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Measurement of gastrointestinal passage rate in Atlantic salmon (*Salmo salar*) fed dry or soaked feed

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ABSTRACT

A method for measurement of gastrointestinal passage rate was tried in Atlantic salmon, *Salmo salar* (body weight 1131 g, temperature 13.5 °C, salinity 32‰). Salmon were force fed one single ration (10 g) extruded feed 'as is' (92% dry matter) or soaked 2 h in sea water (70% dry matter), in triplicate with three individuals per replicate. Content from stomach, small intestine and distal intestine was collected at 2, 6, 12, 18, 24 and 48 h after feeding.

Two hours after feeding, significantly more feed was transferred from the stomach to the small intestine in salmon fed soaked feed than in those fed dry feed. After that, no significant differences in gastrointestinal passage rate were found. Numerically however, soaked feed seemed to pass through the gut faster than dry feed.

The content in stomach declined gradually, and all stomachs were empty after 48 h. Salmon in both treatment groups used 6–12 h on average to empty the stomach 50%. The content in small intestine peaked at the sampling 12 h post feeding for salmon fed both feed types. After 48 h, the small intestines were empty in all three replicates of salmon fed soaked feed, and in one replicate fed dry feed. The largest amounts of dry matter in the distal intestine were found between 12 and 24 h for both feed groups. Some dry matter was still present in the hindgut after 48 h.

Individual variation in the gastrointestinal passage rate was large and few significant differences were revealed. However, soaked feed gave a significantly higher gastric evacuation rate than dry feed shortly (2 h) after feeding. As the fish regurgitated some pellets, the method may be most suitable when feeding small rations. Besides, due to variation a larger number of replicates would increase the power of the test.

1. Introduction

Commercial salmon farming has developed towards increasingly larger units, which require technology and logistics to handle large amounts of fish, and thus, large amounts of feed. Transport and storage of feed pellets in bulk require pellets with robust physical quality to avoid formation of fines and dust during handling and feeding (Aas et al., 2011a; Oehme et al., 2012). However, previous studies have shown that the physical properties of feed affect the biological response in the fish (Aas et al., 2011b; Baeverfjord et al., 2006; Glencross et al., 2011; Morken et al., 2011; Sveier et al., 1999; Venou et al., 2009). If this is not taken into consideration, the physical feed quality may be suboptimal for the salmon's full capacity for growth, resulting in a loss in commercial farming. Feed costs represent more than 50% of the cost in Norwegian aquaculture (Zahirovic, 2012). Furthermore, sustainable food production depends on effective use of feed ingredients. Thus, the optimal feed meets the requirements with regard to both logistic and feeding systems, and to nutritional response in the fish.

Feed utilization in Atlantic salmon is highest at high feed intake (Einen et al., 1995, 1999; Grisdale-Helland et al., 2013). Feed intake may thus be a key factor when searching for the optimal physical feed quality. However, a stable high feed intake depends on a corresponding high transit of material through the gastrointestinal (GI) system. Oehme et al. (2014) reported that feed intake increased in Atlantic salmon when the feed was soaked prior to feeding, particularly when the appetite in general was low. Furthermore, Aas et al. (2011b) showed that

Abbreviations: GI, gastrointestinal; AD, apparent digestibility; RD, relative disappearance

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feed intake was approximately 20% higher in rainbow trout (*Oncorhynchus mykiss*) fed a feed with low water stability compared to a feed with high water stability. This leads to the hypothesis that the feed intake, as long as below maximum feed intake, may be higher in fish fed pellets that absorb water well and disintegrate rapidly in the stomach compared to pellets with the same composition that disintegrate slowly. Thus, increased feed intake in salmon fed soaked pellets (Oehme et al., 2014) may be due to faster disintegration of feed in the stomach, and thus faster passage through the GI tract, than in salmon fed dry pellets.

Appetite regulation in mammals, and particularly in humans, has been studied extensively and involves molecular, hormonal and neural signaling (reviewed by Gibbons and Blundell, 2015). Many of the same signaling substances and mechanisms are also found in fish (e.g. Ji et al., 2015; Murashita et al., 2008; Rønnestad et al., 2010), although proper understanding of how fish regulate feed intake is still missing. Amino acids are among the most studied attractants for fish, and salmonid fish have been shown to respond to a few amino acids. Free amino acid mixes are often used as feeding stimulants at the early life stages of aquatic organisms. In larger fish fed plant-based diets, these compounds are added to provide adequate feed intake response (Dias et al., 1997). Possibly, water soluble components such as amino acids or other feed stimulation components that are released or made more accessible during soaking may thus contribute to increase feed intake in fish fed soaked feed.

The drinking rate varies with fish species, water salinity and temperature (Eddy and Bath, 1979; Evans, 1968; Lega et al., 1992). In salmonid fish, more pronounced in rainbow trout than in Atlantic salmon, a distended stomach is seen in relation to water belly, fat regurgitation and osmotic stress, and rapid disintegration of pellets in the stomach together with stomach distention might be an activator of negative feedback mechanism which slows stomach emptying (Anderson, 2006).

Several methods have been applied to study gastric evacuation rate and GI passage rate in fish (Bromley, 1994; Fänge and Grove, 1979). This includes gravimetric methods, X-radiography, use of isotopes, and dye or inert markers. The GI passage rate is also reflected in the plasma free amino acids after a meal (Karlsson et al., 2006; Yamamoto et al., 1998). The rate of digestive processes is affected by temperature (dos Santos and Jobling, 1991; Miegel et al., 2010) and feed composition (Storebakken et al., 1999; Sveier et al., 1999), but also by meal size (dos Santos and Jobling, 1991; Garber, 1983). Ideally, all fish in the trial should therefore have the equal feed intake when studying GI passage rate. This can only be achieved by force feeding, which causes stress, which again affects the digestive processes in the fish (Bolasina et al., 2007; Peters, 1982). Likewise will sampling of fish from a tank at certain points in time cause stress to all fish in the tank. Use of non-invasive sampling methods, such as x-ray of GI content, theoretically allow the use of the same fish at all sampling points, but repeated sedation and handling will also cause stress. On the other hand, methods which allow each fish to be sampled only once, such as using blood samples, or euthanizing and dissecting the fish, require a large number of sampled fish. Collection of faeces directly from water (Storebakken et al., 1999) is non-invasive and can be performed with undisturbed fish, but give solely information of total GI passage rate, and processes in stomach and intestine cannot be studied with this method. Evidently, the various methods for measuring GI passage rate in fish all have weaknesses and limitations. Such measurements are therefore challenging and involve compromises, and a common, standard method for measurement of GI passage rate in fish is missing.

The present study had two objectives. The main objective was to test whether the increased feed intake seen in salmon fed soaked feed compared to dry feed (Oehme et al., 2014) could be ascribed to an increased GI passage rate. Secondly, a method for measurement of GI passage rate was tried and assessed. Force feeding one defined feed ration, and thereafter sampling fish at given times to collect gut content was chosen as method in the present study. Atlantic salmon were given one meal of dry (as is) or soaked feed. The feeds and the soaking procedure were the same as used in the previous study by Oehme et al. (2014), and to the feeds yttrium oxide was added as digestibility marker. The content of stomach, small intestine (pylorus and mid intestine) and distal intestine was sampled 2, 6, 12, 18, 24 and 48 h post feeding, weighed, and analyzed for digestibility marker, dry matter, nutrients and energy.

2. Materials and methods

2.1. Feeds

A commercial-like feed was used for the experiment. The feed was formulated to contain 20% fishmeal, 15.5% soy protein concentrate, 3% wheat gluten, 15.5% sunflower expeller, 15.2% dehulled bean, 21.1% fish oil, 9% rapeseed oil, 1.26% monocalcium phosphate, 0.36% amino acids, 0.23% mineral and vitamin mix and 0.05% yttrium oxide as an inert digestibility marker (Austreng, 1978; Austreng et al., 2000). The feed resembled an average commercial salmon feed in composition. The feed was fed to fish either as is (also denoted 'dry'), or the ration was soaked in sea water (salinity 32‰) at 4 °C for two hours prior to feeding. The feed was produced by BioMar AS (Tech Centre, Brande, Denmark). The chemical composition of the dry and soaked feed is shown in Table 1, and the physical properties of the feeds are given in Table 2. The feeds are further described by Oehme et al. (2014).

2.2. Fish trial and sampling

Atlantic salmon with mean weight 1131 g (range 900–1449 g) were used for the experiment. Prior to the experiment, the fish were kept under continuous light in 1 m^2 tanks supplied with sea water (salinity 32‰, oxygen saturation > 80%) in a flow through system and fed commercial feed (Skretting, Stavanger, Norway). In order to empty the intestine prior to the trial, the fish were fasted from two days (Storebakken et al., 1999; Sveier et al., 1999) prior to the start of the experimental feeding schedule. During the four days the trial lasted, the water temperature (measured once daily) ranged from 13.3 to 13.6 °C.

Before force feeding, the fish were completely anaesthetized with Finquel MS-222 (tricaine methanesulfonate, 50 mg/L, Scan-Vacc, Hvam, Norway). The anaesthetized fish were force fed with 10 g rations (soaked feed was weighed before soaking), which was gently pushed into the stomach through a tube. For some fish, the stomach appeared full before all feed was fed, and for these fish, the full ration was not forced into the stomach.

Units of three fish were used for each pooled sample. After feeding one unit of three individuals, these three fish were placed in a separate tank (1 m^3) for a given time (2, 6, 12, 18, 24 or 48 h). In the following, 'tank' refers to a unit of three fish placed in the same tank after feeding.

Table 1
Chemical composition of experimental feeds. Data are given as g kg^{-1} or MJ kg^{-1}.

	Dry feed (as is)	Soaked feed
Dry matter	923	695
In dry matter:		
Crude protein (Nx6.25)	371	372
Sum of amino acids[a]	306	294
Crude lipid	351	355
Starch	57.0	55.8
Energy	26.0	26.2
P	10.7	10.4
Mg	2.1	2.5
Na	3.2	5.8
Fe	0.21	0.26
Zn	0.17	0.17
Y_2O_3	0.45	0.45

[a] Amino acids are given as dehydrated residuals.

Table 2
Physical properties of experimental feeds.

	Dry feed (as is)	Soaked feed
Diameter, mm	10.7	10.6
Hardness, N	52.3	54.3
Water stability[a], %	92.1	93.0

[a] Remaining dry matter after 240 min of water stability test.

The trial was run in triplicate. Thus, for each sampling point, three tanks were used for salmon fed dry feed, and three tanks for salmon fed soaked feed, in total 6 tanks and 18 fish at each sampling point. All fish were fasted from the same point of time. To achieve the desired sampling points and replicates within the available 1 m^3 tanks, force feeding took place over two consecutive days. Thus, fish sampled 2, 6 or 24 h after feeding were fed after being fasted 2 days, whereas fish sampled 12 and 18 h after feeding were fed after 3 days fasting.

Some fish regurgitated pellets, which were collected in sieves at the outlet of the water. These pellets, and pellets from the ration that were not fed into the fish, were counted, and average pellet weight (0.875 g) was used to estimate the feed intake for each tank.

At the relevant time (2, 6, 12, 18, 24 or 48 h after feeding), all three fish from a tank were given a lethal dose of anesthetic. The GI tract was removed and closed with artery clamps in both ends and immersed in liquid N to avoid leakage of the content. Subsequently, the GI tract was wrapped in aluminum foil and kept frozen at −20 °C until analysis. Later, the GI tracts were partly thawed, and the content collected. The content was divided in three: content from stomach, content from small intestine (pylorus and mid intestine; from the pyloric sphincter to the appearance of transverse luminal folds and increased diameter), and content from distal intestine (from the appearance of transverse luminal folds and increased diameter to the anus). The samples were pooled by tank and weighed, and the content was frozen before freeze drying and chemical analysis. The GI content was collected as completely as possible. However, some loss was inevitable, and the content of the pyloric caeca could not be collected.

The fish trial was approved by the Norwegian Animal Research Authority.

2.3. Measurements of physical feed quality

Pellet hardness was analyzed by diametrical compression using a texture analyzer (TA-XT2, Model 1000 R, SMS Stable Micro Systems, Blackdown Rural Industries, Surrey, UK) as summarized by Aas et al. (2011a). For each diet, 35 pellets were analyzed, and strength at rapture (N) was recorded when the pellet cracked. The texture analyzer also recorded the diameter on the pellets used for the hardness measurements. Water stability was measured with a modified version of the method described by Baeverfjord et al. (2006). Three replicate samples of 20 g of dry or soaked pellets were placed in a custom made steel-mesh placed inside glass beakers containing 300 ml distilled water. The beakers were shaken (100 shakings per minute, 2 × 4.9 cm swing distance per shaking) in a water bath at 23 °C. After 240 min shaking the retained dry matter was measured.

2.4. Chemical analyses

The samples of GI content were weighed, freeze dried and homogenized prior to analysis. Dry matter was estimated by further drying the samples at 105 °C to constant weight. The water loss during freeze drying was included in dry matter estimation. The samples were analyzed for ash by combustion at 550 °C to constant weight, crude protein (nitrogen × 6.25, Kjeltec Auto Analyser) and crude lipid (SOXTEC hydrolyzing and extraction systems). Gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter), and yttrium oxide and minerals were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS, at Eurofins, Moss, Norway). Feeds were analyzed likewise, but without freeze drying.

The fish were fasted before and after the one experimental feeding, which implies samples of zero or small amounts of gut content at some of the sampling points. Since there was not sufficient material for complete chemical analyses at all sampling points, the analyses were prioritized by the following order: 1) dry matter, yttrium oxide and minerals (all from one sample preparation); 2) nitrogen; 3) energy; and 4) crude lipid (requires the largest sample size). As all samples could not undergo complete analysis, the dataset contains some missing values, not to be confused with the value zero at sampling points with no content present in the gut.

2.5. Calculations

1) Apparent digestibility of nutrients and energy were calculated as

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.

2) The calculation of relative disappearance was calculated equivalent to the calculation of apparent digestibility, except that it was calculated from the concentrations in stomach and in small intestine, whereas apparent digestibility was calculated from the concentrations in distal intestine (faeces).

3) Feed intake for each tank (3 individuals) was estimated as

Feed intake (g) = Feed ration (10 g × 3) − [(No of pellets not fed + No of pellets regurgitated)·0.875 g], where 0.875 g is the average weight of one pellet. For dry matter basis, the feed intake was corrected for dry matter content of the dry (as is) feed (923 g kg^{-1}).

4) For control of the estimated retention of dry matter in the gut, the feed intake was also calculated from total analyzed Y_2O_3:

Feed intake (g) = [(Y in stomach + Y in small intestine + Y in hindgut)(g)]/[Y in feed (%)/100]

Calculation 4 is only valid before Y_2O_3 in faeces is excreted. Digesta appeared in the hindgut 12 h post feeding (very small amounts were found in two out of eighteen sampled fish at 6 h). Thus, this calculation was only used to estimate feed intake at 2, 6 and 12 h after feeding.

2.6. Statistics

Tank (the three fish in each replicate) was the statistical unit in the dataset. The data are given as mean ± S.E.M.

The data were analyzed by comparing the two feed groups with an ANOVA (*t*-test) at each sampling time (2, 6, 12, 18, 24 and 48 h after feeding). Differences were considered significant if $P < 0.05$. If $0.05 < P < 0.1$, this was reported as a trend.

For data given as percentage, an ANOVA was also performed for log-transformed data. Significant differences are given based on the original data, but only if confirmed by a significant difference ($P \leq 0.05$) or trend ($0.05 < P < 0.1$) in the log-transformed data.

The exact time for each sampling was recorded so that any effects of deviation from the intended time schedule could be assessed statistically. A linear regression analysis using exact time after feeding as a continuous variable was performed for data at 2 and 6 h after feeding. After this, small deviations in time were considered not to affect the results. Significant effect of time indicates that deviation in sampling time affected the results. The results from regression analysis are referred to if significant.

The estimated feed intake for salmon fed dry or soaked feed was also analyzed with a linear regression, using time of sampling (hours after feeding) as independent variable.

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

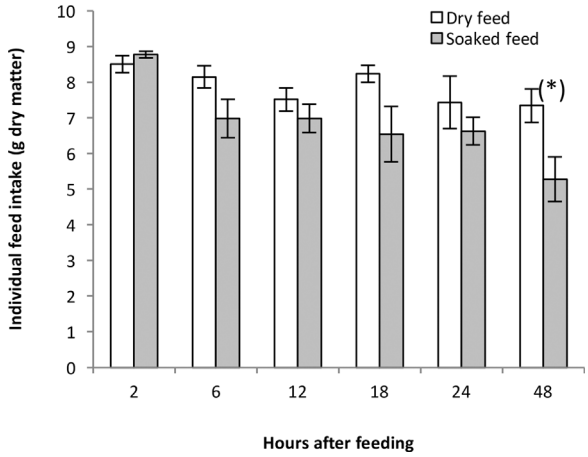

Fig 1. Estimated feed intake in Atlantic salmon fed one meal of dry or soaked feed, given as g dry matter per individual. Calculated by subtracting regurgitated pellets and not fed pellets from the intended ration (10 g). The feed intake in salmon fed dry and soaked pellets was compared with an ANOVA (*t*-test) at each sampling point. Asterisk in brackets indicates a trend ($P < 0.1$). Data are given as mean ± SEM (n = 3).

3. Results

Large individual variation in GI evacuation rate was observed visually. E.g. at 18 h after feeding, one fish fed dry feed still had feed in the stomach, while another fish from the same tank had almost emptied the GI tract.

Regurgitated pellets were collected at all samplings, showing that the salmon regurgitated pellets for more than 24 h after force feeding. Correspondingly, the estimated feed intake decreased throughout the experiment (significant linear regression model). Forty-eight hours after feeding, feed intake tended ($P < 0.1$) to be lower in salmon fed the soaked than in those fed the dry (as is) pellets, indicating that the salmon regurgitated more of the soaked than the dry pellets (Fig. 1).

3.1. GI passage rate of dry matter

The amount of dry matter found in stomach, small intestine, and hindgut is shown in Fig. 2 (left panels), given as % of ingested dry matter (calculation 3). At 2 h, significantly smaller amounts of dry matter were present in stomachs of salmon fed soaked feed than in those fed dry feed (77 ± 3 and 92 ± 1% of ingested dry matter, respectively). Correspondingly, the amount of dry matter in small intestine was significantly higher in fish fed soaked feed compared to those fed dry feed at this time. At 2 h after feeding, a significant linear regression model indicated that the decrease in dry matter in the stomach of fish fed soaked feed compared to fish fed dry feed was influenced by deviation in time of sampling. In the small intestine however, there was no effect of deviation in time. It can thus be concluded that the gastric evacuation rate 2 h after feeding was significantly higher in fish fed soaked feed compared to those fed the dry feed. At the other sampling times, and in the distal intestine, there were no significant differences in dry matter content (% of ingested) between the two treatment groups (Fig. 2).

As a control of the estimates, the dry matter in stomach, small intestine and distal intestine was also calculated using the total analyzed Y_2O_3 as an estimate for feed intake (calculation 4). This calculation was only valid for data from 2, 6 and 12 h after feeding, before any material had evacuated the gut. These estimates (Fig. 2, right panels) corresponded well with the estimates where feed intake was calculated from the feed ration corrected for uneaten pellets (Fig. 2).

3.1.1. Gastric emptying

As Fig. 2 (left panels) shows, the content of dry matter in the stomach declined gradually, and all stomachs were empty after 48 h after feeding. At 6 h, 55 ± 4% and 77 ± 3% of ingested dry and soaked feed, respectively, was still present, whereas at 12 h, less than 30% of ingested dry matter was present in fish fed either feed. This shows that salmon uses between 6 and 12 h on average to empty the stomach 50% after a single meal under the conditions used in this trial. Twenty-four hours after feeding, the amount of dry matter in stomachs of salmon fed dry and soaked feed was 8 ± 4% and 1 ± 0%, respectively, of ingested dry matter (not significantly different).

3.1.2. Small intestine

The content in the small intestine peaked at the sampling 12 h post feeding for both feed groups (Fig. 2, left panels). After 48 h, the small intestine was emptied in fish from all tanks with salmon fed soaked feed, and from one tank fed dry feed. Although not significant, the soaked feed thus seemed to pass completely through the small intestine somewhat faster than the dry feed.

3.1.3. Distal intestine

For both treatment groups, the first appearance of material in the hindgut was observed at the sampling 6 h post feeding (Fig. 2, left panels). The largest amounts of dry matter in the hindgut were found in the samplings from 12 to 24 h after feeding for both feed groups. Some material was still present in the hindgut 48 h after feeding, although many individuals had emptied the GI tract completely at this time. Again, a numerically, but not statistically significant, higher amount of ingested dry matter was found in the hindgut at 48 h after feeding in fish fed dry feed, compared to those fed soaked feed.

3.2. GI passage rate of nutrients and energy

The content of stomach, small intestine and distal intestine was also analyzed for Y_2O_3, energy, N, crude lipid and minerals (Tables 3–5). Significant differences between salmon fed dry or soaked feed were only found in stomach and small intestine 2 h after feeding. The remaining data (content in stomach and small intestine 6–48 h after feeding and in distal intestine at all samplings) in Tables 3–5 are therefore given as overall mean for both treatments. For most of the measured feed components, the picture was similar to that of dry matter (Fig. 2), but in small intestine and distal intestine there was not sufficient material for analyses at all sampling points. However, the amount found (% of ingested materials) varied among the different components. At 2 h after feeding, the amount present in the stomach ranged from 87% of ingested Zn to 99% of ingested Y_2O_3 in salmon fed the dry feed. In those fed the soaked feed, the range was from 73% of ingested lipid to 85% of ingested Y_2O_3. This shows that the different components of the feed left the stomach at different rates.

The relative disappearance from stomach and small intestine, and the apparent digestibility, were estimated for dry matter, lipid, nitrogen, energy, phosphorus and zinc. No significant differences in relative disappearance from stomach or small intestine, or in apparent digestibility, between the two treatment groups were found. Therefore, these data are given as the overall mean for all tanks at each sampling point (Table 6).

For fish kept in salt water, calculations of apparent digestibility and relative disappearance of sodium are violated by the fish drinking the salt water, and therefore these data are not given. The content of sodium (% of ingested) found in the GI tract (Fig. 3) was generally highest in fish fed the dry diet, indicating a higher drinking rate in salmon fed a dry diet compared to a soaked diet. The amount of sodium (% of ingested) in the stomach was significantly higher in salmon fed dry feed than in salmon fed soaked feed 2 and 6 h after feeding, and there was a trend for the same pattern 12 and 24 h after feeding. In the small intestine, the sodium content was significantly higher in salmon fed the dry diet than in those fed the soaked diet 18 h after feeding, and in the distal intestine, the difference was significant 12 h after feeding. These

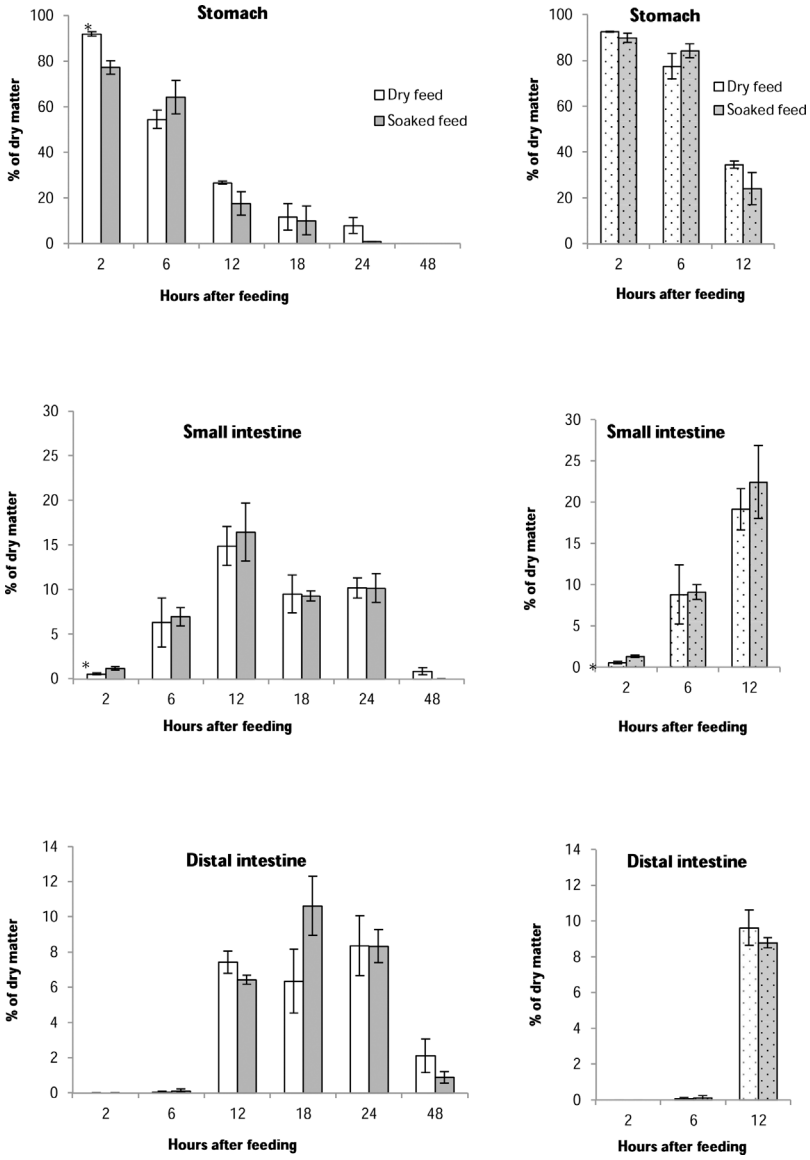

Fig. 2. Amount of dry matter, given as % of ingested dry matter, found in stomach (upper panels), small intestine (middle panels) and hindgut (lower panels) of Atlantic salmon fed one single meal of dry or soaked feed. The left panels show retained dry matter calculated from feed intake estimated from feed ration corrected for uneaten pellets (calculation 3), whereas the right panels (dotted bars) show retained dry matter calculated from total analyzed Y_2O_3 (calculation 4). The latter could not be estimated for samples from 18, 24 and 48 h after feeding, since at these sampling, some Y_2O_3 had evacuated from the distal intestine. The two treatment groups are compared with an ANOVA (*t*-test) at each sampling point. Significant differences ($P < 0.05$) are indicated with an asterisk, *. Data are given as mean ± SEM (n = 3).

data (Fig. 3) are given as % of ingested sodium, i.e. the sodium absorbed by the pellets during soaking in sea water is included in the sodium ingested. There were however no significant differences between salmon fed dry and soaked feed in total sodium amount (mg) found in the stomach, small intestine or distal intestine at any samplings (data not shown). This shows that the sodium from drinking in salmon fed dry feed evened out the sodium absorbed in feed during soaking.

4. Discussion

Gastric evacuation rate in fish has mainly been measured with the purpose to study feed intake and feeding rhythms, whereas the kinetics of the digestion of different feed qualities in salmon is poorly

Table 3
Percentage of nutrients, energy and Y_2O_3 ingested from feed, recovered from stomach 2, 6, 12, 18, 24 and 48 h after feeding Atlantic salmon dry or soaked feed. The two treatments were compared with an ANOVA (*t*-test) at each sampling point. Significant differences were only found 2 h after feeding, data from the remaining samplings are therefore given as overall means for both treatments. Data are given as mean ± SEM (SEM is not included if 0), and n is given for each mean.

Time after feeding (h)	2		6	12	18	24	48
	Dry feed	Soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed
Lipid	89 ± 1[a] (n = 3)	73 ± 2[b] (n = 3)	54 ± 5 (n = 6)	22 ± 1 (n = 4)	11 ± 4 (n = 4)	9 ± 0 (n = 2)	0 (n = 6)
Nitrogen	94 ± 1[a] (n = 3)	78 ± 3[b] (n = 3)	62 ± 4 (n = 6)	23 ± 3 (n = 6)	13 ± 4 (n = 5)	12 ± 1 (n = 2)	0 (n = 6)
Energy	92 ± 1[a] (n = 3)	77 ± 3[b] (n = 3)	58 ± 5 (n = 6)	24 ± 1 (n = 5)	13 ± 5 (n = 4)	11 ± 1 (n = 2)	0 (n = 6)
P	89[a] (n = 3)	79 ± 3[b] (n = 3)	58 ± 5 (n = 6)	19 ± 3 (n = 6)	10 ± 3 (n = 6)	4 ± 2 (n = 6)	0 (n = 6)
Zn	87 ± 1[a] (n = 3)	78 ± 3[b] (n = 3)	59 ± 4 (n = 6)	22 ± 4 (n = 6)	11 ± 4 (n = 6)	5 ± 3 (n = 6)	0 (n = 6)
Y_2O_3	99 ± 1([a]) (n = 3)	85 ± 5([b]) (n = 3)	65 ± 4 (n = 6)	28 ± 4 (n = 6)	15 ± 5 (n = 6)	6 ± 3 (n = 6)	0 (n = 6)

[a,b]Means within a row in data from stomach with different letters are significantly dint ($P < 0.05$). Superscript letter given in brackets indicates a trend ($P < 0.1$).

Table 4
Percentage of nutrients, energy and Y_2O_3 ingested from feed, recovered from small intestine 2, 6, 12, 18, 24 and 48 h after feeding Atlantic salmon dry or soaked feed. The two treatments were compared with an ANOVA (*t*-test) at each sampling point. Significant differences were only found 2 h after feeding, data from the remaining samplings are therefore given as overall means for both treatments. Data are given as mean ± SEM (SEM is not included if 0), and n is given for each mean. Data are given without decimals, therefore significant differences are indicated also at some values that are the same for both treatments.

Time after feeding (h)	2		6	12	18	24	48
	Dry feed	Soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed	Dry and soaked feed
Lipid	–	–	–	9 ± 1 (n = 4)	5 ± 0 (n = 2)	–	0 (n = 4)
Nitrogen	–	–	6 ± 1 (n = 5)	11 ± 1 (n = 6)	5 ± 1 (n = 6)	5 ± 1 (n = 6)	0 (n = 4)
Energy	–	–	10 ± 1 (n = 2)	14 ± 2 (n = 5)	8 ± 1 (n = 4)	9 ± 1 (n = 4)	0 (n = 4)
P	0^b (n = 3)	1^a (n = 3)	7 ± 1 (n = 6)	19 ± 3 (n = 6)	11 ± 1 (n = 6)	8 ± 1 (n = 6)	0 (n = 6)
Zn	1^b(n = 3)	1^a (n = 3)	11 ± 3 (n = 6)	30 ± 4 (n = 6)	14 ± 2 (n = 6)	14 ± 1 (n = 6)	1 ± 1 (n = 6)
Y_2O_3	0^b (n = 3)	1^a (n = 3)	9 ± 2 (n = 6)	27 ± 3 (n = 6)	19 ± 2 (n = 6)	19 ± 1 (n = 6)	1 (n = 6)

[a,b]Means within a row in data from stomach with different letters are significantly dint ($P < 0.05$).
'–' Not sufficient material for analysis.

understood. Measurement of GI passage rate in fish is challenging, and although several methods are used for this purpose (Bromley, 1994; Fänge and Grove, 1979), no good standard method is established. In the present trial, force feeding was chosen in order to both assure that all sampled fish had eaten, and to achieve equal feed intake in all fish. Feed intake affects the apparent digestibility of nutrients, presumably because low feed intake is associated with low GI passage rate, which increases absorption efficiency in the intestine (Aas et al., 2011b; Oehme et al., 2014). The equal feed intake among all fish was violated by regurgitation of pellets and the apparent limited stomach volume in some fish in the present study. This occurred in spite of the fact that the size of the ration was established by a pilot study prior to the trial, with salmon of similar size as in the trial but with different history and at different (lower) temperature. The feed load was also in accordance with the expected growth rate (Austreng et al., 1987). This shows that the maximal amount that can be force fed varies under different conditions, and this method should therefore not be used when feeding the full ration. Except for at 48 h after feeding in the present trial, the feed intake was not significantly different between the two groups, and the differences in feed intake are assumed to have minor impact on the results.

The regurgitation of pellets may be due to the gastric dilatation, which is also seen in GDAS (gastric dilation, air sacculitis) and fat regurgitation in salmonids (Aas et al., 2011b; Anderson, 2006; Baeverfjord et al., 2006; Lumsden et al., 2002). In these conditions, the distended stomach is filled with watery and/or oily content. The processes in the stomach are under both hormonal and neural control. Anderson (2006) suggests that a feedback mechanism slows gastric emptying to protect the intestine from nutrient overload. Furthermore, feeding the fish to full satiation causes an increased metabolic demand for oxygen, which may also induce this feedback mechanism (Anderson, 2006). The regurgitation of pellets may thus be the first reaction to the distention of the stomach or to a nutrient overload.

The individual variation within treatment was large. This variation may be due to individual differences in GI passage rate, but may also be related to individuals reacting differently to the experimental factors such as anesthesia, force feeding and stress. These effects were assumed to be equal for both treatment groups. Due to the large variation among individuals, and, consequently, limited power of the statistical analyses, non-significant patterns should not be dismissed. However, as the individual variation was large, to create a stronger design of the trial, a larger n is required. Also, due to regurgitation of pellets and limited stomach volume of some fish, this method was found not to be optimal for its intended purpose, but may be more suitable in studies where only a small ration is fed, e.g. when using a marker or studying a micronutrient.

After two hours soaking of the feed, the measured pellet hardness was not reduced. Soaking the feed only resulted in a soft outer layer of the pellets, whereas the core of the pellets, which is measured in the hardness measurement, maintained its hardness during soaking. In the stomach, the feed is disintegrated by the surface of the particles being transferred to the liquid phase (Andersen and Beyer, 2005), and how the pellets disintegrates during soaking may affect gastric evacuation rate of the feed. Other feed qualities than the one used in the present trial, may absorb water more evenly throughout the whole pellet during soaking. Thus, feeds with other physical properties may produce different results than the results achieved in the present study.

The gastric emptying in fish varies with temperature, season, osmotic stress, activity, body size, metabolic rate, satiety, feed type and physical pellet properties, but effect of meal size on the gastric evacuation rate shows contradictory results (Anderson, 2006; De Silva and Anderson, 1995; Smith, 1980). In the present study, the gastric evacuation rate was significantly higher in salmon fed the soaked diet than in those fed the dry diet two hours after feeding. Accordingly, Sveier et al. (1999) reported that gastric evacuation rate in Atlantic salmon was affected by physical characteristics of the feeds. Storebakken et al. (1999) found that gastrointestinal passage rate in salmon was lower when feeding a diet containing soy bean meal than when feeding a fish meal control diet. This was assumed to be caused by a slower onset of evacuation of the diet containing soy bean meal, an ingredient that

Table 5
Percentage of nutrients, energy and Y_2O_3 ingested from feed, recovered from distal intestine 2, 6, 12, 18, 24 and 48 h after feeding Atlantic salmon dry or soaked feed. The two treatments were compared with an ANOVA (*t*-test) at each sampling point. No significant differences were found, data are therefore given as overall means for both treatments. Data are given as mean ± SEM (SEM is not included if 0 or if n = 1), and n is given for each mean.

Time after feeding (h)	2 Dry and soaked feed	6 Dry and soaked feed	12 Dry and soaked feed	18 Dry and soaked feed	24 Dry and soaked feed	48 Dry and soaked feed
Lipid	0 (n = 6)	0 (n = 4)	–	3 (n = 3)	3 ± 1 (n = 2)	–
Nitrogen	0 (n = 6)	0 (n = 4)	3 (n = 6)	5 (n = 5)	4 ± 1 (n = 5)	2 (n = 1)
Energy	0 (n = 6)	0 (n = 4)	6 (n = 1)	7 ± 1 (n = 4)	7 ± 1 (n = 3)	–
P	0 (n = 6)	0 (n = 6)	14 ± 1 (n = 6)	15 ± 3 (n = 6)	12 ± 1 (n = 6)	1 ± 1 (n = 6)
Zn	0 (n = 6)	0 (n = 6)	19 ± 1 (n = 6)	21 ± 4 (n = 6)	19 ± 2 (n = 6)	4 ± 2 (n = 6)
Y_2O_3	0 (n = 6)	0 (n = 6)	20 ± 1 (n = 6)	24 ± 4 (n = 6)	23 ± 2 (n = 6)	3 ± 1 (n = 6)

Table 6
Relative disappearance (%) from stomach and small intestine and apparent digestibility (%) of dry matter, energy and analyzed nutrients from Atlantic salmon 2, 6, 12, 18, 24 and 48 h after feeding dry or soaked feed. No significant differences were revealed and therefore data are given as overall means for both treatments. Total number of tanks at each sampling was 6, but since there was not sufficient material for complete analyses at each sampling, n for each mean is given in the table. The data include an inaccuracy due to different evacuation rates of nutrients and yttrium. Data are given as mean ± S.E.M.

Time after feeding (h)	2 Dry and soaked feed	6 Dry and soaked feed	12 Dry and soaked feed	18 Dry and soaked feed	24 Dry and soaked feed	48 Dry and soaked feed
Relative disappearance (%) from stomach:						
Dry matter	8 ± 1 (n = 6)	8 ± 2 (n = 6)	22 ± 2 (n = 6)	28 ± 3 (n = 5)	28 ± 4 (n = 6)	–
Lipid	12 ± 1 (n = 6)	18 ± 3 (n = 6)	32 ± 3 (n = 4)	42 ± 1 (n = 3)	37 ± 7 (n = 2)	–
Nitrogen	7 ± 1 (n = 6)	5 ± 2 (n = 6)	20 ± 2 (n = 6)	27 ± 8 (n = 4)	13 ± 2 (n = 2)	–
Energy	8 ± 1 (n = 6)	10 ± 2 (n = 6)	25 ± 2 (n = 5)	29 ± 1 (n = 3)	25 ± 3 (n = 2)	–
Phosphorus	9 ± 1 (n = 6)	10 ± 2 (n = 6)	37 ± 4 (n = 6)	34 ± 3 (n = 5)	48 ± 9 (n = 6)	–
Zinc	10 ± 1 (n = 6)	9 ± 2 (n = 6)	24 ± 4 (n = 6)	19 ± 7 (n = 5)	37 ± 11 (n = 6)	–
Relative disappearance (%) from small intestine:						
Dry matter	−23 ± 9 (n = 6)	21 ± 6 (n = 6)	43 ± 2 (n = 6)	48 ± 3 (n = 6)	47 ± 3 (n = 6)	37 ± 1 (n = 2)
Lipid	–	–	67 ± 4 (n = 4)	77 ± 1 (n = 2)	–	–
Nitrogen	–	40 ± 6 (n = 5)	61 ± 3 (n = 6)	70 ± 3 (n = 6)	73 ± 4 (n = 6)	–
Energy	–	12 ± 23 (n = 2)	50 ± 3 (n = 5)	63 ± 1 (n = 4)	51 ± 5 (n = 4)	–
Phosphorus	8 ± 3 (n = 6)	22 ± 2 (n = 6)	32 ± 3 (n = 6)	40 ± 4 (n = 6)	57 ± 2 (n = 6)	29 ± 7 (n = 2)
Zinc	−52 ± 11 (n = 6)	−19 ± 8 (n = 6)	−12 ± 9 (n = 6)	19 ± 7 (n = 6)	28 ± 5 (n = 6)	−39 ± 26 (n = 2)
Apparent digestibility (%):						
Dry matter	–	41 ± 6 (n = 2)	64 ± 2 (n = 6)	65 ± 1 (n = 6)	62 ± 3 (n = 6)	38 ± 8 (n = 6)
Lipid	–	–	–	87 ± 2 (n = 3)	90 ± 0 (n = 2)	–
Nitrogen	–	–	82 ± 3 (n = 6)	83 ± 1 (n = 5)	83 ± 4 (n = 5)	66 (n = 1)
Energy	–	–	73 (n = 1)	74 ± 1 (n = 4)	73 ± 6 (n = 3)	–
Phosphorus	–	18 ± 4 (n = 2)	30 ± 3 (n = 6)	36 ± 2 (n = 6)	45 ± 2 (n = 6)	51 ± 5 (n = 6)
Zinc	–	−21 ± 28 (n = 2)	4 ± 6 (n = 6)	14 ± 6 (n = 6)	14 ± 6 (n = 6)	3 ± 24 (n = 6)

–No material present, or insufficient quantity for chemical analysis.

affects the physical properties of feed (Øverland et al., 2009). Likewise, Yamamoto et al. (1998) found the peak in free amino acid concentration in blood in rainbow trout 12 h after feeding a fish meal diet, and at 21 h after feeding diets with soy bean meal or malt protein flour. In the present trial, soaking resulted in a soft outer layer but no reduction in hardness of the core of the pellets. Thus, the increased gastric evacuation rate as an effect of soaking found on the earliest sampling after the meal, may reflect a reduced processing time of the soft part of the pellets in the stomach in salmon fed the soaked feed. It should be emphasized that the suggested potential for increasing feed intake by optimizing the feed quality is based on the assumption that today's commercial salmon farming is not utilizing the salmon's full capacity for growth. The feed intake can not be increased beyond the salmon's capacity for processing and metabolizing the feed.

Emptying of the stomach followed a pattern that was suited for curve fitting. When including data from 2 to 24 h, curves with reasonable R^2 values could be fitted (Fig. 4). According to these fitted curves, the stomach of the fish was 50% emptied after 6 h and 38 min for fish fed dry feed, and after 5 h and 55 min for fish fed the soaked feed. With relatively few sampling points and variation within groups, the data do not provide precision to establish such an exact point in time for 50% emptying of the stomach. However, these estimates support the indications that soaking the feed increases gastric emptying rate.

After the feed is disintegrated in the stomach and transferred to the small intestine, there is probably no difference in the GI passage rate among different physical feed qualities. Thus, for feeds with identical composition, the gastric evacuation rate is probably the determinant for GI passage rate. Feeds which disintegrate easily in the stomach, and are quickly transferred to the small intestine so that the stomach is emptied quickly, are probably beneficial for achieving a high feed intake and growth in farmed salmon. Soaking the feed increased the gastric evacuation rate under the conditions used in the present trial. Thus, the present data indicate that the increased feed intake in salmon fed soaked feed compared to dry feed observed by Oehme et al. (2014) may be, at least in part, caused by increased gastric evacuation rate in salmon fed soaked feed. Correspondingly, Sveier et al. (1999) found that both gastric evacuation rate and feed intake was higher in Atlantic salmon fed diets containing standard fish meal compared to diets containing coarse or micro ground fish meal.

Since the GI tract was divided in the three chosen segments for sampling, the data from the present trial mainly gives information about the gastric evacuation rate, and the total GI passage rate. The gastric evacuation rate is not only important to find the optimal feed quality, but can also be used to plan the feeding routines in commercial salmon farming. In the present trial, half of the ingested feed had evacuated from the stomach after 6–12 h, and after 18 h, approximately 10% of the ingested feed was still in the stomach in fish in both treatment groups. The data thus show that under the conditions used in this trial, and when regarding only digestive processes, salmon of this size may have the capacity for eating two large meals per day. The gastric evacuation rates found in the present study corresponded well with data from other studies of salmonids (Burton and Boisclair, 2013; Grove et al., 1978; Sveier et al., 1999). However, the salmon in the present study was starved for 2–3 days prior to the trial and was thus in starvation mode when the meal was given. This may have affected the dynamics of the gut evacuation compared to when the salmon is fed daily.

Collection of faeces from the distal intestine is commonly used in e.g. digestibility studies. Successful sample collection for such studies depends on faecal material being present. In the present study, the amount of content in the distal intestine was largest between 12 and 24 h after feeding, which shows the ideal time interval for sampling faeces under the conditions used in this trial. The total GI passage rates found was in accordance with results from other studies (Storebakken et al., 1999; Sveier et al., 1999).

The amount (% of ingested) of the marker (Y_2O_3) and nutrients in the samples varied. This is an indication that nutrients dissolve from the feed and leave the stomach at different rates, either the transfer to the small intestine at various rates, or by absorption of nutrients from the stomach. Y_2O_3 was the slowest among the analyzed components to leave the stomach. Also, soaking the feed affected the rate and order in which nutrients left the stomach. The marker:nutrient ratio in faeces is commonly used to estimate apparent digestibility of nutrients in feeds.

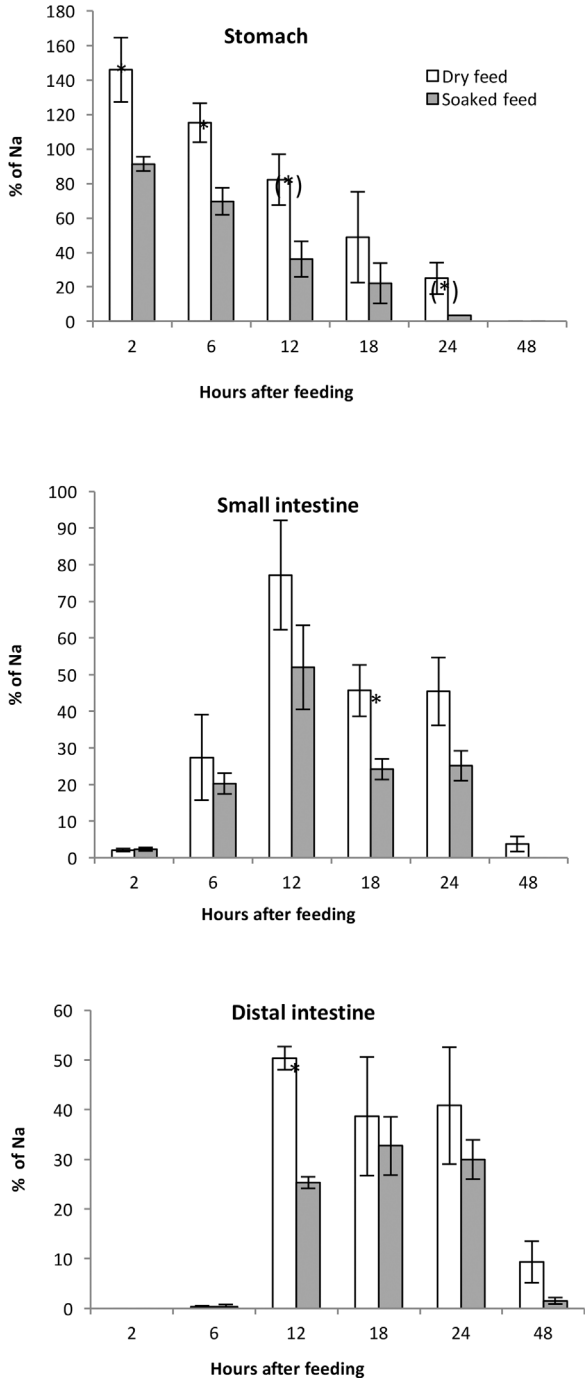

Fig. 3. Amount of sodium, given as % of sodium ingested from feed, found in stomach (upper panel), small intestine (middle panel) and hindgut (lower panel) of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA (*t*-test) at each sampling point. Significant differences ($P < 0.05$) are indicated with an asterisk, *, and trends ($P < 0.1$) with an asterisk in brackets, (*). Data are given as mean ± SEM (n = 3).

Our data show that Y_2O_3 and the various nutrients are transported through the GI tract at different rates, which is also supported by other studies (Aas et al., 2011b; Hatlen et al., 2015; Jørgensen and Jobling, 1988). Thus, a steady feed intake over time that creates a constant flow of feed through the gut prior to sampling is required to obtain reliable data in digestibility studies based on marker:nutrient ratios in faeces (Hatlen et al., 2015). Our data on relative disappearance and apparent digestibility include this inaccuracy, but give an estimate of the overall picture of evacuation of nutrient from stomach and intestine.

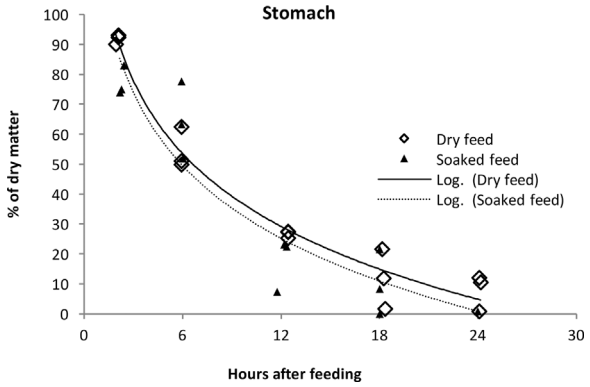

Fig. 4. Logarithmic curves fit (performed in Microsoft Excel 14.0) of amount of dry matter, given as % of ingested dry matter, versus time (h) after feeding, found in stomach of Atlantic salmon fed one single meal of dry or soaked feed (n = 3, time 2–24 h after feeding included). For dry feed, $y = -35.07\ln(x) + 116.31$ and $R^2 = 0.9706$. For soaked feed, $y = -35.08\ln(x) + 112.35$ and $R^2 = 0.8833$.

The sodium intake related to a meal was similar in salmon fed dry feed or feed soaked in sea water. This shows that sodium intake from increased drinking rate in salmon fed the dry diet evened out the feed's sodium absorption during soaking, and thus soaking the feed in sea water did not reduce the sodium load in the salmon. Sea water was chosen for soaking since this is available in unlimited amounts at the fish farming site. However, the data indicate that by soaking feed in fresh water, the sodium intake related to a meal can be reduced, which may be relevant in situations where is it desirable to reduce the sodium load.

The main weaknesses of the method described were regurgitation of pellets and large variation among individuals. Except for at one measuring point, the regurgitation was similar for salmon fed dry and soaked feed, and regurgitation is therefore not considered to have affected the comparison of the two feed types. The individual variation reduced the power of the design, i.e. reduced the probability of revealing significant differences. The differences found however were real, and the dataset produced in the study is considered to be valid although the method used was not good enough to be chosen as a standard method to develop further for studies of this kind. Obviously, to increase the insight in the dynamics of gastrointestinal passage in salmon, there is still need for method development.

In conclusion, the individual variation in measured GI passage rate was large. Despite the fact that only the outer layer of the pellets was softened by soaking, the soaking significantly increased the gastric evacuation rate shortly (2 h) after feeding. This increase of gastric evacuation rate in salmon fed soaked feed may increase the feed intake. Soaking may not be a relevant technique in intensive fish farming, but the present data and the data from Oehme et al. (2014) show that there is potential for increasing feed intake by improving the physical properties of commercial salmon feed.

The method tested for studying the gastrointestinal passage rate is more suitable for trials with feed rations well below maximum daily intake and with larger numbers of replicates. Although the method had its weaknesses, the impact of these were the same for both treatments and the dataset and it's interpretation are considered to be valid.

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