Report 38/2013 • Published September 2013

Gastrointestinal passage rate in Atlantic salmon (*Salmo salar*) fed dry or soaked feed

A CREATE project

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Report

ISBN: 978-82-8296-120-2 (printed)
ISBN: 978-82-8296-121-9 (pdf)
ISSN 1890-579X

Title:	*Report No.:*
Gastrointestinal passage rate in Atlantic salmon (*Salmo salar*) fed dry or soaked feed. A CREATE project	38/2013
	Accessibility: **Open**
Author(s)/Project manager:	*Date:*
Turid Synnøve Aas, Trine Ytrestøyl, Bjarne Hatlen, Hanne Jorun Sixten, Marie Hillestad and Torbjørn Åsgård	10 September 2013
Department:	*Number of pages and appendixes:*
Nutrition and feed technology	27
Client:	*Client's ref.:*
SINTEF Fiskeri og havbruk AS	Arne Fredheim
Keywords:	*Project No.:*
Pellet quality, Pellet moisture, Gastrointestinal evacuation rate	2424

Summary/recommendation:

Atlantic salmon was fed a commercial-like diet either as is (92% dry matter) or soaked for 2 h in sea water prior to feeding (70% dry matter). The salmon showed large individual variation in gastrointestinal passage rate and few significant differences between the two diets were revealed. However, the soaked feed resulted in a significantly higher gastric evacuation rate than the dry diet.

Summary/recommendation in Norwegian:

Atlantisk laks ble fôret enten med vanlig, ekstrudert fôr (92 % tørrstoff), eller fôret ble bløtlagt i 2 t i sjøvann før fôring (70 % tørrstoff). Fôret var laget spesielt til forsøket, men var av tilsvarende kvalitet som kommersielle fôr. Det var stor individuell variasjon i passasjehastighet i mage og tarm, og det ble funnet få signifikante forskjeller mellom de to fôrene. Men magetømmingshastigheten var signifikant høyere når fôret var bløtlagt enn for tørt fôr.

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1 Introduction

The development in salmon farming has moved towards large units, which require technology and logistics to handle large amounts of fish, and thus, large amounts of feed. Transport and storage of feed in bulk require feeds with high physical quality. However, previous studies have shown that physical properties of feed affect the biological response in the fish (Sveier *et al.*, 1999;Baeverfjord *et al.*, 2006;Venou *et al.*, 2009;Aas *et al.*, 2011;Morken *et al.*, 2011;Glencross *et al.*, 2011). If this is not taken into consideration the feed produced for salmon farming may be suboptimal for the fish and result in suboptimal growth.

In a previous trial, feed was soaked prior to feeding to Atlantic salmon. Soaking the feed increased feed intake, particularly when the appetite in general was low (Oehme *et al.*, 2012). Furthermore, Aas et al. (2011) showed that feed intake was approximately 20 % higher in rainbow trout fed a feed with low water stability compared to a feed with high water stability.

This leads to the hypothesis that feed intake may be higher in fish fed diets that disintegrates rapidly in the stomach compared to diets that disintegrates slowly. Thus, increased feed intake of soaked pellets in the previous study may have been due to faster disintegration of feed in the stomach, and thus faster passage through the gastrointestinal tract.

In the present study therefore, Atlantic salmon was given one meal of dry (as is) or soaked feed. The feed and soaking procedure were the same as used in the previous study (Oehme *et al.*, 2012). The content of digestibility marker, dry matter, nutrients and energy in the stomach, small intestine (pylorus and mid intestine) and distal intestine was analysed 2, 6, 12, 18, 24 and 48 hours post feeding.

2 Materials and methods

2.1 Feeds

A commercial-like feed was used for the experiment. The feed was formulated as 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. The feed was fed to fish either as is, or the ration was soaked in sea water at 4 ºC for two hours prior to feeding.

The same feed had previously been used in another experiment (Oehme *et al.*, 2012), and in that study the dry and soaked feeds were denoted D92 and D70, respectively, reflecting the dry matter content. The feed was produced by BioMar AS (Tech Centre, Brande, Denmark) approximately one year prior to the present experiment, and was stored at 4 ºC. The chemical composition of the feeds is shown in Table 1.

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[1]	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

[1] Amino acids are given as dehydrated residuals

The physical properties of the feeds are given in Table 2. The feeds are further described by Oehme et al. (2012).

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[1], %	92.1	93.0

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

2.2 Fish trial

Atlantic salmon with mean weight 1131 g (range 900-1449) g was used for the experiment. The fish was kept under continuous light in 1 m^2 tanks supplied with sea water with and fed commercial feed (Skretting) prior to the experiment. During the trial, the water temperature ranged from 13.3 to 13.6 ºC.

In the trial, groups of three fish were used for pooled samples, and after feeding, each group of three fish was placed in a separate tank (1 m^3) for a given time (2, 6, 12, 18, 24 or 48 hours).

Before feeding, the fish was completely anaesthetized with Finquel MS-222 (tricaine methanesulfonate, 50 mg/L). The fish was 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.

The trial was run in triplicate. Thus, for each sampling point, three tanks were used for fish fed dry feed, and three tanks for fish fed soaked feed. In the following, 'tank' refers to a group of three fish placed in the same tank after feeding. Some fish regurgitated pellets, which were collected in sieves at the outlet of the water. These pellets, and pellets from the ration that was not fed into the fish, were counted, and average pellet weight (0.875 g) was used to calculate the feed intake for each group of three fish.

At the relevant time, all three fish from a tank was anaesthetized with a lethal dose of anaesthetic. The gastrointestinal tract was removed and closed with artery clamps in both ends and immersed in liquid N to avoid leakage of the content. Subsequently, the gastrointestinal tract was wrapped in aluminum foil and frozen at -20 ºC. Later, the gastrointestinal 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) (Fig. 1). Samples were pooled by tank, and the content was frozen before freeze drying. The gastrointestinal content was collected as completely as possible. However, some loss is inevitable, and the content of the pylorus secae could not be collected.

In order to empty the intestine prior to the trial, all fish were fasted from the same point of time. Force feeding took place over two consecutive days: fish sampled 2, 6 or 24 h after feeding were fed after being fasted 2 days, whereas fish sampled 12 and 18 h were fed after 3 days fasting.

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

2.3 Measurements of physical feed quality

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 described in Aas et al. (Aas *et al.*, 2011). For each diet, 35 pellets were analyzed, and strength at rapture (N) were recorded when the pellet cracked. The texture analyzer also recorded the diameter on the pellets used for the hardness measurements. Water stability was measured in three replicate samples of 20 g of pellets placed in a custom made steel-mash placed inside a glass beakers containing 300 ml distilled water. The beakers were shaken at 100 rpm in a water bath at 25 °C. After 240 min the retained dry matter was measured.

2.4 Chemical analyses

Gastrointestinal content was weighed, freeze dried and homogenised prior to analysis. The water loss during freeze drying was included in dry matter estimation. Dry matter was estimated by further drying the samples at 105 °C to constant weight. The samples were analysed 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), and minerals were analysed by inductively coupled plasma mass spectroscopy (ICP-MS, at Eurofins, Moss, Norway).

Since there was not sufficient material for complete chemical analyses at all sampling points, the analyses was prioritised by the following order: 1) dry matter, yttrium oxide and minerals (all from one sample preparation), 2) nitrogen 3) energy and 4) fat. Since all samples could not be analysed completely, the dataset contains some missing values, not to be confused with the value 0 at sampling points with no content present in the gut.

2.5 Calculations

Apparent digestibility and nutrients and energy were calculated as

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

The calculation of relative disappearance was calculated equivalent to the calculation of AD, except that it was calculated from the concentrations in stomachs and in small intestine, whereas AD was calculated from the faeces concentrations.

Feed intake, used in several calculations, was estimated in two ways.

1. Feed intake estimated by correcting the ration (estimated at all sampling points):

Feed intake (g)=Feed ration (10 g)–(No of pellets not fed+No of pellets regurgitated)x0.875, where 0.875 is the average weight of one pellet.

2. Feed intake estimated from analysed yttrium, Y:

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

Calculation 2 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 six 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 pooled sample) was the statistical unit in the dataset. 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 (2, 6, 12, 18, 24 and 48 hours after feeding). Differences were considered significant if $P \leq 0.05$, and 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. For these 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 sampling deviated from the intended schedule. Therefore, a 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 affects the results. The results from regression analysis are only included if significant.

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

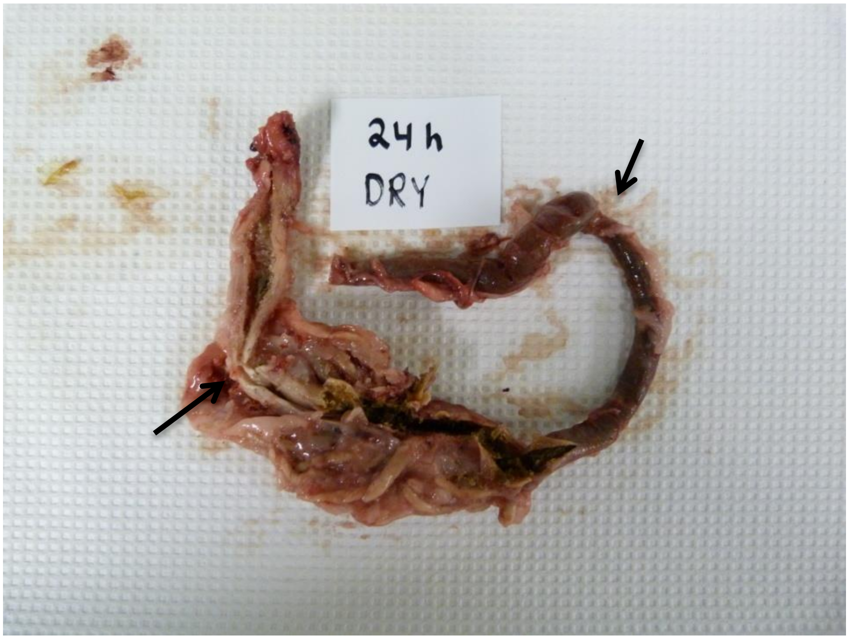

Figure 1 An example of a sampled gut, from a fish sampled 24 h after a meal of dry feed. The arrows indicate where the gut was divided to obtain samples from stomach, from small intestine, and from hindgut.

3 Results

Large variation in gastrointestinal evacuation rate between individuals was observed visually. E.g at 18 hours, one fish fed dry feed still had feed in the stomach, while another fish from the same tank had almost emptied the gastrointestinal tract.

The feed intake (estimated with calculation 1) seemed to decrease throughout the experiment (not tested statistically), indicating that pellets were regurgitated for a long period after the feeding (Table 3). Furthermore, at 48 hours after feeding, there was a tendency (P<0.1) for lower feed intake in salmon fed soaked feed compared to those fed the dry (as is) feed, showing that fish regurgitated more of the soaked than the dry feed.

Table 3 Feed intake, given as g dry matter per individual, calculated by subtracting regurgitated pellets and not fed pellets from the ration (Calculation 1). (Mean ± SEM, n=3).

Time after feeding, h		2	6	12	18	24	48
Feed intake	Dry feed	8.5±0.2	8.1±0.3	7.5±0.3	8.2±0.2	7.4±0.7	7.3±0.5
	Soaked feed	8.8±0.1	7.0±0.5	7.0±0.4	6.5±0.8	6.6±0.4	5.3±0.6
	P-value	0.3486	0.1369	0.3486	0.1039	0.3868	0.0577

ns not significantly different at P<0.05
(*) Trend, P<0.1

3.1 Amount (% of ingested) of nutrients and energy in the gastrointestinal tract

3.1.1 Content of dry matter in stomach, small intestine, and hindgut

The amount of dry matter found in stomach, small intestine, and hindgut is shown in Fig. 2, given as % of ingested dry matter. Data are shown both when estimating feed intake from feed ration corrected for losses (calculation 1, left panels), and from total analysed Y_2O_3 (calculation 2, right panels). When estimating feed intake by correcting the ration by lost pellets (calculation 1), the amount of dry matter in stomachs of salmon fed soaked feed was significantly lower than in fish fed dry feed 2 h after feeding. Correspondingly, the amount of dry matter in small intestine was higher in fish fed soaked feed compared to those fed dry feed at this time.

At 2 h after feeding, regression analyses showed that the decrease in dry matter in stomach of fish fed soaked feed compared to fish fed dry fed was influenced by deviation in time of sampling. In small intestine however, there was no effect of deviation in time.

When using Y_2O_3 to estimate feed intake (calculation 2), there was no significant differences in stomach content between fish fed dry or soaked feed. The higher content of chyme in small intestine after 2 hours in salmon fed soaked feed compared to those fed dry feed was confirmed (Fig. 2). Regression analysis showed however, that there was a trend (p<0.0808) that this difference was affected by deviation in sampling time.

At the other sampling points, and in hindgut, no significant differences between the two diets were revealed. However, the content in stomach declined gradually, and all stomachs were empty after 48 hours. 24 hours after feeding, the amount of dry matter in stomachs of fish fed dry and soaked feed was 8±4% and 1 %, respectively, of ingested dry matter (not significantly different).

The content in small intestine peaked at the sampling 12 hours post feeding for both feeds. After 48 hours, small intestines were empty in all tanks fed soaked feed, and for one tank fed dry feed. Although not significantly different, the soaked feed thus seemed to completely pass through the small intestine somewhat faster than the dry feed.

The amount of dry matter in the hindgut was highest at 12, 18 and 24 hours for both feed groups. 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.

At 2 h, 92±1 and 77±3 % of ingested dry matter from dry and soaked feed, respectively, was still present in stomach, whereas at 6 h, 55±4% and 77±3 %, respectively, was present. At 12 h, less than 30% of ingested dry matter was present in all fish. This shows that under the conditions present in this study, salmon uses between 6 and 12 h on average to empty the stomach 50 % after a single meal. (Feed intake estimated with calculation 1.)

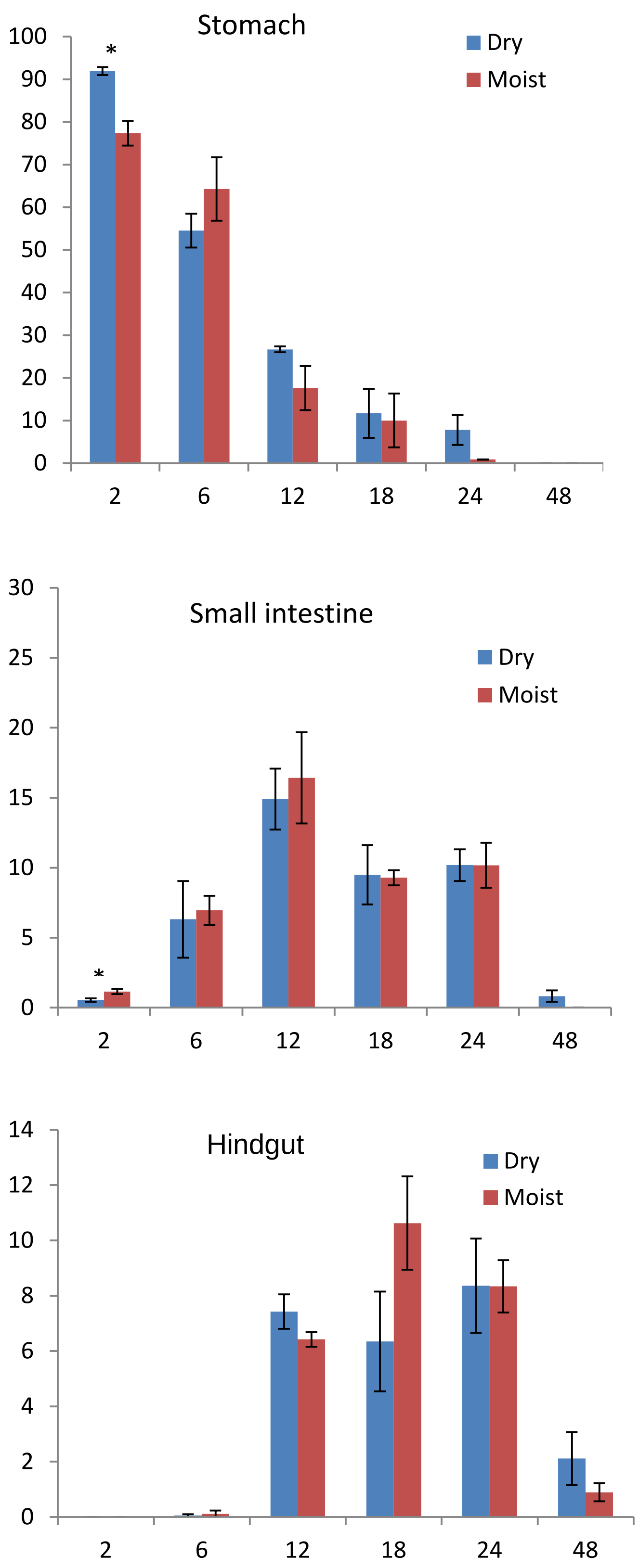

Figure 2 Amount of dry matter, given as % of ingested dry matter, in stomach (upper panels), small intestine (middle panels) and hindgut (lower panels) of Atlantic salmon fed one single meal of dry or soaked feed. X-axis represents time (h) after feeding. Calculations where feed intake is estimated by correcting the full ration by lost pellets are shown at left (dark colours), whereas the corresponding figures, based on feed intake estimated from total analysed Y_2O_3, are shown at right (bright colours). The latter is only calculated at 2, 6, and 12 h after feeding since digesta appeared in the hindgut at 12h. The two treatment groups are compared with an ANOVA at each sampling point. Significant differences (P<0.05) are indicated with an asterisk. (n=3, mean ± SEM).

3.1.2 Content of nutrients, energy and Y_2O_3 in stomach

The % of ingested Y_2O_3, nutrients and energy found in stomach, is shown in Table 4 using feed ration corrected for losses (calculation 1) to estimate feed intake. The same data, using Y_2O_3 to calculate feed intake is shown in Table 5.

The content of fat, N, energy and most of the analysed minerals in the stomach, given as % of ingested amount, followed a similar pattern as dry matter content, with declining content over time, and significantly lower content at 2 h (or a trend, $P<0.1$) in fish fed soaked feed compared to fish fed dry feed (Table 4).

For Na however, which is present in sea water, stomachs of fish fed dry and soaked feed contained 146±19 % and 91±4 %, respectively, of the Na ingested via the feed two hours post feeding, indicating a reduced drinking rate in salmon when soaking the feed. The content of Na in stomachs of salmon fed dry feed was also significantly higher than in those fed soaked feed after 6 hours (Table 4).

The % of ingested material present in stomachs after 2 h varied among the analyzed nutrients. In fish fed dry feed, the amount present ranged from 87 % of ingested Zn, to 99 % of Y_2O_3. For soaked feed, the range was from 73 % of ingested fat, to 85 % of Y_2O_3. Some of this variation may be due to inaccuracy in chemical analysis, but the data indicate that nutrients are dissolved from feed and leave the stomach at different rates, with Y_2O_3 being the slowest among the analyzed components. Also, soaking the feed affected the rate and order at which nutrients left the stomach.

3.1.3 Content of nutrients, energy and Y_2O_3 in small intestine

The content of nutrients and energy in small intestine also followed a similar pattern to that of dry matter (Table 6; feed intake estimated with calculation 1). However, due to insufficient amount of sample, fat, N and energy was not analyzed in all samples.

The same data, but with feed intake estimated from Y_2O_3 are given in Table 7.

3.1.4 Content of nutrients, energy and Y_2O_3 in hindgut

The % of ingested Y_2O_3, nutrients and energy found in hindgut, is shown in Table 8 using feed ration corrected for losses (calculation 1) to estimate feed intake. The same data, using Y_2O_3 to calculate feed intake is shown in Table 9.

In hindgut, there was not found any content in any fish at 2 h. Also, the amount of material remaining in the hindgut was low that fat, energy and N was not analyzed in several of the samples.

The first appearance of material in hindgut was at the sampling 6 h after feeding for both feed groups, and at 48 h some material was still present in some fish, whereas many individuals had emptied the gastrointestinal tract completely after 48 h.

Table 4 *Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in **stomach** of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences ($P<0.05$) are indicated with red, trends ($P<0.1$) with blue. The data are based on calculation of feed intake by correcting the feed ration for loss (calculation 1). Corresponding values based on feed intake estimated with calculation 2 are shown in Table 5.*

Time after feeding, h		2	6	12	18	24	48
Amount of **fat** in stomach content	Dry feed	89±1 (3)	49±4 (3)	22±1 (3)	13±4 (2)	9 (2)	0 (3)
	Soaked feed	73±2 (3)	59±9 (3)	21 (1)	9±9 (2)	- (0)	0 (3)
	P-value	0.0047	0.3810	0.5962	0.7103	-	-
Amount of **N** in stomach content	Dry feed	94±1 (3)	56±4 (3)	28±1 (3)	18±4 (2)	12±1 (2)	0 (3)
	Soaked feed	78±3 (3)	67±7 (3)	18±5 (3)	10±7 (3)	- (0)	0 (3)
	P-value	0.0065	0.2373	0.1317	0.4539	-	-
Amount of **energy** in stomach content	Dry feed	92±1 (3)	54±4 (3)	26±1 (3)	15±4 (2)	11±1 (2)	0 (3)
	Soaked feed	77±3 (3)	63±8 (3)	22 (2)	10±10 (2)	- (0)	0 (3)
	P-value	0.0065	0.3459	0.0507	0.6779	-	-
Amount of **Y_2O_3** in stomach content	Dry feed	99±1 (3)	61±4 (3)	34 (3)	15±7 (3)	10±4 (3)	0 (3)
	Soaked feed	85±5 (3)	68±7 (3)	23±7 (3)	14±8 (3)	1 (3)	0 (3)
	P-value	0.0581	0.4368	0.1931	0.8888	0.1267	-
Amount of **P** in stomach content	Dry feed	89 (3)	52±5 (3)	23±2 (3)	10±5 (3)	7±3 (3)	0 (3)
	Soaked feed	79±3 (3)	64±7 (3)	14±4 (3)	9±5 (3)	1 (3)	0 (3)
	P-value	0.0279	0.2585	0.1129	0.8546	0.1277	-
Amount of **Ca** in stomach content	Dry feed	91±1 (3)	57±4 (3)	27±1 (3)	12±5 (3)	8±4 (3)	0 (3)
	Soaked feed	82±4 (3)	66±7 (3)	17±5 (3)	12±6 (3)	1 (3)	0 (3)
	P-value	0.0597	0.3378	0.1625	0.9565	0.1044	-
Amount of **Mg** in stomach content	Dry feed	95±1 (3)	64±3 (3)	33±2 (3)	16±8 (3)	8±2 (3)	0 (3)
	Soaked feed	78±5 (3)	54±4 (3)	19±6 (3)	16±9 (3)	1 (3)	0 (3)
	P-value	0.0202	0.1388	0.0876	0.9537	0.0402	-
Amount of **Na** in stomach content	Dry feed	146±19 (3)	115±11 (3)	82±15 (3)	49±26 (3)	25±9 (3)	0 (3)
	Soaked feed	91±4 (3)	70±8 (3)	36±10 (3)	22±12 (3)	4 (3)	0 (3)
	P-value	0.0460	0.0296	0.0625	0.4081	0.0781	-
Amount of **Zn** in stomach content	Dry feed	87±1 (3)	53±4 (3)	28±2 (3)	12±5 (3)	9±4 (3)	0 (3)
	Soaked feed	78±3 (3)	64±7 (3)	17±5 (3)	10±6 (3)	1 (3)	0 (3)
	P-value	0.0481	0.2679	0.1262	0.8147	0.1301	-

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

Table 5 Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in ***stomach*** *of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, trends (P<0.1) with blue. The data are based on calculation of feed intake from analysed value of Y_2O_3 (calculation 2). Corresponding values based on feed intake estimated with calculation 1 is shown in Table 4.*

Time after feeding, h		**2**	**6**	**12**
Amount of **fat** in stomach content	Dry feed	89±1 (3)	69±6 (3)	29±2 (3)
	Soaked feed	84±2 (3)	76±5 (3)	28 (1)
	P-value	0.1080	0.4145	0.8134
Amount of **N** in stomach content	Dry feed	95±0 (3)	80±6 (3)	36±2 (3)
	Soaked feed	90±2 (3)	88±3 (3)	24±7 (3)
	P-value	0.0877	0.2649	0.1894
Amount of **energy** in stomach content	Dry feed	93±1 (3)	76±6 (3)	33±2 (3)
	Soaked feed	89±2 (3)	83±4 (3)	30±0 (2)
	P-value	0.2221	0.3945	0.2312
Amount of **P** in stomach content	Dry feed	90±1 (3)	75±6 (3)	30±3 (3)
	Soaked feed	91±2 (3)	84±3 (3)	19±6 (3)
	P-value	0.4738	0.2675	0.1623
Amount of **Ca** in stomach content	Dry feed	92±1 (3)	81±6 (3)	34±2 (3)
	Soaked feed	95±2 (3)	86±3 (3)	24±7 (3)
	P-value	0.1528	0.4739	0.2278
Amount of **Mg** in stomach content	Dry feed	96±2 (3)	90±5 (3)	42±2 (3)
	Soaked feed	90±0 (3)	71±1 (3)	26±8 (3)
	P-value	0.0426	0.0181	0.1119
Amount of **Na** in stomach content	Dry feed	147±19 (3)	165±21 (3)	105±16 (3)
	Soaked feed	106±2 (3)	91±1 (3)	49±14 (3)
	P-value	0.0958	0.0253	0.0597
Amount of **Zn** in stomach content	Dry feed	87±0 (3)	76±6 (3)	36±3 (3)
	Soaked feed	90±2 (3)	84±3 (3)	23±7 (3)
	P-value	0.2297	0.3056	0.1791

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

Table 6 *Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in **small intestine** of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.*

Time after feeding, h		2	6	12	18	24	48
Amount of fat in content of small intestine	Dry feed	- (0)	- (0)	9±3 (2)	5 (2)	- (0)	0 (1)
	Soaked feed	- (0)	- (0)	9 (2)	- (0)	- (0)	0 (3)
	P-value	-	-	0.9967	-	-	-
Amount of N in content of small intestine	Dry feed	- (0)	6±3 (2)	11±2 (3)	6±1 (3)	6±1 (3)	0 (1)
	Soaked feed	- (0)	5±0 (3)	10±2 (3)	5±0 (3)	4±0 (3)	0±0 (3)
	P-value	-	0.7512	0.8826	0.6519	0.3545	-
Amount of energy in content of small intestine	Dry feed	- (0)	11 (1)	13±2 (3)	9±0 (2)	10±2 (2)	0 (1)
	Soaked feed	- (0)	9 (1)	15±3 (2)	7 (2)	9±3 (2)	0 (3)
	P-value	-	-	0.6088	0.0499	0.9348	-
Amount of Y_2O_3 in content of small intestine	Dry feed	0 (3)	9±4 (3)	25±3 (3)	18±5 (3)	19 (3)	1±1 (3)
	Soaked feed	1 (3)	8±1 (3)	30±6 (3)	19±2 (3)	20±2 (3)	0 (3)
	P-value	0.0461	0.7225	0.4664	0.9531	0.8126	0.1162
Amount of P in content of small intestine	Dry feed	0 (3)	7±3 (3)	18±3 (3)	11±3 (3)	8±1 (3)	1 (3)
	Soaked feed	1 (3)	6 (3)	20±5 (3)	10±2 (3)	8±1 (3)	0 (3)
	P-value	0.0497	0.8183	0.6650	0.7264	0.8741	0.1207
Amount of Ca in content of small intestine	Dry feed	1±0 (3)	9±4 (3)	28±4 (3)	23±5 (3)	23±1 (3)	2±1 (3)
	Soaked feed	1±0 (3)	8±0 (3)	35±8 (3)	25±4 (3)	25±3 (3)	0±0 (3)
	P-value	0.0336	0.7464	0.4687	0.7418	0.6055	0.1241
Amount of Mg in content of small intestine	Dry feed	2±0 (3)	17±7 (3)	59±6 (3)	75±20 (3)	83±24 (3)	4±3 (3)
	Soaked feed	6±1 (3)	9±0 (3)	80±22 (3)	76±14 (3)	66±4 (3)	0±0 (3)
	P-value	0.0421	0.3606	0.4222	0.9730	0.5130	0.3432
Amount of Na in content of small intestine	Dry feed	2 (3)	27±12 (3)	77±15 (3)	46±7 (3)	45±9 (3)	4±2 (3)
	Soaked feed	2 (3)	20±3 (3)	52±11 (3)	24±3 (3)	25±4 (3)	0 (3)
	P-value	0.7350	0.5853	0.2532	0.0463	0.1161	0.1381
Amount of Zn in content of small intestine	Dry feed	1 (3)	11±6 (3)	28±1 (3)	15±3 (3)	13±2 (3)	2±1 (3)
	Soaked feed	1 (3)	10±1 (3)	33±8 (3)	14±2 (3)	14±2 (3)	0 (3)
	P-value	0.0230	0.9211	0.5733	0.9439	0.7055	0.1311

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

*Table 7 Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in **small intestine** of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.*

Time after feeding, h		**2**	**6**	**12**
Amount of fat in content of small intestine	Dry feed	- (0)	- (0)	12±4 (2)
	Soaked feed	- (0)	- (0)	12±0 (2)
	P-value	-	-	0.8549
Amount of N in content of small intestine	Dry feed	- (0)	9±4 (2)	14±2 (3)
	Soaked feed	- (0)	7±1 (3)	14±3 (3)
	P-value	-	0.6615	0.9484
Amount of energy in content of small intestine	Dry feed	- (0)	15 (1)	17±3 (3)
	Soaked feed	- (0)	10 (1)	20±4 (2)
	P-value	-	-	0.4725
Amount of P in content of small intestine	Dry feed	0±0 (3)	10±4 (3)	23±3 (3)
	Soaked feed	1±0 (3)	8±1 (3)	28±7 (3)
	P-value	0.0191	0.7290	0.5458
Amount of Ca in content of small intestine	Dry feed	1±0 (3)	13±5 (3)	36±4 (3)
	Soaked feed	2±0 (3)	10±1 (3)	47±11 (3)
	P-value	0.0154	0.6670	0.3638
Amount of Mg in content of small intestine	Dry feed	2±0 (3)	23±9 (3)	76±6 (3)
	Soaked feed	7±2 (3)	13±1 (3)	109±31 (3)
	P-value	0.0472	0.3164	0.3508
Amount of Na in content of small intestine	Dry feed	2±0 (3)	38±15 (3)	99±17 (3)
	Soaked feed	3±0 (3)	27±5 (3)	71±15 (3)
	P-value	0.4102	0.5277	0.2899
Amount of Zn in content of small intestine	Dry feed	1±0 (3)	15±8 (3)	36±1 (3)
	Soaked feed	2±0 (3)	14±1 (3)	45±11 (3)
	P-value	0.0154	0.8486	0.4669

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

*Table 8 Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in **hindgut** of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.*

Time after feeding, h		2	6	12	18	24	48
Amount of fat in content of hindgut	Dry feed	0 (3)	0 (2)	- (0)	3 (2)	2 (1)	- (0)
	Soaked feed	0 (3)	0 (2)	- (0)	4 (1)	3 (1)	- (0)
	P-value	-	-	-	0.2047	-	-
Amount of N in content of hindgut	Dry feed	0 (3)	0 (2)	4±1 (3)	4 (2)	6±2 (2)	2 (1)
	Soaked feed	0 (3)	0 (2)	3 (3)	5±1 (3)	3 (3)	- (0)
	P-value	-	-	0.0865	0.4919	0.2036	-
Amount of energy in content of hindgut	Dry feed	0 (3)	0 (2)	6 (1)	6 (2)	7±2 (2)	- (0)
	Soaked feed	0 (3)	0 (2)	- (0)	8±1 (2)	7 (1)	- (0)
	P-value	-	-	-	0.0535	0.9468	-
Amount of Y_2O_3 in content of hindgut	Dry feed	0 (3)	0 (3)	19±2 (3)	18±5 (3)	20±3 (3)	4±2 (3)
	Soaked feed	0 (3)	0 (3)	20±1 (3)	30±5 (3)	25±3 (3)	1±1 (3)
	P-value	-	0.7137	0.8338	0.1594	0.3370	0.1858
Amount of P in content of hindgut	Dry feed	0 (3)	0 (3)	13±2 (3)	11±3 (3)	11±2 (3)	2±1 (3)
	Soaked feed	0 (3)	0 (3)	14±1 (3)	20±3 (3)	13±2 (3)	1 (3)
	P-value	-	0.7568	0.5901	0.0935	0.4716	0.2518
Amount of Ca in content of hindgut	Dry feed	0 (3)	0 (3)	20±2 (3)	18±5 (3)	24±4 (3)	7±1 (3)
	Soaked feed	0 (3)	0 (3)	22 (3)	34±4 (3)	24±3 (3)	7±2 (3)
	P-value	-	0.6069	0.3822	0.0604	0.9190	0.8170
Amount of Mg in content of hindgut	Dry feed	0 (3)	0 (3)	31±6 (3)	33±15 (3)	74±4 (3)	17±5 (3)
	Soaked feed	0 (3)	1±1 (3)	26±5 (3)	61±14 (3)	34±1 (3)	16±5 (3)
	P-value	-	0.6731	0.4860	0.2556	0.0007	0.8517
Amount of Na in content of hindgut	Dry feed	0 (3)	0 (3)	50±2 (3)	39±12 (3)	41±12 (3)	9±4 (3)
	Soaked feed	0 (3)	0 (3)	25±1 (3)	33±6 (3)	30±4 (3)	2±1 (3)
	P-value	-	0.8641	0.0006	0.6792	0.4295	0.1398
Amount of Zn in content of hindgut	Dry feed	0 (3)	0 (3)	20±2 (3)	16±5 (3)	20±5 (3)	6±4 (3)
	Soaked feed	0 (3)	0 (3)	17±1 (3)	26±4 (3)	19±2 (3)	1±1 (3)
	P-value	-	0.9312	0.2301	0.2256	0.8711	0.3214

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

Table 9 *Percentage of nutrient, energy or Y_2O_3 ingested from feed, recovered in **hindgut** of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences ($P<0.05$) are indicated with red, and trends ($P<0.1$) are indicated with blue.*

Time after feeding, h		**2**	**6**	**12**
Amount of fat in content of hindgut	Dry feed	0±0 (3)	0±0 (2)	- (0)
	Soaked feed	0±0 (3)	0±0 (2)	- (0)
	P-value	-	-	-
Amount of N in content of hindgut	Dry feed	0±0 (3)	0±0 (2)	5±1 (3)
	Soaked feed	0±0 (3)	0±0 (2)	4±0 (3)
	P-value	-	-	0.1300
Amount of energy in content of hindgut	Dry feed	0±0 (3)	0±0 (2)	8 (1)
	Soaked feed	0±0 (3)	0±0 (2)	- (0)
	P-value	-	-	-
Amount of P in content of hindgut	Dry feed	0±0 (3)	0±0 (3)	17±2 (3)
	Soaked feed	0±0 (3)	0±0 (3)	19±1 (3)
	P-value	-	0.8558	0.4023
Amount of Ca in content of hindgut	Dry feed	0±0 (3)	0±0 (3)	25±4 (3)
	Soaked feed	0±0 (3)	0±0 (3)	30±1 (3)
	P-value	-	0.6742	0.2684
Amount of Mg in content of hindgut	Dry feed	0±0 (3)	0±0 (3)	41±8 (3)
	Soaked feed	0±0 (3)	1±1 (3)	35±6 (3)
	P-value	-	0.7566	0.6226
Amount of Na in content of hindgut	Dry feed	0±0 (3)	0±0 (3)	65±4 (3)
	Soaked feed	0±0 (3)	0±0 (3)	35±1 (3)
	P-value	-	0.9746	0.0017
Amount of Zn in content of hindgut	Dry feed	0±0 (3)	0±0 (3)	26±3 (3)
	Soaked feed	0±0 (3)	0±0 (3)	23±2 (3)
	P-value	-	0.9559	0.4118

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

3.1.5 Content of digestibility marker in stomach, small intestine and hindgut

The fraction of total analysed Y_2O_3 found in stomach, small intestine and gut, respectively is shown in Fig. 3. When comparing the two treatment groups with an ANOVA at each sampling point (n=3), salmon fed the soaked diet showed significantly lower fraction of the total Y_2O_3 in the stomach and significantly larger fraction in the small intestine compared to fish fed dry feed after 2 hours. The regression analysis showed that there was a trend (P=0.08) that this difference was affected by deviation in sampling time. After 24 h, the amount of total Y_2O_3 found in the hindgut was significantly higher in fish fed soaked diet than in fish fed dry diet.

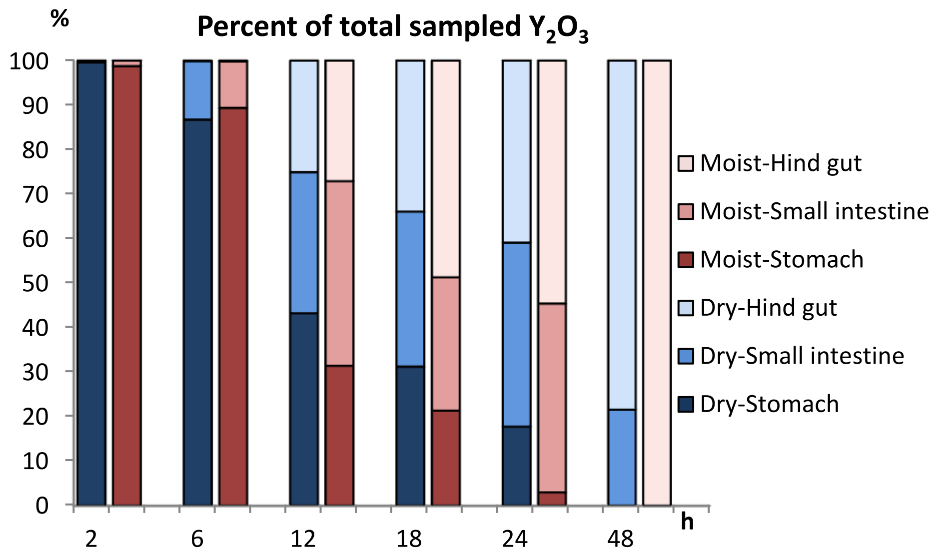

Figure 3 *Amount (%) of the total analyzed Y_2O_3 found in stomach, small intestine and hindgut of Atlantic salmon fed one single meal of dry or soaked feed. X-axis represents time (h) after feeding. Content of hindgut appeared at 12 h post feeding, and from this point of time, Y_2O_3 may be excreted in the faeces.*

3.2 Relative disappearance from stomach

The relative disappearance (RD, %) of nutrients from stomach is calculated equivalently to the apparent digestibility, except that concentration of nutrients and marker from stomach content is used instead of concentrations in faeces. The RD in stomach reflects the rate at which the nutrients, relative to Y_2O_3, are transferred from stomach into the pylorus.

No significant differences in the RD of dry matter from stomach were revealed (Fig. 4). Generally, the RD of dry matter from both feeds increased over time, except RD of dry matter from dry feed at 6 h. At 48 h, stomachs were empty or contained insufficient sample material for chemical analysis, thus n=0 for measurements of relative disappearance at 48 h.

The RD of N was similar to that of dry matter, with non-significantly higher RD of N from soaked feed than dry feed at 2, 12 and 18 h after feeding. For fat however, the RD was non-significantly lower in soaked feed than dry feed at 12 and 18 h, indicating that soaking the feed may have increased passage time of dry matter and N from stomach, whereas it delays the passage time of fat (Fig 4).

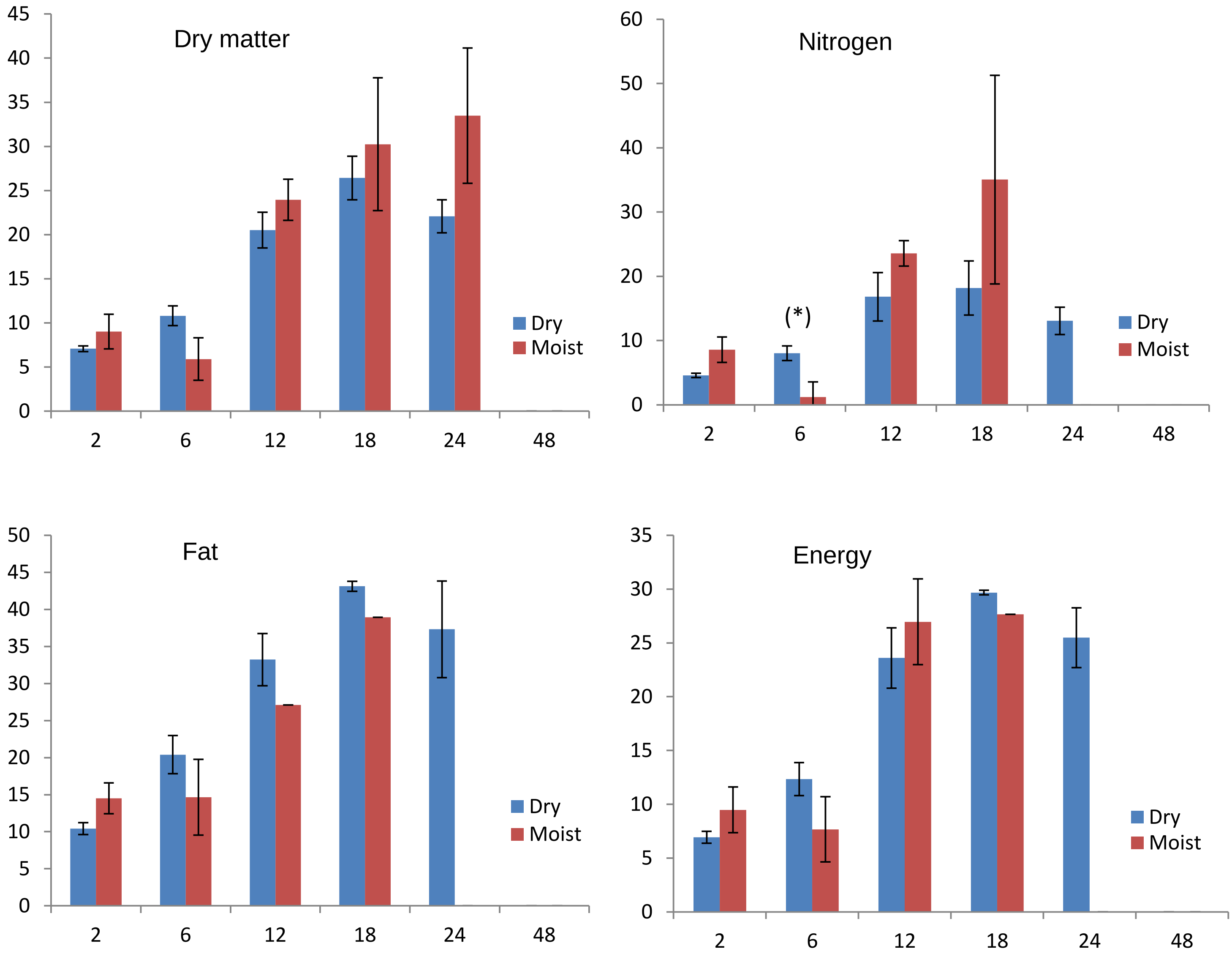

Figure 4 *Relative disappearance (%) of dry matter, N, fat and energy from stomach of Atlantic salmon fed one single meal of dry or soaked feed. X-axis represents time (h) after feeding. The two treatment groups are compared with an ANOVA at each sampling point. Data are given as mean ± SEM. n, given in the order as the bars appear along the x-axis (2h dry, 2 h moist, 6h dry, 6 h moist etc) were as follows:*

- Dry matter: 3, 3; 3, 3; 3, 3; 3, 2; 3, 3; 0 and 0

- Nitrogen: 3, 3; 3, 3; 3, 3; 2, 2; 2, 0; 0 and 0

- Fat: 3, 3; 3, 3; 3, 1; 2, 1; 2, 0; 0 and 0

- Energy: 3, 3; 3, 3; 3, 2; 2, 1; 2, 0; 0 and 0

(*) Trend (P<0.1)

The RD of minerals is shown in Table 10. P and Zn followed a pattern resembling that of RD of dry matter, except that at 2 h after feeding, the RD from soaked feed was lower (not significantly) than RD from dry feed. Furthermore, as for RD of N, there was a trend (P<0.1) to lower RD of P and Zn from soaked feed than dry feed at 6 h after feeding.

The negative values of Mg and Na reflect the fish drinking sea water. For both elements, the RD values show larger drinking rate for fish fed dry feed, although only significantly different at 6 h after feeding.

Table 10 Relative disappearance (RD, %) of minerals form stomach of Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.

Time after feeding, h		2	6	12	18	24	48
RD of P in stomach	Dry feed	10±1 (3)	14±2 (3)	30±5 (3)	31±4 (3)	37±11 (3)	- (0)
	Soaked feed	8±2 (3)	6±2 (3)	43±4 (3)	37±5 (2)	58±12 (3)	- (0)
	P-value	0.2957	0.0720	0.1236	0.4202	0.2660	-
RD of Ca in stomach	Dry feed	8±1 (3)	7±1 (3)	21±3 (3)	14±7 (3)	6±10 (3)	- (0)
	Soaked feed	4±2 (3)	4±3 (3)	27±3 (3)	10±15 (2)	47±18 (3)	- (0)
	P-value	0.0857	0.3606	0.2585	0.7714	0.1176	-
RD of Mg in stomach	Dry feed	4±2 (3)	-4±2 (3)	2±6 (3)	-16±22 (3)	-60±89 (3)	- (0)
	Soaked feed	9±0 (3)	21±2 (3)	16±5 (3)	-44±71 (2)	-18±20 (3)	- (0)
	P-value	0.0714	0.0008	0.1453	0.6732	0.6670	-
RD of Na in stomach	Dry feed	-48±19 (3)	-92±28 (3)	-145±44 (3)	-263±86 (3)	-269±128 (3)	- (0)
	Soaked feed	-7±2 (3)	-2±2 (3)	-60±14 (3)	-72±31 (2)	-175±31 (3)	- (0)
	P-value	0.1013	0.0315	0.1382	0.1905	0.5156	-
RD of Zn in stomach	Dry feed	12±0 (3)	13±2 (3)	17±5 (3)	13±9 (3)	25±15 (3)	- (0)
	Soaked feed	9±2 (3)	6±2 (3)	30±5 (3)	29±8 (2)	49±15 (3)	- (0)
	P-value	0.1230	0.0976	0.1391	0.2735	0.3272	-

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

3.3 Relative disappearance from small intestine

The relative disappearance (RD, %) of nutrients from small intestine is calculated equivalently to the apparent digestibility, except that concentration of nutrients and marker from content of small intestine is used instead of concentrations in faeces. The RD of small intestine reflects the rate at which the nutrients, relative to Y_2O_3, are absorbed, or transferred to the hindgut.

The RD of dry matter from small intestine was numerically, but not significantly lower for dry feed than moist feed at almost all sampling points (Fig. 5). The exceptions were at 6 h after feeding, where there was a trend to higher RD of dry matter from dry feed than moist feed, and at 48 h when only fish fed moist feed had sufficient content for chemical analyses in this segment of the gut.

Negative values for RD of dry matter in small intestine were found at 2 h after feeding. Since RD is the disappearance of, in this case dry matter, relative to Y_2O_3, the negative values reflect a slower rate of Y_2O_3 than dry matter from the stomach to pylorus, as also indicated in the data in Fig 2 and Tables 4-5. Similarly, a negative value of RD of energy was found in soaked feed at 6 h (Fig. 5).

There was a trend ($P<0.1$) towards lower RD of N from soaked than dry feed in this gut segment at 6 h after feeding, but oppositely at 12 h.

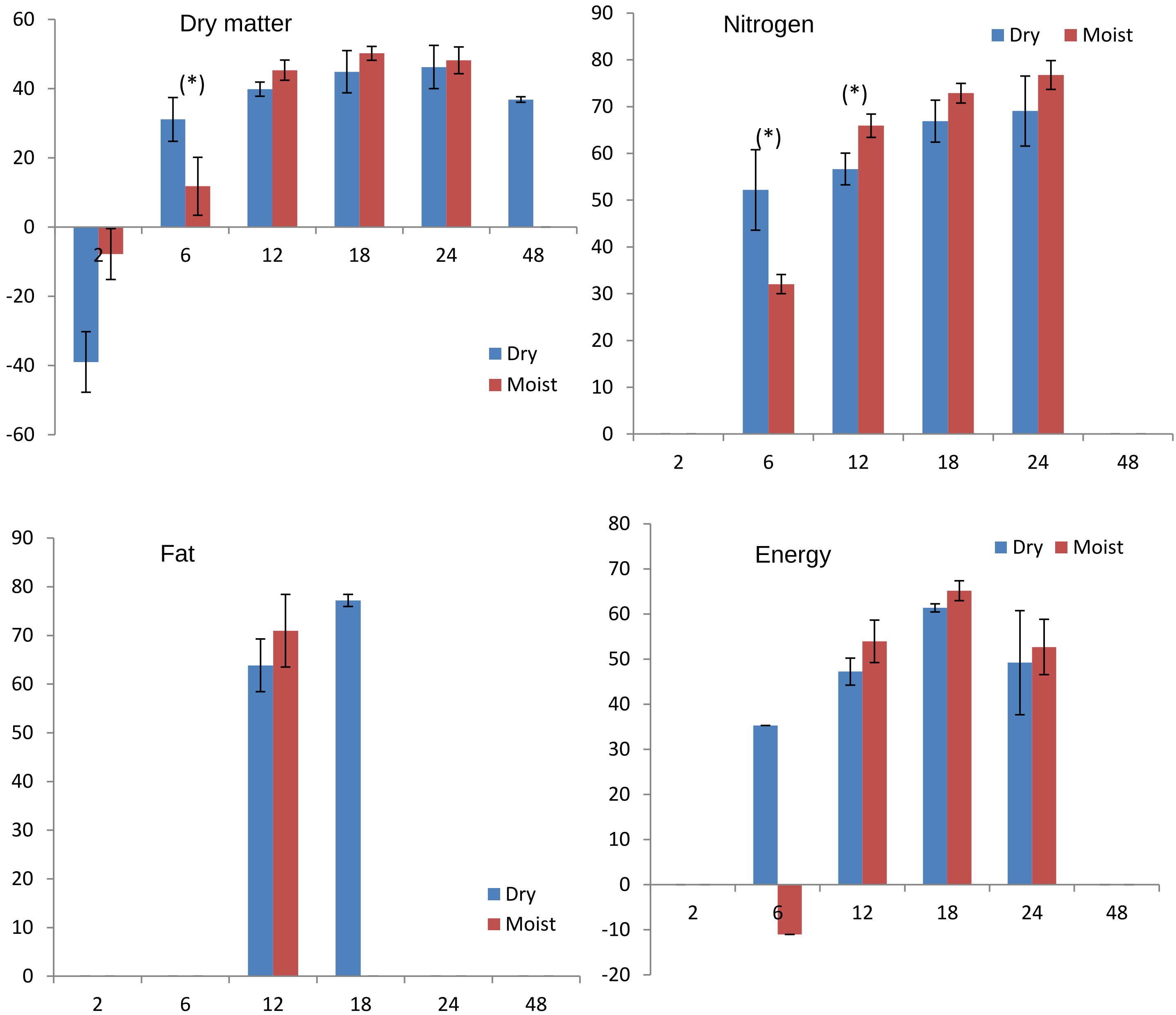

Figure 5 Relative disappearance (%) of dry matter, N, fat and energy from small intestine of Atlantic salmon fed one single meal of dry or soaked feed. X-axis represents time (h) after feeding. The two treatment groups are compared with an ANOVA at each sampling point. Data are given as mean ± SEM. n, given in the order as the bars appear along the x-axis (2h dry, 2 h moist, 6h dry, 6 h moist etc) were as follows:

- *Dry matter: 3, 3; 3, 3; 3, 3; 3, 3; 3, 3; 2 and 0*
- *Nitrogen: 0, 0; 2, 3; 3, 3; 3, 3; 3, 3; 0 and 0*
- *Fat: 0, 0; 0, 0; 2, 2; 2, 0; 0, 0; 0 and 0*
- *Energy: 0, 0; 1, 1; 3, 2; 2, 2; 2, 2; 0 and 0*

** Significantly different (P<0.05)*

(*) Trend (P<0.1)

No significant differences in RD of P between the two feeds were revealed, but numerically, the RD of P in small intestine was higher in soaked than dry feed, except at 6 h after feeding. The RD of P increased over time, except that lower RD of P from dry feed was found at 48 h than at 24 h (Table 11).

There was neither any significant difference in the RD of Ca. and Zn from dry and soaked feed in small intestine. However, negative values were observed.

Negative values of RD of Mg and Na were also observed, significantly ($P<0.05$) lower negative values for dry than soaked feed at 6 h after feeding for Mg, and at 2 and 6 h for Na.

Negative RD values may be due to both drinking sea water (Na, Mg, Ca), and a gastrointestinal passage rate that differs from that of Y_2O_3.

Table 11 Relative disappearance (RD, %) of minerals form small intestine of Atlantic salmon fed one singlemeal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.

Time after feeding, h		**2**	**6**	**12**	**18**	**24**	**48**
RD of P in small intestine	Dry feed	4±4 (3)	26±2 (3)	29±4 (3)	35±6 (3)	56±4 (3)	29±7 (2)
	Soaked feed	12±1 (3)	19±3 (3)	34±6 (3)	46±4 (3)	59±2 (3)	- (0)
	P-value	0.1503	0.1176	0.5837	0.2049	0.6365	-
RD of Ca in small intestine	Dry feed	-97±85 (3)	6±4 (3)	-12±3 (3)	-30±11 (3)	-22±9 (3)	-55±20 (2)
	Soaked feed	-41±38 (3)	2±3 (3)	-14±3 (3)	-32±10 (3)	-29±11 (3)	- (0)
	P-value	0.5800	0.5094	0.6636	0.8710	0.6306	-
RD of Mg in small intestine	Dry feed	-501±240 (3)	-82±16 (3)	-144±27 (3)	-322±31 (3)	-340±127 (3)	-169±243 (2)
	Soaked feed	-500±252 (3)	-21±9 (3)	-157±16 (3)	-299±48 (3)	-253±64 (3)	- (0)
	P-value	0.9976	0.0290	0.6988	0.7024	0.5748	-
RD of Na in small intestine	Dry feed	-446±32 (3)	-194±1 (3)	-211±31 (3)	-184±61 (3)	-140±50 (3)	-188±71 (2)
	Soaked feed	-113±5 (3)	-157±20 (3)	-73±20 (3)	-30±14 (3)	-29±15 (3)	- (0)
	P-value	0.0005	0.1486	0.0201	0.0706	0.1015	-
RD of Zn in small intestine	Dry feed	-58±10 (3)	-6±12 (3)	-16±10 (3)	14±12 (3)	30±9 (3)	-39±26 (2)
	Soaked feed	-45±23 (3)	-32±5 (3)	-8±16 (3)	24±7 (3)	26±8 (3)	- (0)
	P-value	0.6174	0.11182	0.7179	0.4957	0.7575	-

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

3.4 Apparent digestibility

The apparent digestibility (AD, %) of nutrients reflects the disappearance of nutrients, presumably due to absorption, relative to the digestibility marker (Y_2O_3). The AD of nutrients and energy is shown in Fig. 6 and Table 12.

There was a trend ($P<0.1$) to higher AD of DM from soaked feed than dry feed 12 h after feeding (Fig. 6). Except for this there were no significant differences in AD of dry matter between the two feeds. At 48 h, the AD of dry matter was lower than at 24 h for both feeds.

No significant difference in AD of N, fat or energy between dry and soaked feed were observed (Fig. 6).

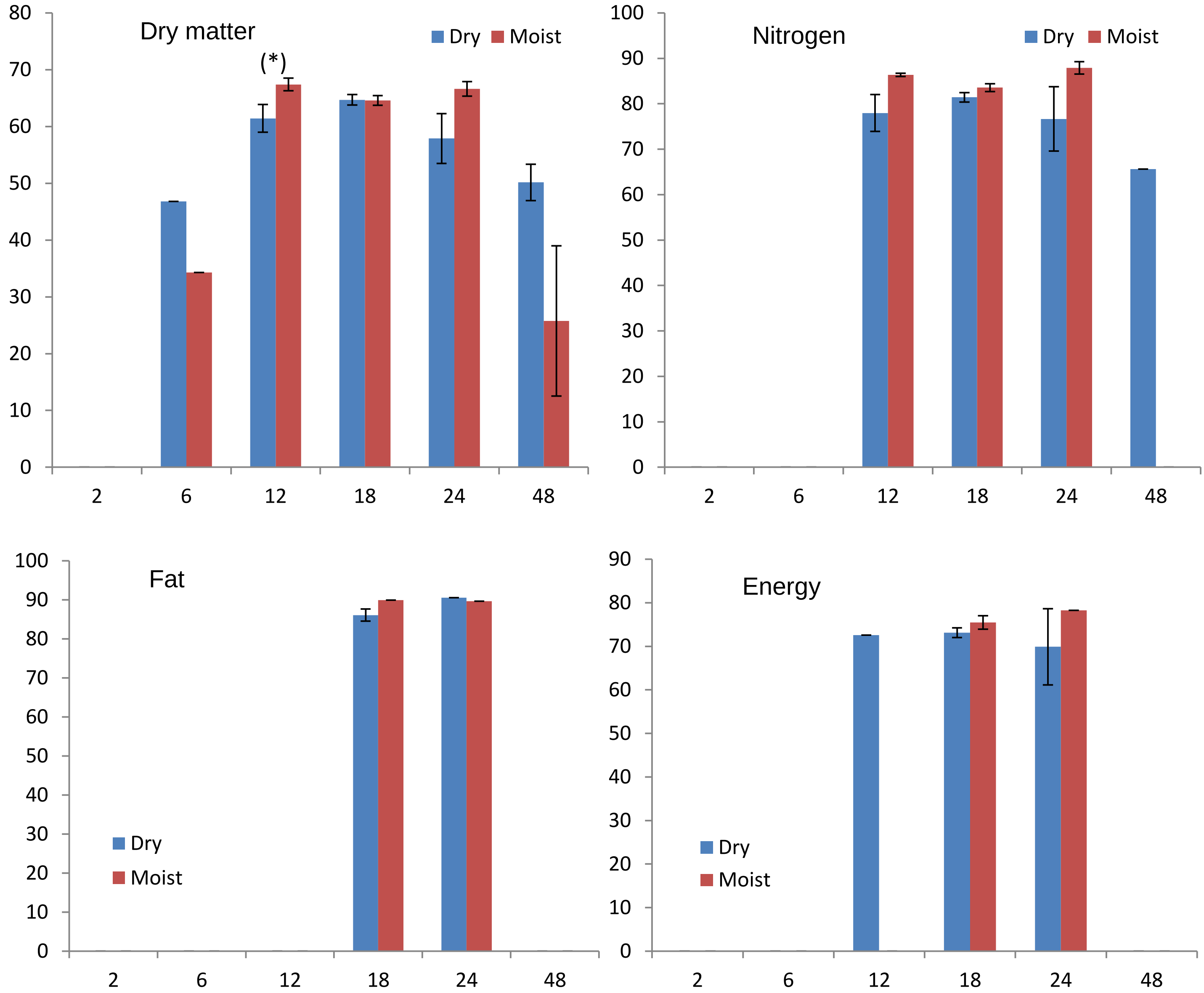

Figure 6 *Apparent digestibility (%) of dry matter, N, fat and energy in Atlantic salmon fed one single meal of dry or soaked feed. X-axis represents time (h) after feeding. The two treatment groups are compared with an ANOVA at each sampling point. Data are given as mean ± SEM. n, given in the order as the bars appear along the x-axis (2h dry, 2 h moist, 6h dry, 6 h moist etc) were as follows:*

- Dry matter: 0, 0; 1, 1; 3, 3; 3, 3; 3, 3; 3 and 3

- Nitrogen: 0, 0; 0, 0; 3, 3; 2, 3; 2, 3; 1 and 0

- Fat: 0, 0; 0, 0; 0, 0; 2, 1; 1, 1; 0 and 0

- Energy: 0, 0; 0, 0; 1, 0; 2, 2; 2, 1; 0 and 0

** Significantly different (P<0.05)*

(*) Trend (P<0.1)

There was no significant difference between the two feeds in AD of P, and as opposed to AD of dry matter, the AD of P from both dry and soaked feed increased throughout the sampling period (Table 12).

Negative AD-values were observed for Na, Mg and Ca, which are present in sea water. For Na, the higher negative AD-values were observed in dry feed compared to soaked feed, reflecting a reduced drinking rate when soaking the feed before feeding. The AD of Mg and Ca varied at the different sampling points, but at 24 h after feeding, the AD of both of these was significantly lower, with negative values, in dry feed than in soaked feed (Table 12).

Table 12 *Apparent digestibility (AD, %) of minerals in Atlantic salmon fed one single meal of dry or soaked feed. The two treatment groups are compared with an ANOVA at each sampling point. (Mean ± SEM, n is given in brackets.) Significant differences (P<0.05) are indicated with red, and trends (P<0.1) are indicated with blue.*

Time after feeding, h		**2**	**6**	**12**	**18**	**24**	**48**
AD of P	Dry feed	- (0)	13 (1)	32±4 (3)	38±1 (3)	44±3 (3)	49±7 (3)
	Soaked feed	- (0)	22 (1)	28±3 (3)	33±4 (3)	47±1 (3)	53±8 (3)
	P-value	-	-	0.5292	0.3108	0.3799	0.7326
AD of Ca	Dry feed	- (0)	-18 (1)	-1±7 (3)	1±4 (3)	-18±3 (3)	-98±62 (3)
	Soaked feed	- (0)	-62 (1)	-12±8 (3)	-17±10 (3)	3±2 (3)	-634±263 (3)
	P-value	-	-	0.3400	0.1608	0.0030	0.1184
AD of Mg	Dry feed	- (0)	-159 (1)	-61±20 (3)	-79±46 (3)	-289±59 (3)	-404±214 (3)
	Soaked feed	- (0)	-189 (1)	-32±24 (3)	-105±35 (3)	-41±19 (3)	-1 367±445 (3)
	P-value	-	-	0.4105	0.6803	0.0163	0.1229
AD of Na	Dry feed	- (0)	-207 (1)	-162±10 (3)	-123±38 (3)	-99±30 (3)	-125±59 (3)
	Soaked feed	- (0)	-120 (1)	-28±4 (3)	-11±17 (3)	-19±7 (3)	-16±11 (3)
	P-value	-	-	0.0002	0.0535	0.0636	0.1437
AD of Zn	Dry feed	- (0)	-48 (1)	-6±10 (3)	14±12 (3)	3±8 (3)	-15±46 (3)
	Soaked feed	- (0)	7 (1)	13±3 (3)	14±4 (3)	24±1 (3)	22±18 (3)
	P-value	-	-	0.1283	0.9902	0.0625	0.4987

- Not sufficient material for chemical analysis, or not sufficient replicates for ANOVA.

4 Discussion

The individual variation within treatment in this trial was large. The variation may be due to individual/genetic differences in gastrointestinal passage rate, but may also be related to experimental factors such as anaesthesia and stress. Due to the large variation and, consequently, limited power of the statistical analyses, non-significant patterns should not be dismissed, but instead, investigated further.

Besides, soaking the feed for 2 h resulted in softening the outer layer of the pellet, whereas the core was still hard after soaking. As reported by Oehme et al. (2012), soaking the feed did not reduce pellet hardness. Thus, if using pellet qualities that change more during soaking, the effect of soaking may be larger than in the present study.

The gastrointestinal passage rate may be affected by anaesthesia and stress, thus the present data may not represent absolute data for passage rate, but was intended as a comparison between the two feeds used, assuming that anaesthesia and stress affected both feed groups equally.

Dividing the gastrointestinal tract into 1) stomach, 2) small intestine and 3) hindgut makes it possible to study processes in the stomach, and the overall gastrointestinal transit time. In the small intestine, nutrients are both absorbed and released to the hindgut, and our data do not give detailed information about the processes in this segment of the gut.

Although it is impossible to empty the gastrointestinal tract completely to collect the samples, the collected amount of nutrients (in Fig. 2 and Tables 4, 6, and 8, given as % of ingested nutrient) gives valuable information about the passage through the gastrointestinal tract. Overall, these data suggest that that soaking the feed increased the gastrointestinal passage rate somewhat, although few significant differences were found. Shortly after feeding however, a significant increase of soaking was found on the rate at which feed was transferred from stomach to pylorus (Fig. 2). Some of the difference in amount of DM and nutrients in stomach after 2 h was explained by deviation in sampling time, however, in gut there was no effect of deviation in sampling time on difference in dry matter. Thus, this difference is explained by feed only, which confirms that the gastric evacuation rate increased when feed was soaked. This was also confirmed by using Y_2O_3 to calculate feed intake and shows that overall, the gastric evacuation rate increased when feed was soaked.

The data also show clearly that the various components of the feed have different passage rate. In the stomach, fluid and peristaltics cause feed to disintegrate, and the chyme is released into the pylorus, controlled by the pyloric sphincter. The solubility and disintegration rate of feed components in the stomach are important factors for the rate at which the components enters the pylorus. It has also been shown that gastric evacuation rate is impeded as particle size increases (Sveier *et al.*, 1999). Two h after feeding, the highest amount of eaten material still present in the stomach was found for Y_2O_3 in both dry and soaked feed, whereas the gastrointestinal evacuation rate was considerably higher for e.g. fat (Table 4). The slow rate of Y_2O_3 to be released from stomach to pylorus, results in an underestimation of the RD of nutrients from small intestine at the first measurements after feeding, seen as negative values for RD of dry matter at 2 h.

Different digestibility markers have different solubilities and follow different fractions of the gut content, and thus, result in different estimates of apparent digestibility (Austreng *et al.*, 2000). In digestibility studies where apparent digestibility is estimated from chemical analysis of faeces, fish is normally fed daily over a period of at least two weeks before collection of faeces. Thus, the flow of nutrients and digestibility marker through the gastrointestinal tract is assumed to be constant so that differences in solubilities even out, provided that there is a constant feed intake prior to sampling. In the present study however, the gut was empty before feeding the one meal. Thus, the different feed components move through the gut at different rates and the calculated RD and AD ares therefore only a comparison of the rate of the nutrients with the rate of Y_2O_3.

Although soaking of the feed did not have a very large effect on the physical quality of the whole pellet, and there was some individual variation also in the trial performed by Oehme et al (2012), where soaking of the feed had a positive effect on feed intake, particularly at low feed intake. Correspondingly, in spite of relatively small differences in physical feed quality and individual variation in the present trial, soaking of the feed increased gastric evacuation rate in salmon. Thus, the increased gastric evacuation rate in salmon fed a soaked feed compared to a dry feed, may at least in part explain the corresponding increased feed intake in salmon fed soaked diet found by Oehme et al. (2012). In the present trial, soaking the feed did not have a vast effect on the gastric evacuation rate and the difference in gastric evacuation rate in salmon fed dry or soaked feed was small. However, our data indicate that today's commercial salmon feeds may not have the optimal pellet quality with regard to optimal feed intake, at least in periods when feed intake is low.

4.1 Conclusion

The individual variation in gastrointestinal passage rate was large. Few significant differences between the two feeds were revealed, however, the gastric evacuation rate was increased by soaking the feed. At the conditions present, stomachs were nearly emptied 24 h after feeding, and 48 h after feeding, some content was still present in hindgut. Although not significantly different, both stomach and hindgut appeared to empty earlier in salmon fed soaked feed compared to salmon fed dry feed.

4.2 Acknowledgement

The work was funded by the Centre for Research-based Innovation in Aquaculture Technology (CREATE) and was done in collaboration with BioMar, Lerøy, Marine Harvest and Salmar. The authors wish to thank Asbjørn Valset and Ane Maria Guttu for contributing with knowledge and hard work during the trial, and Britt Seljebø and Kristin Skei Nerdal for skillfully carrying out the chemical analyses.

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ISBN 978-82-8296-120-2 (printed)
ISBN 978-82-8296-121-9 (pdf)
ISSN 1890-579X