected 8, 16, 24, 32, 40 and 48 hr after feeding on day 27 and analysed. La, Yb and Y in faeces originated from feed eaten on days 26, 27 and 28, respectively. Marker concentrations in faeces showed that some Yb appeared in faeces 8 hr after feeding. After 16 hr, the passage rate was higher for Diet 1 than Diet 2. After 48 hr, both feeds had passed almost completely through the gastrointestinal system. The apparent digestibility of lipid was higher in Diet 1 than in Diet 2. There was no significant difference in feed intake.

KEYWORDS
apparent digestibility, Atlantic salmon, digestibility markers, gastric evacuation rate, gastrointestinal passage rate, physical feed quality

1 | INTRODUCTION

Effective utilization of feed and minimal loss of feed and nutrients is fundamental for economic and environmental sustainability of aquaculture. This requires not only optimal composition of feeds, but also optimal physical quality of the feed pellets. In Norwegian salmon farming, the feed is transported and stored in bulk and distributed to the sea cages with pneumatic systems. From the factory to the sea cage, the feed is subjected to forces that cause formation of varying amounts of dust and small particles and high physical feed quality is required (Aas, Oehme, et al., 2011).

Physical feed properties also interact with the nutritional response in fish (Aas et al., 2017, 2020; Aas, Terjesen, et al., 2011; Baeverfjord et al., 2006; Glencross, Hawkins, et al., 2011; Morken et al., 2011; Oehme et al., 2014; Sveier et al., 1999; Venou et al., 2009), but the knowledge is limited and data are somewhat conflicting. Feed ingredients and process parameters during feed production influence the physical properties of the feed (Draganovic et al., 2011; Glencross et al., 2010, 2011; Kraugerud et al., 2011; Kraugerud & Svihus, 2011; Morken et al., 2012; Oterhals & Samuelsen, 2015; Samuelsen et al., 2013, 2014; Sørensen, 2012; Sørensen et al., 2009, 2010, 2011; Thomas

et al., 1997, 1998). Salmon feed can therefore be produced with a wide range of different physical properties, which can be measured as differences in, for example, hardness, durability and water stability. The optimal pellet quality should have properties appropriate for the logistic systems and for the fish.

In Norwegian salmon farming, feed represents more than 50% of the production cost (Zahirovic, 2012). The salmon farming industry consumes a large amount of highly valuable feed ingredients such as fishmeal, fish oil and soy protein concentrate (Aas et al., 2019; Ytrestøyl et al., 2015). Atlantic salmon utilizes the feed most effectively at high feed intake when the growth rate is high (Einen et al., 1995, 1999; Grisdale-Helland et al., 2013). High feed intake is essential for high feed utilization and to achieve maximum growth of the salmon. The feed intake has been shown to increase when the feed is soaked in water, particularly in situations when feed intake is low (Oehme et al., 2014). The causes of increased intake of soaked feed are unknown, but substances that stimulate feed intake may be released to the water, or the soaked pellets may have a consistency that salmon prefers, or that causes fast processing of the feed in the stomach. The latter will result in higher gastrointestinal (GI) passage rate of soaked feed than of dry feed (Aas et al., 2017). When the feed passes through the GI system at a high rate, the salmon may be able to process a larger amount of feed through the GI system. The fish can only ingest a certain amount of feed, and the aim to increase feed intake is under the assumption that the feed intake is below the fish's maximum capacity for digesting and metabolizing the feed components.

Several methods are used to measure the physical properties of feeds (Baeverfjord et al., 2006; Kaliyan & Vance Morey, 2009; Sørensen, 2012; Thomas & van der Poel, 1996). None of the commonly used method predicts pellet breakage in feeding systems very well (Aas, Oehme, et al., 2011). Durability measurements are often given for commercial feeds, and feeds within a certain range are expected to function well in logistic systems. There are currently no defined measurements of the physical feed quality that specify the expected feed intake or feed utilization in the fish.

Data from previous studies lead to the hypothesis that feed intake in salmon is affected by the physical properties of the feed and of the GI passage rate of the feed. In the present study, the GI passage rate in salmon fed two feeds with different water stabilities was compared. Each feed was produced in three batches added La, Yb or Y as inert markers (Austreng et al., 2000). The fish were fed one meal daily, and during sampling, each meal was labelled with a different marker. The faeces was collected over 48 hr, and gastrointestinal passage rate was measured from marker concentrations in faeces. The feed intake and the apparent digestibility of dry matter, nitrogen, lipid, ash and energy in the two feeds were also measured.

2 | MATERIALS AND METHODS

The salmon were fed one meal daily. To measure the GI passage rate, feeds from the different meals were labelled with different inert markers, lanthanum (La), ytterbium (Yb) or yttrium (Y). The markers were

TABLE 1 Conditions and settings in the preconditioner, extruder and drier at production of the two feeds with different physical properties

	Diet 1	Diet 2
Preconditioner:		
Temperature (°C)	80	90
Steam: moisture ratio	1.2	2.3
Extruder:		
Feeding rate (kg/h)	218	200
Rotations per minute, RPM	536	403
Shaft pressure (bar)	52	37
Temperature, average (°C)	93	90
Steam: moisture ratio	13.1	1.9
Specific mechanical energy, SME (kWh/ton)	52	46
Specific thermal energy, STE (kcal/kg)	97	100
Water at die (g/kg)	240	250
Drier:		
Temperature, average (°C)	94	84
Cycle time (s)	131	145

added as trivalent oxides (Austreng et al., 2000). Days 1–26, feeds labelled with lanthanum were fed to the salmon. During this time, feed intake was measured and faeces for digestibility measurements was collected. On day 27, the salmon were fed the diets added ytterbium. From day 28, the diets added yttrium were used. Faeces were collected at 8 hr' intervals on days 27–29 and analysed chemically. The ratio of concentrations of La, Yb and Y in faeces was used to measure the GI passage rate of the meal given on day 27.

2.1 | Feed production

Two different feeds (Diet 1 and Diet 2) were produced for the trial (at a pilot line by BioMar AS, Tech Centre, Brande, Denmark). The two feeds were produced to have identical formulation but different physical properties, aiming at a difference in water stability. The differences in physical properties were achieved by varying the conditions in the preconditioner, extruder and drier (Table 1). Each feed was produced with three different markers (La, Yb and Y; 0.4 g/kg) and was therefore produced in three batches (in total 6 feeds). The three batches of Diet 1 were denoted Diet 1La, Diet 1Yb and Diet 1Y and the three batches of Diet 2 were denoted Diet 2La, Diet 2Yb and Diet 2Y, indicating which marker was added to the diets. The feeds were produced with commercial-like quality regarding both composition (Table 2 and Table 3) and physical properties (Table 4).

2.2 | Fish trial

The trial was carried out at Nofima's Research Station for Sustainable Aquaculture at Sunndalsøra, Norway. Ten weeks prior

to the experiment, on average 159 salmon were allocated to each of eight tanks (3.3 m³, salinity 32 g L⁻¹, flow-through system, continuous light) for acclimation to the experimental conditions. During this period, the salmon were fed a test feed similar to the experimental feeds.

At start of the trial, four tanks were randomly allocated to each feed. The salmon were fed one meal of one hour's duration per day, from 7 a.m. to 8 a.m. for 29 days. The feeds added La were fed to the

TABLE 2 Formulation of experimental feeds (g/kg feed, "as is")

	Diet 1 and Diet 2
North Atlantic fishmeal	99
South American fishmeal	99
Soy protein concentrate	158
Sunflower expeller	158
Wheat gluten	30
Micronized pea starch	70
Wheat	70
Standard fish oil	208
Rapeseed oil	89
Monocalcium phosphate	9.6
Amino acid mix[a]	6.0
Vitamin and mineral premix[b]	3.2
Marker (La, Yb or Y)	0.4

[a]Balancing the diet according to requirements (National Research Council (NRC), 2011).
[b]BioMar commercial vitamin and mineral premix. Content of vitamins and minerals in feed is in accordance with requirements (National Research Council (NRC), 2011).

salmon days 1–26. On day 27, the feeds added Yb were fed. After that specific meal, the salmon were fed the feeds added Y (days 28–29). Chemical analysis of the three markers (La, Yb and Y) in faeces collected on days 27–29 was used to distinguish which meal the faeces originated from.

During days 1–25, the uneaten feed was collected, and feed intake was estimated as described by Helland et al. (1996). The daily ration for each tank during days 1–25 was decided from the last three day's estimated feed intake, aiming at 20% overfeeding. To assure sufficient feed available for all fish, and sufficient faecal material produced, 50% overfeeding was aimed at from day 26 to day 29.

From day 30, the salmon was fasted before bulk weight was registered on day 33. The mean body weight was 1,047 g.

On days 27, 28 and 29, when faecal material was sampled for the measurement of GI passage rate, the temperature was 11.5, 12.0 and 12.3°C, respectively.

2.3 | Sampling

Faeces for digestibility estimation were collected (Austreng, 1978; Austreng et al., 2000) days 25–26, and the sampled material was pooled by tank. The end of the feeding of day 27 (8.00 a.m.) was denoted time 0. Collection of samples for estimation of GI passage rate started at 8, 16, 24, 32, 40 and 48 hr after the feeding on day 27. Each of these samplings lasted for 30 min. Time 0 was represented by faeces sampled on days 25 and 26, assuming that marker ratios in faeces were constant until the switch of feeds on day 27.

Faeces were collected in a wire mesh (openings < 1 mm) container at each tank outlet. The containers were quickly emptied, rinsed and returned to the tank outlet at 5-min intervals. All faecal samples were at stored at −20°C until freeze-drying.

TABLE 3 Chemical composition of experimental feeds

	Diet 1La	Diet 1Yb	Diet 1Y	Diet 2La	Diet 2Yb	Diet 2Y
Dry matter (g/kg)	941	944	932	949	949	956
In dry matter:						
Lipid (g/kg)	365	353	359	354	347	335
Nitrogen (g/kg)	54.9	55.9	54.8	55.8	55.2	56.3
Crude protein (Nx6.25, g/kg)	343	350	343	349	345	352
Ash (g/kg)	59.8	61.6	61.0	62.2	63.0	63.5
Energy (MJ/kg)	267.8	262.2	264.4	261.2	261.3	258.4
Phosphorus (g/kg)	11.5	11.7	11.7	11.8	11.9	12.0
Zinc (g/kg)	0.16	0.15	0.16	0.16	0.19	0.17
Digestibility markers, in dry matter):						
Lanthanum (g/kg)	0.35	0.07	0.01	0.39	0.03	–
Ytterbium (g/kg)	–	0.37	0.02	–	0.37	–
Yttrium (g/kg)	0.02	0.01	0.39	0.02	0.02	0.41

Abbreviation: -, not detectable.

TABLE 4 Physical quality of experimental feeds

	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^1)	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)	128.5 (18.9)	140.5 (17.5)	152.1 (16.4)	148.2 (11.8)	172.0 (14.2)	153.3 (14.0)
Water stability test (remaining dry matter, g/kg):	786 (28)	769 (65)	779 (23)	849 (14)	85.2 (13)	818 (54)
Durability:						
Ligno test (g/kg)	982 (6)	982 (1)	992 (0.4)	982 (1)	983 (0.4)	981 (1)
DORIS test:						
Whole pellets (g/kg)	646 (40)	659 (27)	816 (21)	801 (6)	814 (7)	783 (4)
Fracture (g/kg)	300 (39)	290 (21)	159 (16)	166 (12)	153 (9)	186 (4)
Fines (g/kg)	54 (4)	51 (7)	25 (5)	33 (6)	32 (3)	31 (3)
Fat leakage (g/kg)	62 (5)	64 (2)	66 (4)	36 (5)	35 (6)	35 (3)

Data are given as mean with standard deviation in brackets.

2.4 | Chemical analysis

Faeces were freeze-dried prior to analysis. Feeds and faecal samples used for digestibility estimation (collected on days 25 and 26) 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, nitrogen (Kjeltec Auto System, Tecator, Höganäs, Sweden) and crude lipid (Soxtec hydrolysing and extraction systems, Tecator, Höganäs, Sweden). Gross energy was measured by bomb calorimetry (Parr 1,271 Bomb Calorimeter; Parr Instrument Company, Moline, Il, USA). Minerals and markers (La, Yb and Y) in feeds and all faecal samples were analysed with ICP-OES (Perkin Elmer Optima 5,300 DV; Perkin Elmer, Inc, 2004 Shelton, USA) or ICP-MS (8,800 Triple Quad, Agilent; Santa Clara, CA, USA) after decomposition in concentrated HNO_3 at 260°C (UltraClave, Milestone Microwave Ultraclave III; Milestone, Sorisole, Italy) and thereafter diluted to 10% HNO_3.

2.5 | Measurement of physical feed properties

Diameter and length of 20 pellets per feed were measured with an electronic calliper. Bulk density was measured three times pre-feed by loosely pouring the into a 1,000-ml measuring cylinder. The top was gently flattened before measuring the weight. 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/s and the penetration depth 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 measured major break of the pellet (the peak force) was given in Newton (N). Twenty pellets from each of feed were measured. The pellets were abraded with sandpaper P120 prior to measurements in order to make the pellet stand in an upright position. The water stability of

the feeds was measured with a modified version of the method of Baeverfjord et al. (2006). Briefly, four replicates of 20 g of each feed were 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, 2.4.9 cm swing distance) for 120 min at 23°C, and remaining dry matter (g/kg) was measured. The mechanical pellet durability was measured in a Ligno tester (LT-II; Borregaard Lignotech, Sarpsborg, Norway). Feed samples (100 g feed, in triplicate) without dust or broken pellets were run for 90 s in the Ligno tester. Subsequently, the samples were sifted and weight of the intact pellets was recorded. The durability was given as g per kg of intact sample. The Doris Durability Index (DDI) was measured in an AkvaMarina DORIS Feed Tester (Aquasmart ASA, Bryne, Norway) as described by Aas, Oehme, et al. (2011). Briefly, presieved samples (triplicate) of 350 g pellets were placed in the DORIS Feed Tester, conveyed by a screw onto a rotating paddle and collected in an accumulation container. The different size fractions were separated on three sieves (8.00, 5.60 and 2.36 mm) and given as g per kg of whole pellet (>8.00 mm), fracture (2.36–8.00 mm) and fines (<2.36 mm). The test was run in triplicate. Fat leakage was given as the loss of fat from the feeds. Triplicate samples of 75 g feed in a plastic container lined with blotting paper were incubated at 40°C for 24 hr. The amount of fat leaked was given as g per kg of the sample.

2.6 | Calculations

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

Feed intake (DM basis) = Feed fed (g, DM) – [Uneaten feed (g, DM)/ Recovery], where.

Recovery = Feed spill (g, DM)/ Feed used (g, DM).

Recovery was estimated by following the experimental feeding routines, but with no fish in the tanks. DM = dry matter.

Apparent digestibility (%) = 100 · (a − b)/ a, 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 [X]/ ([La] + [Yb] + [Y]), where X represents one marker (La, Yb or Y).

2.7 | Statistical analysis

Tank was used as the statistical unit. Data are given as mean ± SEM unless otherwise is specified. Data were analysed by comparing the two feed groups with a one-way ANOVA (t test) at each sampling time. Differences were considered significant if $p \leq .05$ and were reported as a trend if $.05 < p < .1$. All statistical analyses were performed with the SAS computer software (SAS 1985; SAS Institute Inc, Cary, USA).

3 | RESULTS

3.1 | Feed intake and body weight

The mean final body weight of the fish, measured on day 33, was almost identical for salmon fed Diet 1 and Diet 2 (1,046 and 1,048 g, respectively). No significant differences in feed intake between salmon fed Diet 1 and Diet 2 were found (Table 5). On day 27, when the sampling started, the feed intake for salmon fed both feeds was estimated to 8 g per individual.

3.2 | Apparent digestibility

The apparent digestibility was measured in faeces from days 25 and 26 of the trial when feeds added La were used (Table 6). The apparent digestibility of lipid was significantly higher in Diet 1La (94.1 ± 0.5%) than in Diet 2La (92.3 ± 0.3%; Table 6). No other significant differences in digestibility were found.

3.3 | Gastrointestinal passage rate

Marker levels in collected faeces are shown in Figure 1, given as the ratio between the concentration of each single marker relative to the concentration of sum of all three markers (La, Yb and Y). Prior to these measurements, the salmon were fed diets added La. Correspondingly, at time 0 faeces from salmon fed both feeds contained mainly La (Figure 1a).

At time 0, salmon was fed the feeds added Yb, and eight hours later, Yb was found in faeces from salmon fed both Diet 1 and Diet 2 (Figure 1a,b). At this time, the amount of Yb in faeces had increased slightly and the amount of La decreased correspondingly. At time

TABLE 5 Individual feed intake (g per individual, dry matter) in Atlantic salmon fed two feeds with different physical properties.

	Diet 1La	Diet 2La	p-value
Total feed intake days 1–26	104 ± 4	108 ± 6	.5680

The feed intake is given as total feed intake for 26 days with feeds added La (Mean ± SEM, n = 4).

TABLE 6 Apparent digestibility (%) of nutrients and energy in Diet 1La and Diet 2La fed to Atlantic salmon (Mean ± SEM, n = 4)

	Diet 1La	Diet 2La	p-value
Dry matter	66.7 ± 1.5	66.8 ± 0.8	.9648
Lipid	94.1 ± 0.5[a,b]	92.3 ± 0.3[a,b]	.0175
Nitrogen	91.4 ± 0.5	90.7 ± 0.5	.3067
Energy	85.2 ± 0.9	84.2 ± 0.5	.3491
P	39.2 ± 2.8	39.7 ± 2.4	.9030
Zn	21.0 ± 4.8	22.8 ± 3.4	.7678

[a,b]Significant differences (p ≤ .05) of means within a row are indicated with different letters.

16 hr, there was a large increase in the Yb levels in faeces, significantly higher in salmon fed Diet 1 than those fed Diet 2. The La levels dropped correspondingly. Thus, in the time interval 8–16 hr after switching to feeds containing Yb, and when the concentrations in marker ratios change fastest, Diet 1 passed faster through the GI system than Diet 2 (Figure 1a,b).

The Yb levels peaked at the sampling 24 hr after feeding feeds containing Yb, showing that at this sampling, the faeces contained the highest amount of material from the meal given at time 0 (Figure 1a,b). At this time, the highest Yb level and lowest La levels were found in faeces of salmon fed Diet 2.

For the time interval from time 24 hr (when the feeds added Y were administered) to 48 hr, (which corresponds to the time interval 0–24 hr), the picture was more blurred (Figure 1a–c). The difference in several of the measured physical properties between Diet 1Y and Diet 2Y was smaller than the difference between Diet 1Yb and Diet 2Yb. Thus, the feeds added Y did not produce the same results as the feeds added Yb.

For salmon fed both feeds, Yb was almost completely evacuated from the intestine after 48 hr.

4 | DISCUSSION

The method used in this trial, labelling the feeds with different markers and measure marker concentration in faeces, allowed studying GI passage rate in undisturbed fish. Stress has an impact on digestive processes (Bolasina et al., 2007; Chen & Fernald, 2008; Oxley et al., 2010; Peters, 1982). Results in studies of GI passage rate are therefore affected by sampling and handling the fish. The salmon were fed throughout the sampling period, maintaining the normal
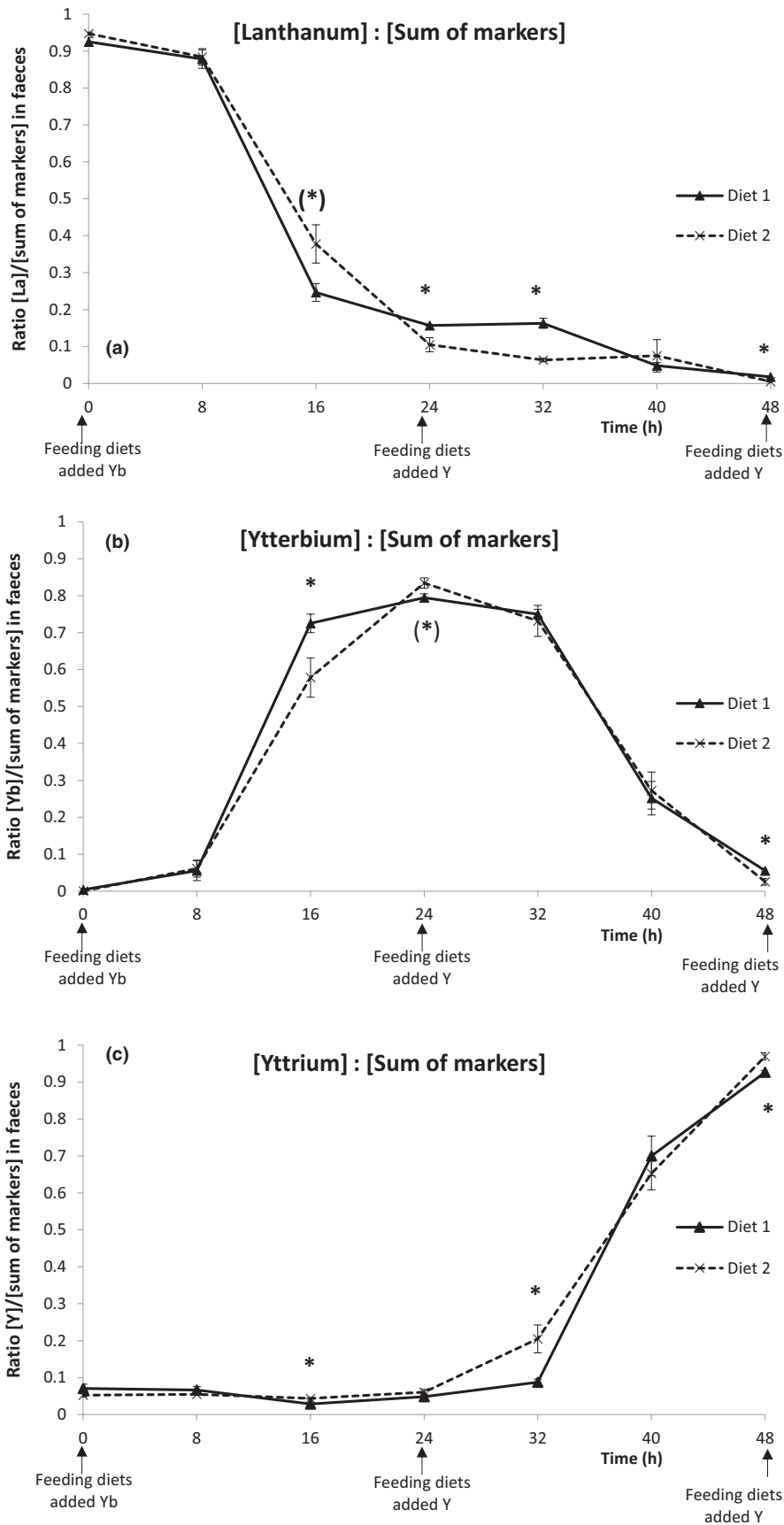
FIGURE 1 Marker ratios in faeces. Ratio of concentration of lanthanum (a), ytterbium (b) and yttrium (c) relative to the total concentration of markers in faeces from Atlantic salmon sampled 8, 16, 24, 32, 40 and 48 hr after a meal with feeds added Yb (time 0). The salmon were fed feeds added Y at 24 and 48 hr. Prior to time 0, the salmon was fed feeds added La. (mean $\pm$ SEM, $n = 4$). Significant differences ($p \leq .05$) are indicated with an asterisk. Trends ($0.05 < p < .1$) are indicated with an asterisk in brackets

nutritional state. The main limitation of the method is that only the total GI passage rate could be measured. Digestive processes in the different segments of the GI system could not be studied separately.

Production of feeds with different markers implies either producing several batches of each feed with the marker mixed in the ingredients (as was chosen in the present study) or coating the feed with the markers postextrusion. Coating introduces a source of error from inhomogeneous distribution of marker in the single pellet, presumably with the highest concentration near the pellet surface (Hatlen et al., 2015). Feed pellets are processed in the salmon's stomach by disintegration and removal of particles from the surface layer (Aas et al., 2017; Aas, Terjesen, et al., 2011; Andersen & Beyer, 2005). Coated markers may thus leave the stomach at a higher rate than other feed components (Hatlen et al., 2015). When producing each feed in separate batches, a certain variation in physical properties between the batches is inevitable. In the present trial, similar water stability among the three batches was obtained for both feeds, and lower in Diet 1 than in Diet 2, as intended. Hardness and Doris durability of Diet 1Y were similar to the measured values of the three batches of Diet 2. The feeds containing Y were therefore not expected to have the same properties in the salmon's GI system as the feeds added Yb. The feeds added Y were fed from time 24 hr. The results from the samplings after that therefore represent GI passage rate in general and do not show the effects of different physical feed properties.

It should be noted that due to the complexity of the extrusion process, obtaining the specified physical characteristics of the feed pellet as desired in this study is challenging even for the most experienced operators. To be able to study the effect of physical pellet quality, some compromises between what is desirable and what is achievable have to be accepted.

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

When collecting faeces at the water outlet, there is a risk of nutrients leaking from faeces to the water (Storebakken et al., 1998). In the present trial, the sieves were emptied every five minutes to minimize loss of material from the collected faeces. The error from leakage was assumed to be equal for all samples.

It has been shown previously that feed intake in salmonids is affected by the physical properties of feed, possibly caused by the gastric evacuation rate (Aas et al., 2017, 2020; Aas, Terjesen et al., 2011; Oehme et al., 2014; Sveier et al., 1999). Feed intake seems to be a key factor when searching for the optimal pellet quality. It has also been shown that mineral utilization in salmonids is affected by the physical properties of the feed (Aas et al., 2017, 2020; Aas, Terjesen, et al., 2011). Feeds can be produced with an infinite number of physical pellet qualities, and several different methods are used for measuring different properties of the pellets. It is poorly documented which of the measurements of physical pellet properties that can predict feed intake, and the available data are conflicting. In rainbow trout, a feed with low water stability resulted in approximately 20% higher feed intake compared to a feed with high water stability (Aas, Terjesen, et al., 2011). Those data indicated that water stability may be a key parameter for feed intake in rainbow trout, which was confirmed in Atlantic salmon (Oehme et al., 2014). Diet 1 in the present study was therefore produced to have lower water stability than Diet 2. This also gave lower bulk density, lower hardness and higher fat leakage in Diet 1 than in Diet 2. The durability measured with the Ligno test was similar among the two diets, whereas the pellet breakage was larger in Diet 1 than in Diet 2 in the DORIS test.

In the present study, no significant difference in feed intake was found although the two feeds differed in water stability. In the above-mentioned study with rainbow trout (Aas, Terjesen, et al., 2011), the difference in water stability between the two feeds was considerably larger than in the present study.

The apparent digestibility of lipid was approximately 2% higher in Diet 1La than in Diet 2La in the present study. Aas, Terjesen, et al. (2011) found differences in apparent digestibility of nutrients in rainbow trout fed two feeds with different water stability. In that study, the difference in feed intake was large, and it was unknown whether the difference in nutrient digestibility was caused by the pellet quality itself or by the difference in feed intake. Oehme et al. (2014) found that high feed intake may reduce the apparent digestibility of nutrients in Atlantic salmon. Since there were no differences in feed intake in the present study, the difference in lipid digestibility can be ascribed to the feeds. For the specific feeds used in this trial, the lipid digestibility was highest in Diet 1, which had the lowest water stability and pellet hardness.

The apparent digestibility of minerals is generally variable and depends on the mineral status of the fish. In the present trial, the apparent digestibility of phosphorus and zinc was similar for the two feeds, indicating no difference in mineral utilization in the fish. The present trial was not designed to measure digestibility of minerals, and these data should be used with care.

At the time when passage rate was measured, the feed intake was close to 0.8% of body weight per day. Assuming an expected feed conversion ratio (g dry matter of feed eaten/ g fish growth) of 0.9 for salmon of this size, the growth would be close to expected value according to Austreng et al. (1987) and relevant for normal feed intake under commercial farming conditions. The feed intake during days 1–26 was lower than this, indicating an initial low feed intake, which is normal in Atlantic salmon when introduced to a new feed.

The GI passage rate varies among fish species and with temperature (Fänge & Grove, 1979). The GI passage rates found in the present study correspond with data from a similar study (Sveier et al., 1999). In that study, Atlantic salmon were fed feeds produced from coarse, standard or microground fishmeal. Twelve hours after feeding, marker concentrations indicated that gastric emptying was fastest for feed with standard fishmeal and slowest for feed with coarse fishmeal. Correspondingly, marker concentration in hind gut was highest in salmon fed feeds with standard ground fishmeal and lowest in fish fed feed with coarsely ground fishmeal 12 hr after feeding (Sveier et al., 1999). In the present

trial, a difference in GI passage rate between the two feeds was found in the interval from 8 to 16 hr after a meal. During this time interval, the faeces shifted from being dominated by La, to being dominated by Yb from the meal at time 0 hr. This indicates that a substantial portion of the feeds fed at time 0 hr has passed through the GI system within 16 hr after feeding, and Diet 1 at a significantly higher rate than Diet 2.

5 | CONCLUSION

Two feeds with different physical properties had similar passage rates through the gastrointestinal system of Atlantic salmon, but the passage rate was significantly different for the two feeds at samplings 8 and 16 hr after feeding. The difference in apparent digestibility was only found for lipid. There was no difference in feed intake. The study shows that the physical properties of salmon feed may have an impact on the gastrointestinal passage rate and on lipid digestibility. Therefore, the physical feed properties are important in evaluation of commercial feed and in studies of fish nutrition.

DATA AVAILABILITY STATEMENT
The data used to support the findings of this study are available within the paper.

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