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Nutritional value of feeds with different physical qualities

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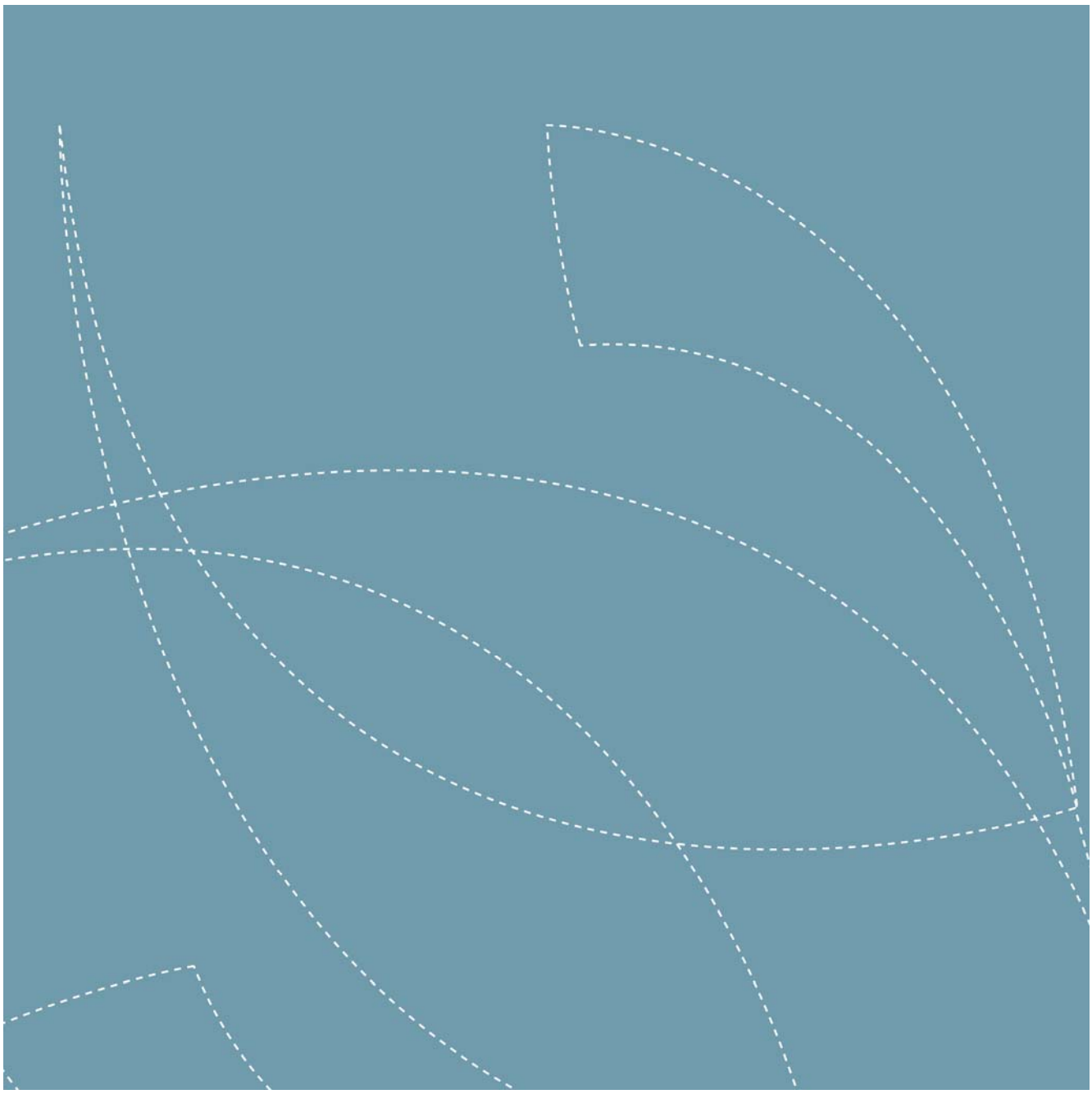

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Summary:
In a previously performed study, rainbow trout with mean initial weight 1144 g was fed two experimental feeds with similar chemical composition, but differing in physical properties (high and low water stability, denoted Feed A and B, respectively). The fish was also exposed to stable or fluctuating environment (salinity, temperature and O_2 saturation). The feed intake was highest in trout fed Feed B, and when kept at stable environment. The growth tended to follow the same pattern (not significantly though). At termination of the trial, the stomachs of trout fed Feed B contained the largest amounts of feed material. The stomachs of these fish also contained large amounts of free water and oil, most severely in trout kept at stable environment, coinciding with the highest feed intake.

In the present experiment, the physical properties of the two feeds were further investigated. Feed A had less dust, more unbroken pellets, higher hardness, larger diameter and shorter pellets than Feed B. The apparent digestibility (ADC, %) of protein (the sum of amino acids) and most individual amino acids, starch, dry matter, energy and phosphorus was highest in Feed A. The environmental treatment also affected the ADC of some amino acids and minerals. In general however, the ADC appeared to be reduced at high feed intake. The plasma concentrations of sodium and chloride ions were not affected by the feed, but were elevated in individuals kept at the constant environment.

The results clearly demonstrate that the nutritional value of a feed is affected by its physical properties. Furthermore, several of the treatment effects in the fish caused by physical feed properties, may be related to the feed intake.

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1 Introduction

In commercial fish farming, the feed is commonly transported from a storage silo to the sea pens by means of a pneumatic conveying system, i.e. the feed pellets are carried by an air stream through a pipe system. This may cause some pellet breakage and formation of undersized particles, which in turn represent economic loss and unintended addition of nutrients to the water. Considering the large amounts of feed used in intensive fish farming, even small fractions lost will sum up to large costs. In 2007, approximately 7,000,000,000 NOK was spent on feed in Norwegian fish farming, meaning that each percent loss costs 70,000,000 NOK. Consequently, feed pellets with high technical quality are preferred.

High prices of fish meal and fish oil due to limited availability of these ingredients have forced the aqua feed producers to use several different feed ingredients. The ingredient composition however, affects the technical quality of the feed (Refstie et al., 2006, Sørensen et al., 2009), resulting in variation in the technical properties of commercial feeds. Pellet breakage is easily visible and quantifiable, and a fish farmer is likely to return a shipment of feed with poor pellet quality. However, the technical properties of a feed may influence its biological value, and thus the growth of the fish. And, since pellet breakage in the conveying system to a certain degree can be minimised by adjusting the settings (Sørensen et al., 2008), the nutritional value may be of larger interest to the fish farmer. Reduced growth of the fish represents a substantial economic loss, although losses due to suboptimal pellet quality are not easy to detect and quantify.

Some research has been carried out in order to study the interaction between technical and nutritional qualities of salmonid feeds. However, Sørensen et al. (Sørensen et al., 2007) showed that the growth rate was significantly higher in rainbow trout (*Oncorhynchus mykiss*) fed a feed with a “soft” pellet than in trout fed a feed with harder pellet. Baeverfjord et al. (2006) found water- and oil separation in the stomach of rainbow trout fed pellets with low water stability. This may potentially lead to fat belching, which in addition to aesthetic and welfare concerns, represents loss of highly valuable dietary fat.

Fat belching is associated with abdominal distension syndrome, which is characterised by enlarged stomach with water and oil accumulation (Staurnes et al., 1990). Among the Norwegian farmed species, this condition is a problem in rainbow trout, and, to a lesser extent Atlantic salmon, reared in sea water. Feed quality, high lipid content, high feed intake, high water salinity, high temperature and low oxygen saturation are factors which affects the development of distended abdomen (Anderson, 2006).

A previous study was carried out aiming at investigating the effect of technical quality of the feed on fat belching in rainbow trout kept at stable or fluctuating environment (Terjesen et al., 2008). The results from this experiment showed that the feed intake was highest in fish fed the feed with low water stability. The growth rate also tended to be greater for trout fed this feed, compared to trout fed a feed with high water stability. Although no fat belching was observed, separation of water and oil was found in the stomach of these fish. Neither technical feed quality nor nutrient digestibility was analysed in the experiment of Terjesen et al. (2008). In the present experiment, samples from this trial were analysed in order to estimate nutrient digestibility and technical properties of the two feeds. The aim was to obtain more knowledge about interaction between technical quality and nutritional value of extruded feed, when fed to rainbow trout at standardised experimental conditions, and at conditions mimicking the fluctuating environment in Norwegian fjords.

2 Materials and methods

2.1.1 Experimental feeds

Two test feeds (denoted Feed A and B) were designed by BioMar Ltd, and different extrusion parameters and starch contents were used to achieve different physical properties of the feeds. The two feeds were intended to have high (Feed A) and low (Feed B) water stability. Except for the starch content, the feeds were formulated to be similar in chemical composition (Table 1). Yttrium oxide (Y_2O_3) was added to both feeds for digestibility estimation.

Both feeds had pellet length and diameter of approximately 9 mm.

Fatty acid analyses (data not shown) revealed only minor differences between Feed A and B in the quantitatively important fatty acids. Some fatty acids present in smaller amounts (particularly C20:1 n-9, C 22:1 n-11 and C 22:4 n-6) was higher in Feed A than in Feed B however.

The feeds were stored at 4ºC until use.

2.1.2 Fish trial

Twenty-four to twenty-six rainbow trout (*Oncorhynchus mykiss*) of 1144±24 (SD) g, fasted for 24 hours prior to the start of the trial, were distributed to each of 8 quadratic tanks (1 m^3). Feed A and B was allocated to four tanks each, and two tanks from each dietary treatments were equipped for daily change in environment (temperature, oxygen saturation and salinity). Because of the manual work required for the environmental treatment groups, the tanks at the end of a tank row were chosen for these groups in order to reduce stress on the experimental fish. Thus, tanks and treatments were not randomly distributed.

Biomass per tank ranged between 27.5 and 29.4 kg. The fish had not previously been used in experiments, and was fed Skretting Orion LB U 200-60A (Skretting, Stavanger, Norway) the last month prior to the present experiment. Water temperature was increased from 7-8°C to about 12°C the last weeks before the trial.

The tanks were supplied with 10 µm filtered and UV-treated seawater with salinity 32-34 ppt, temperature 12-14°C and with oxygen level >80%, except for the fish that were exposed to fluctuations in water, salinity and temperature (environmental changes). The fish was exposed to 24 h continuous light, and were fed one meal per day using disc feeders. The feeding period lasted for one hour from 07:15. The amount of feed was adjusted every three days aiming at feeding 20% excess. Feed intake was estimated from amount of feed allotted and feed waste collected, correcting for recovery of each feed (Helland et al., 1996). Collected feed waste was stored at -20ºC for each tank, and at termination of the feeding experiment, the waste was homogenized, and dry matter content measured. When feed intake during Period 1 had stabilized at the expected level for trout of this size held at 12°C (calculated according to Skretting ARC, Norway), the fish was exposed to changes in the environmental conditions, and Period 2 commenced (Table 2).

Table 1 Chemical composition of diet A and B. Amino acids are given as dehydrated residuals.

	Feed A	**Feed B**
Dry matter (g/kg)	957	958
In dry matter:		
N (g/kg)	62	66
Sum of amino acids (g/kg) [1]	313	330
Crude lipid (g/kg)	377	379
Phospholipids (g/kg)	12	12
Mono- and diacylglycerols (g/kg)	12	11
Free fatty acids (g/kg)	18	18
Triacylglycerols (g/kg)	335	338
Ash (g/kg)	88	94
Starch (g/kg)	90	36
Energy (MJ/kg)	26.3	26.5
Minerals (mg/kg):		
P	13023	13897
Ca	19435	20958
Mg	2076	2180
Na	5160	5602
Fe	275	339
Mn	47	60
Zn	167	184
Cu	10	11
Y_2O_3	488	497
Essential amino acids and Cys and Tyr (g/kg) [1]:		
Arg	20.6	23.1
His	7.9	8.6
Ile	14.6	15.8
Leu	24.8	26.5
Lys	25.2	27.3
Met	9.7	10.1
Phe	14.4	15.2
Thr	13.6	14.3
Trp	3.5	3.6
Val	15.8	16.9
Cys	3.2	3.2
Tyr	12.3	12.3
Non-essential amino acids (g/kg) [1]:		
Ala	18.1	19.3
Asp [2]	31.5	34.5
Glu [2]	53.1	53.3
Gly	17.3	18.3
Pro	14.3	14.0
Ser	13.5	14.1

[1] Amino acids are given as dehydrated residuals.

[2] Asn and Gln are oxidised to Asp and Glu, respectively, during analysis. Therefore, Asp and Glu represents both Asp and Asn, and Glu and Gln, respectively.

Oxygen saturation, temperature and salinity was monitored in the tanks with changing environment at 08:10, 09:10, 14:50 and at 22:00 hours each day, while the other four tanks were measured once daily at 14:50 hours (Fig. 2). Thus, water properties were monitored before and after the change in environmental conditions occurred. In addition, one tank in the variable environment group were monitored semi-continuously (each 10 min.) for temperature and oxygen saturation (data not shown). The environmental treatments lasted for 1 week, followed by a limited sampling (outlined below) to ascertain if stomach oil and water separation had occurred. Period 3 subsequently started, using the same treatment as in Period 2, and the trial terminated after the final sampling at day 43 and day 44.

In Table 2, an outline of the trout trial, as well as samples analysed in the previous and present project, are shown. In Period 1 (adaptation period), the trout was only subjected to the two different feeds. In Period 2 and 3, the temperature, oxygen saturation and salinity were reduced daily for a period (Table 2), imitating the fluctuating environment in Norwegian fjords. After four days of Period 2 however, the drop in O_2-level was left out due to a dramatic reduction in feed intake in the trout given the variable environment. As seen from Table 2, the samples available for the present project was stomach content, faeces and plasma samples from the final sampling.

Table 2 Overview over treatments and sampling of the fish trial, and samples available for analysis or analysed in the previous project.

	Treatment 1 Feed A	**Treatment 2** Feed B	**Treatment 3** Feed A	**Treatment 4** Feed B
Period 1 (28 days)	Adaptation	Adaptation	Adaptation	Adaptation
Period 2 (7 days)	Stable environment Salinity: 32-34 ppm Temp: 12 °C O_2-Saturation: >80%	Stable environment Salinity: 32-34 ppm Temp: 12 °C O_2-Saturation: >80%	8:15-15:00 Daily: Salinity: < 10 ppm Temp: 6 °C O_2-Saturation: 50-60% 15:00-8:15 Daily: Back to the same conditions as tanks with stable environment	8:15-15:00 Daily: Salinity: < 10 ppm Temp: 6 °C O_2-Saturation: 50-60% 15:00-8:15 Daily: Back to the same conditions as tanks with stable environment
Sampling	All tanks: Stomach content, 5 fish per tank (analysed in the previous project)			
Period 3 (7 days)	Treatment as in Period 2	Treatment as in Period 2	Treatment as in Period 2, except for stable O_2-level	Treatment as in Period 2, except for stable O_2-level
Sampling	All tanks: Stripping for faeces, pooled by tank (samples not analysed in the previous project) Plasma from 5 fish per tank (samples not analysed in the previous project) X-Ray, 5 fish per tank Stomach content, 15 fish per tank (5 of them analysed in the previous project)			

2.2 Sampling and weighing

At start of the trial, only bulk biomass was registered to reduce stress for the fish. Individual weight of sampled fish was registered at the sampling points, however. At the end of Period 2, stomachs were sampled to ascertain if stomach oil and water separation had occurred. The sampling after Period 3 was performed over two days. To achieve sampling at an equal post-prandial time and time after changing environment, a tank-specific staggered sampling schedule was employed. The days of sampling, for the relevant tanks, the feeding and environmental change took place at time intervals which allowed sampling from each tank 4 hours into the environmental treatment, which equals 5 hours after feeding began. At the sampling after period 2, the system was set to a 30 minute interval between tanks, while 50 minute intervals were used after Period 3 due to the more extensive sampling. Only four tanks could be sampled per day, therefore the variable environment groups (both feeds) were sampled the first day, followed by the constant environment groups (both feeds) the next day. The fish to be sampled was given a lethal dose of tricaine methane sulfonate (MS-222, Argent Chemical Laboratories, Redmond, WA, USA). Body weight and length was registered, and subsequently (Period 3 only), blood was collected from five fish from the caudal vein into heparinized vials, spun for 10 min at 2 200 rpm, and plasma stored at -80ºC. After Period 3, 5 fish per tank were also x-rayed in a custom-made crib (abdomen downwards, left and right hand side) using a Giotto 6020 instrument. The stomachs of 5 fish (Period 2 and 3) were excised and the stomach contents were weighed, the number of whole and kernel pellets counted, and a score (0-10) given in terms of crushed pellets, free oil and free water according to Bæverfjord *et al.* (2006). pH was measured (Orion meter) in a slit opening of the stomach before emptying the contents (Period 3 only). A digital picture or video was taken (Samsung DigiMax V4 or Fujifilm FinePix M603). The remaining 10 stomachs (Period 3) were wrapped in aluminum foil after closing the esophagus and hindgut with clips, frozen in liquid nitrogen and stored at -80ºC.

2.3 Chemical analysis

2.3.1 Samples analysed in the previous project:

Samples of the two feeds were analyzed for proximate composition. Dry matter was measured after drying to constant weight, ash was analyzed by combustion at 550°C to constant weight, crude protein by nitrogen x 6.25 (Kjeltec Auto Analyser), crude lipid (SOXTEC hydrolyzing and extraction systems), while gross energy was measured by bomb calorimetry (Parr 1271 Bomb calorimeter). Starch was determined as glucose after enzymatic hydrolysis, by a commercial kit employing the GODPOD method (Megazyme). The concentration of the different lipid classes in the feeds and their fatty acid profiles were determined by thin layer chromatography followed by gas chromatography.

2.3.2 Samples analysed in the present project:

The stomach content and faeces was analysed for dry matter, ash, nitrogen, crude lipid, gross energy and starch as described above. I addition, these samples as well as feeds were analysed for yttrium oxide (inert digestibility marker) and minerals by inductively coupled plasma mass spectroscopy (ICP-MS, at AnalyCen, Ås, Norway), and for amino acids using a Biochrom 30 amino acid analyser (Biochrom, Cambridge, UK). Tryptophan was analysed after basic hydrolysis (Hugli & Moore, 1972), and the remaining amino acids according to Davies (Davies, 2002). During sample preparation, Gln and Asn are converted to Glu and Asp, respectively. Thus, the given value for Glu and Asp in the following represents the sum of Glu and Gln, and Asp and Asn, respectively.

The plasma ions (Na^+ and Cl^-) were assayed using a Biolyte 2000 titrator. Triacylglycerols in plasma were analysed by ABX Pentra Triglycerides CP reagent kit (Horiba ABX, Montpellier, France) and Cobas Mira S analyser (Hoffman-La Roche & Co., Basel, Switzerland).

2.4 Physical properties of feed

2.4.1 Analyses performed in the previous project:

The feeds were subjected to a water stability test according to Bæverfjord et al. (2006). Briefly, triplicate samples of 10 g feed were placed in custom made steel-mesh buckets placed inside glass beakers filled with 300 ml distilled water. The beakers were shaken (100 shakings/min) in a thermostatic controlled water bath (23ºC) for 0-240 minutes. Samples were taken after 30, 60, 120 and 240 minutes for dry matter (triplicate) and lipid content analysis (one pooled sample per sampling point and feed).

2.4.2 Analyses performed in the present project:

Strength at rupture (hardness) was measured by diametral compression using a Texture-Analyser (TA-XT2®, Model 1000 R; SMS Stable Micro Systems, Blackdown Rural Industries, Surrey, UK), fitted with a 25 kg load cell and a PC-operated remote control. The analyses were carried out by pressing a cylinder (50 mm probe) onto the pellets (one by one) at a constant speed of 2 mm s- 1 to achieve 60% compression. The strength applied on the pellet was progressively increased until the pellet cracked. The strength–time graphs were recorded by a computer and analysed using the Texture Expert for Windows (version 1.15, Stable Micro Systems), and strength at rupture was recorded on 30 pellets, and was reported as the average of 3 x 10 pellets.

Length and diameter was measured on the same pellets that were used for the hardness analysis. Length was manually measured by use of an electronic digital calliper. The diameter of the pellets was automatically measured by the texture analyser.

Bulk density was determined by loose pouring the feed in to a 50-ml measuring-cylinder. Three replicates of each feed were prepared by pouring the pellets until a pile of feed had developed on the top. Then excess feed was gently removed by pulling a scrape one time over the edge of the cylinder. The sample was weighed on an electronic scale, and the bulk density was recorded as g l^{-1}.

Existing quality (amount of unbroken pellets in the feed bags) was measured on representative feed samples with a Retsch AS 200 Control (Retsch GmbH, Haan, Germany). The three following standard sieves were used: 8.0mm (1st sieve), 5.6 mm (2nd sieve), 2.36 mm, and 0.0 mm (collector). The sample (350 g) was sieved for 30 sec at 1.5 mm amplitude in 3 replicates from each feed. The fraction of pellets or dust in each sieve was weighed on an electronic scale (SJ-H/SJ-HS, A&D Co., Japan) and existing quality was calculated as the difference between full and empty 1st sieve.

DORIS durability was measured using a DORIS tester, manufactured by AKVAsmart (Bryne, Norway). Sifted pellets (350 g) were loaded into the DORIS tester and emptied afterwards at a collector on a set of 8.0 mm (1^{st} sieve), 5.6 mm (2^{nd} sieve), 2.36 mm (3^{rd} sieve), and 0.0 mm (collector) screens. Sieving was carried out for 30 sec at amplitude of 1.5 with a Retsch AS 200 Control sieving machine. The DORIS analysis was carried out in 3 replicates. Amount of dust collected from the bottom pan was weighed and reported as DORIS dust, fracture collected on the 2.36 mm screen was reported as DORIS small fracture, whereas fracture collected on the 5.6 mm screen was reported as DORIS large fracture. DORIS value

was calculated as the sum of DORIS dust and DORIS fracture (small and large). Pellets collected on the 8 mm screen were evaluated as whole pellets.

2.5 Calculations

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

$$\text{Thermal growht coefficient}\,(\text{TGC}) = 1000 \cdot \frac{\text{Final weight}^{\frac{1}{3}} - \text{Start weight}^{\frac{1}{3}}}{\text{Sum daydegrees}}$$

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

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

$\text{Relative disappearance}\,(\%) = 100 \cdot \frac{a - b}{a}$, where a represents the nutrient to marker ratio in feed and b represents the nutrient to marker ratio in stomach content. This calculation is equivalent to the calculation of ADC, except that it is calculated from the stomach concentrations, whereas ADC is calculated from the faeces concentrations.

2.6 Statistics

Tank mean was used as the statistical unit in the data from the fish trail. The trial was run with duplicate tanks, thus the data are presented as mean±range unless otherwise stated. The data from the present project was analysed with the “Proc Mixed” procedure in the SAS statistical software (SAS 1985, SAS institute Inc, Cary, USA). Period 1 was run with only one factor (feed), whereas data from period 2 and 3 was analysed as a 2x2 factorial design, fitting the main effects of feed and environment, and the interaction effect of feed x environment. The results from the measurements of the physical properties of the feeds were analysed by a Student’s t-test (SAS, 1990). These results are presented as mean values for each feed and standard errors of the means (SEM). In all statistical analysis, differences were considered significant if $P \leq 0.05$.

3 Results

3.1 Main results from the previously performed trial

The results from the first part of the project are fully reported by Terjesen et al. (2008). The main findings are also given below.

3.1.1 Water stability of the feeds

The shaking test showed large differences in the water stability between the two feeds, Feed B having very low water stability compared to Feed A (Fig. 1 and 2). The pellets from Feed A swelled and increased in wet weight, but were still almost intact after 240 min shaking. The Feed B pellets on the other hand, also swelled, but the remaining dry matter decreased rapidly, leaving only a small amount of pellet particles after 240 min.

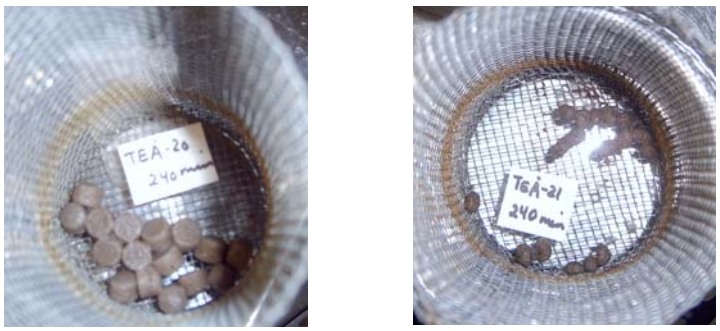

Figure 1 Feed A (left) and B (right) after 240 min in the water stability test

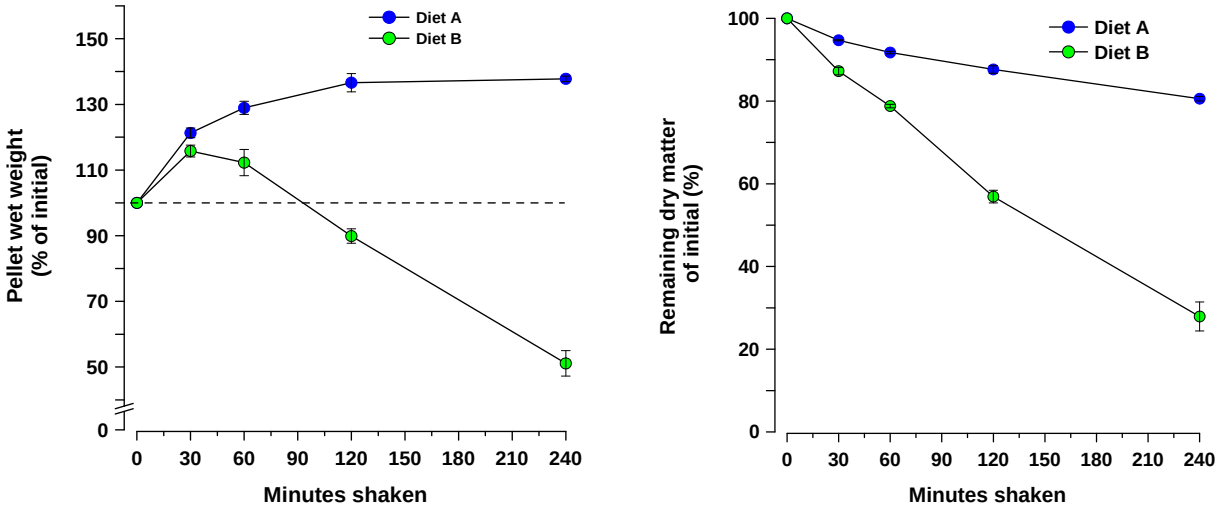

Figure 2 Wet weight (left panel) and dry weight (right panel) of remaining pellets at the sampling points in the water stability test.

3.1.2 Feed intake

In Period 1, the feed intake was significantly higher in trout fed Feed B compared to those fed Feed A (Fig. 3 and Table 3). The differences in feed intake was clear from the start of the trial, and around day 10, the feed intake stabilised, with fish fed Feed B eating approximately expected amounts, while those fed Feed A had approximately 80% of expected feed intake. Immediately when starting Period 2, the feed intake in trout held at fluctuating environment dropped radically for both dietary treatment groups, consuming 40-60% of expected intake throughout the rest of the trial, although the oxygen reduction was discontinued from day four of Period 2.

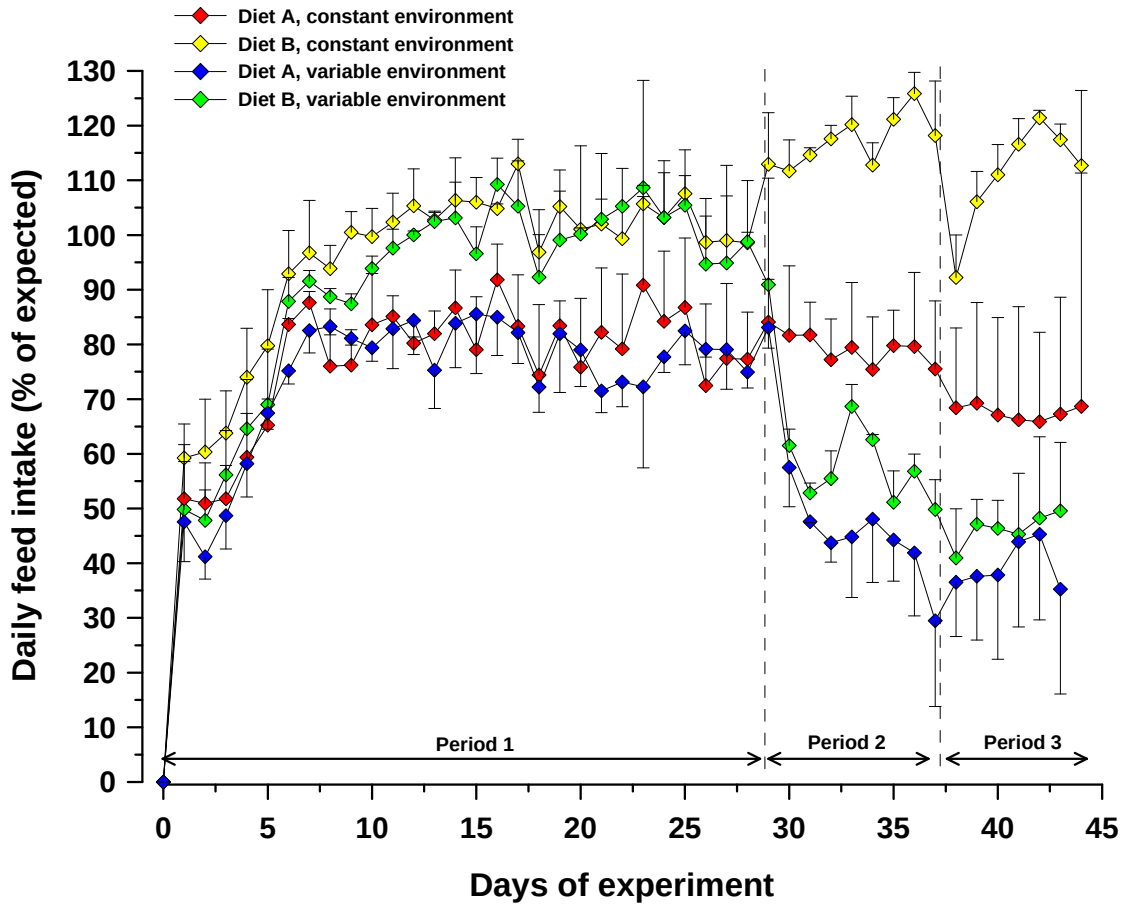

Figure 3 *Daily feed intake in % of expected intake (means ± range, n=2) based on commercial growth rates and feed conversion ratios for rainbow trout in seawater (Skretting ARC, 2006). The datasets were constructed by measuring daily feed intake per tank, and relating this to the expected intake for the current water temperature and fish weight, based on growth curves for each tank. The water temperature used in these calculations for the variable environment groups were the same as for the constant environment groups.*

Table 3 Feed intake (g dry matter per individ) for the three periods of the trial (tank means ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	Feed B	Feed A	Feed B	Feed	Env	Env x Feed
Period 1	374	451	360	450	*		
Period 2	117	168	61	77	*	*	NS
Period 3	91	146	51	64	NS	*	NS

Env = Environment
NS = not significantly different.
* = significantly different at p≤0.05

3.1.3 Growth

The trial was not designed as a growth trial, but as seen from the data in Table 4, the final weight was significantly higher in trout fed Feed B compared to trout fed Feed A. Although not significant, the values for weight gain, SGR and TGC were all highest in trout fed Feed B, and in the fish kept at stable environment.

Table 4 Growth and feed conversion ratio in the rainbow trout as measured following Period 3 (tank means ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	Feed B	Feed A	Feed B	Feed	Env	Feed x Env
Initial weight (g ind^{-1})	1 149±18	1 117±15	1 141±6	1 170±4	NS	NS	NS
Final weight (g ind^{-1})	1 773±2	1 913±57	1 690±65	1 790±1	*	NS	NS
Weight gain (g ind^{-1})	625±17	797±72	549±71	619±3	NS	NS	NS
SGR (% day^{-1})	1.0±0.0	1.2±0.1	0.9±0.1	1.0±0.0	NS	NS	NS
TGC	2.8±0.1	3.5±0.3	2.5±0.3	2.8±0.0	NS	NS	NS
FCR	1.0±0.1	1.0±0.1	0.9±0.0	1.0±0.1	NS	NS	NS

Env = Environment
NS = not significantly different.
* = significantly different at p≤0.05

Analyses of the stomach contents after Period 2 and 3 revealed considerable differences in both amount and appearance of the content (Fig. 4 and Table 4), mainly due to dietary treatment. For both environmental treatment groups, the stomachs from fish fed Feed B contained larger amounts of feed, the pellets were crushed, and large amounts of separated oil and water were present. The stomach contents of trout fed Feed B, ranged from extreme cases where almost the entire stomach was filled with red oil and only a few kernels, to just a mash of pellets. The stomachs from fish fed Feed A contained whole pellets, and in some individuals, the X-ray pictures showed the pellets as beads on a string (not shown).

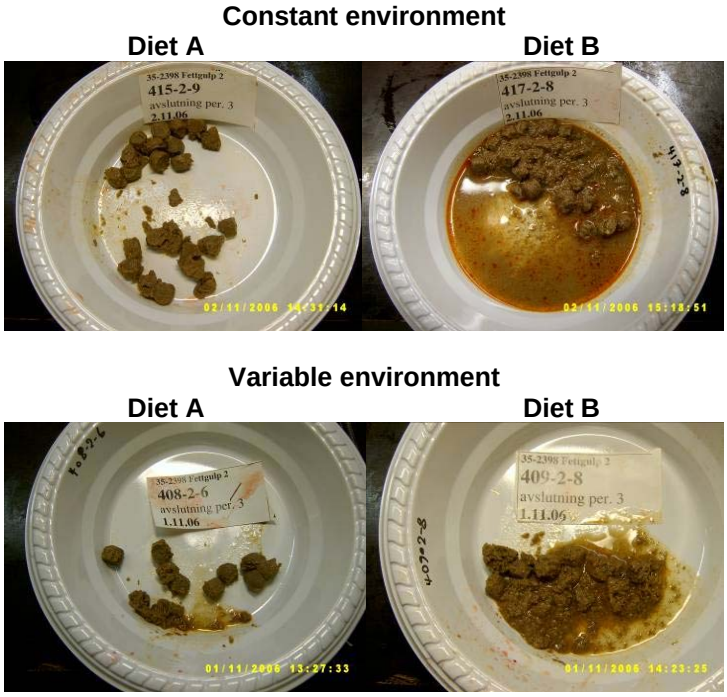

Figure 4 Digital pictures of stomach contents of rainbow trout sampled after Period 3 (5 hours post-prandial).

Table 4 Characteristics of stomach contents of the rainbow trout after Period 2 and 3 (tank means ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	Feed B	Feed A	Feed B	Feed	Env	Feed x Env
Stomach contents (g ind^{-1})							
Period 2	23.4±6.9	28.1±0.3	12.2±4.4	21.0±4.4	NS	NS	NS
Period 3	16.3±0.8	32.7±2.9	11.9±5.2	21.9±3.6	*	NS	NS
pH[1]							
Period 3	4.7±0.2	4.5±0.2	5.8±0.1	6.2±0.0	NS	NS	NS
Whole pellets (pellets ind^{-1})							
Period 2	16.7±7.5	1.5±0.1	8.9±2.9	0.3±0.3	*	NS	NS
Period 3	7.5±1.1	0.0±0.0	7.0±3.8	0.6±0.2	*	NS	NS
Pellet kernels (kernels ind^{-1})							
Period 2	5.3±0.1	13.6±1.6	2.8±1.4	9.5±3.1	*	NS	NS
Period 3	8.1±0.3	18.9±2.1	4.3±1.9	11.3±3.3	*	NS	NS
Crushed pellets (score)[2]							
Period 2	1.0±0.3	3.2±0.4	1.1±0.1	2.0±0.2	*	NS	NS
Period 3	1.0±0.2	3.0±0.0	0.9±0.1	2.4±0.2	*	NS	NS

[1] Tested statistically on H^+ concentration data
[2] The degree of crushed pellets were assigned a score from 0 (no crushed pellets) to 10 (extreme amount of crushed pellets)
NS = not significantly different
* = significantly different at p≤0.05

3.2 Results from present trial

3.2.1 Physical properties of the feeds

The physical properties of the feeds are shown in Table 5. The data show that the feed with high water stability (Feed A) had less dust, more unbroken pellets, higher hardness, larger diameter and shorter pellets compared to the feed with low water stability (Feed B).

Table 5 Physical quality parameters of the two feeds. Data are given as mean ± SEM.

	Feed A	Feed B
Hardness, N	27.3±1.3[a]	20.1±1.1[b]
Diameter, millimetre	9.8±0.1[a]	9.2±0.1[b]
Length, millimetre	8.5±0.0[b]	8.8±0.0[a]
Bulk density, g/l	689.6±14.6	676.0±5.8
Excisting quality:		
Unbroken pellets, g	154.0±2.2[a]	75.7±3.7[b]
Doris test:		
DORIS value, g	195.7±2.3[b]	271.6±3.0[a]
DORIS fracture large, g	120.9±2.9[a]	108.7±2.2[b]
DORIS fracture small, g	42.9±0.8[b]	116.3±1.5[a]
DORIS dust, g	17.7±0.1[b]	46.5±0.9[a]

[a b] Means in the same row with the same letter are not significantly different at P≤0.05.

3.2.2 Relative disappearance of nutrients from the stomach

The calculated values for relative disappearance of nutrients from the stomach are given in Table 6. Relative to yttrium (digestibility marker), nitrogen, the sum of amino acids (all individual amino acids except Cys and Trp) and some of the minerals disappeared in the stomach in fish from all treatment groups. Starch, Trp and some minerals had negative relative disappearance values (i.e their concentrations increased compared to yttrium) in all treatment groups. Fat, dry matter and energy had negative values in fish fed Feed B at continuous environment but were “absorbed” (relative to yttrium) from the stomachs of trout from the remaining groups. The relative disappearance values were negative for ash, Cys and some minerals in trout fed Feed B at both environmental treatments, and positive for those fed Feed A.

Statistical analysis showed that the relative disappearance of nitrogen, the sum of amino acids and all individual amino acids, starch, dry matter, ash, phosphorus, calcium, magnesium and sodium was significantly higher from Feed A (both environmental treatments) compared to Feed B, whereas no significant differences were observed in the relative disappearance of fat, energy, iron, manganese, zinc and copper between trout fed the two diets (Table 6). The relative disappearance of Met, Ala and zinc was highest in trout kept at constant environment. The relative disappearance of ash, magnesium and sodium however, was significantly higher (or with lower negative relative disappearance values) in fish kept at variable environment and fed Feed A, with an interaction effect between diet and environment (Table 6).

Table 6 Relative disappearance (%) of nutrients from stomach (mean ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	**Feed B**	**Feed A**	**Feed B**	**Feed**	**Env**	**Feed x Env**
Nitrogen	11.1±0.7	5.7±0.5	9.7±1.7	4.3±0.8	*	NS	NS
Sum of amino acids	10.0±0.3	3.5±0.0	9.1±0.8	2.4±0.2	*	(*)	NS
Crude lipids	17.0±0.9	-24.2±22.6	15.5±3.0	7.5±4.3	NS	NS	NS
Starch	-4.4±1.0	-14.6±1.8	-1.3±0.8	-16.4±2.2	*	NS	NS
Ash	0.2±1.1	-27.0±1.3	13.9±0.8	-5.4±1.5	*	*	*
Energy	11.7±0.8	-13.0±13.4	10.7±2.1	4.0±3.4	(*)	NS	NS
Dry matter	10.0±0.6	-11.2±9.2	10.6±1.7	2.6±2.5	*	NS	NS
Minerals:							
Phosphorus	11.7±1.5	2.6±0.4	11.4±2.2	2.5±1.1	*	NS	NS
Zinc	30.1±0.8	32.0±8.5	8.9±2.4	15.6±5.1	NS	*	NS
Essential amino acids:							
Arg	8.7±0.4	4.1±0.1	8.2±0.6	2.7±0.2	*	(*)	NS
His	9.2±0.1	3.9±0.6	8.9±0.2	3.5±0.0	*	NS	NS
Ile	6.9±1.2	2.5±0.3	7.0±0.3	1.7±0.2	*	NS	NS
Leu	8.1±0.2	2.3±0.1	7.8±0.3	1.5±0.3	*	(*)	NS
Lys	7.5±0.6	2.5±0.3	7.0±0.1	1.7±0.2	*	NS	NS
Met	9.9±0.4	6.7±1.5	7.7±0.5	4.1±0.1	*	*	NS
Phe	6.9±1.1	1.4±0.3	7.3±0.2	0.4±0.2	*	NS	NS
Thr	8.9±0.5	1.4±0.7	8.2±0.6	0.5±0.2	*	NS	NS
Trp	-7.4±1.9	-13.1±0.6	-2.9±3.4	-14.0±0.2	*	NS	NS
Val	8.5±0.6	4.7±1.2	8.7±1.3	3.2±0.4	*	NS	NS
Cys	7.8±1.4	-0.5±0.8	6.9±0.9	-0.8±0.8	*	NS	NS
Tyr	19.0±0.4	7.6±1.0	16.7±3.1	7.2±1.1	*	NS	NS
Non-essential amino acids:							
Ala	9.8±0.1	4.2±0.0	9.0±0.6	3.1±0.0	*	*	NS
Asp	8.6±0.6	3.2±0.3	7.8±0.8	2.0±0.1	*	NS	NS
Glu	12.8±0.1	3.8±0.4	10.5±1.4	2.3±0.3	*	(*)	NS
Gly	12.8±0.6	6.3±0.5	11.5±0.9	4.9±0.4	*	NS	NS
Pro	15.1±0.4	5.9±1.9	13.5±2.1	6.8±1.6	*	NS	NS

NS = not significantly different
* Significant at P≤0.05
(*) Trend (0.05<P<0.1)

3.2.3 Apparent digestibility

The apparent digestibilities (ADC, %), estimated from nutrients remaining in the faeces, are given in Table 7. No significant treatment effect was found on the ADC of lipids, nitrogen and ash. The ADC of the sum of amino acids, all individual amino acids except Asp, His, Lys and Arg, starch, dry matter and energy was highest in Feed A. The environment also affected the ADC of some of the minerals and amino acids significantly. The ADC of Thr and Glu was highest in fish kept at variable environment, whereas for Gly and His, the highest ADC values were found in fish kept at constant environment. For the sum of amino acids, and most of the individual amino acids, there was also a significant interaction effect between diet and environment, generally resulting in smaller difference in ADC values between the two dietary treatment groups when kept at variable environment, compared to when kept at constant environment. The ADC estimates of minerals present in the water are disturbed by the fish drinking water. However, the absorption of the two nutritionally important minerals P and Zn were highest at continuous environment, and for P, the absorption was highest from Feed A (Table 7).

Table 7 Apparent digestibility (ADC, %) of nutrients (mean ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	**Feed B**	**Feed A**	**Feed B**	**Feed**	**Env**	**Feed x Env**
Nitrogen	89.9±0.2	88.7±0.5	89.6±0.1	89.5±0.1	(*)	NS	(*)
Sum of amino acids	92.9±0.2	91.7±0.1	92.4±0.0	92.0±0.0	*	NS	*
Crude lipid	96.5±0.3	95.1±0.0	96.0±0.8	96.7±0.0	NS	NS	(*)
Starch	97.6±0.7	93.7±0.3	97.6±0.3	93.5±0.9	*	NS	NS
Ash	4.1±6.4	1.2±0.7	0.3±2.7	-7.0±0.1	NS	NS	NS
Energy	89.7±0.5	86.9±0.3	88.9±0.4	88.2±0.1	*	NS	*
Dry matter	78.8±0.4	74.7±0.2	77.9±0.1	75.1±0.3	*	NS	(*)
Minerals:							
P	36.9±1.6	29.7±2.3	27.7±1.2	22.7±3.1	*	*	NS
Zn	31.3±2.5	24.7±1.6	18.7±2.8	19.0±4.5	NS	*	NS
Essential amino acids:							
Arg	94.1±0.0	94.0±0.3	93.8±0.2	93.5±0.0	NS	NS	NS
His	93.4±0.3	92.7±0.3	92.4±0.1	92.3±0.0	(*)	*	NS
Ile	94.0±0.2	93.0±0.1	93.8±0.0	93.5±0.1	*	NS	*
Leu	94.6±0.2	93.6±0.0	94.4±0.0	94.0±0.1	*	NS	*
Lys	95.5±0.1	95.0±0.3	95.0±0.0	94.9±0.0	(*)	NS	NS
Met	93.3±0.2	92.0±0.0	92.9±0.1	92.3±0.1	*	NS	(*)
Phe	92.8±0.0	92.0±0.3	92.5±0.0	91.7±0.0	*	NS	NS
Thr	92.3±0.1	91.1±0.1	92.4±0.0	91.9±0.2	*	*	*
Trp	89.3±0.1	87.9±0.5	88.7±0.0	88.5±0.0	*	NS	(*)
Val	93.8±0.2	92.8±0.1	93.4±0.1	93.1±0.1	*	NS	*
Cys	85.4±0.7	80.8±0.1	84.8±0.2	83.2±0.1	*	(*)	*
Tyr	92.7±0.2	90.9±0.2	92.6±0.0	91.3±0.0	*	NS	(*)
Non-essential amino acids:							
Ala	92.8±0.1	92.0±0.0	92.4±0.1	92.4±0.0	*	NS	*
Asp	89.8±0.6	87.9±0.5	87.9±0.4	88.6±0.2	NS	NS	*
Glu	95.3±0.2	93.9±0.0	95.2±0.0	94.7±0.1	*	*	*
Gly	87.3±0.1	86.2±0.1	86.1±0.2	86.3±0.1	*	*	*
Pro	90.1±0.3	87.6±1.0	89.3±0.9	86.7±0.5	*	NS	NS

NS = not significantly different
* Significant at P≤0.05
(*) Trend (0.05<P<0.1)

3.2.4 Ions and triacylglycerols in plasma

No significant treatment effects were observed in the plasma concentrations of sodium ions, chloride and triacylglycerols (Table 8). However, the concentrations of sodium and chloride ions of plasma were significantly higher in trout kept at constant environment than in those held at variable environment. There were no significant differences in the plasma concentrations of triacylglycerols, but numerically, the highest levels were found in fish held at constant environment.

Table 8 Na^+, Cl^- and triacylglycerols (mM) in plasma after Period 3 (mean ± range).

	Constant environment		Variable environment		Main effects		Interactions
	Feed A	**Feed B**	**Feed A**	**Feed B**	**Feed**	**Env**	**Feed x Env**
Na^+	175±1	176±1	164±0	164±1	NS	*	NS
Cl^-	141±0	141±0	131±2	130±2	NS	*	NS
Triacyl glycerols	7.0±1.5	7.3±0.3	5.2±0.5	5.2±1.1	NS	NS	NS

NS = not significantly different
* Significant at P≤0.05

4 Discussion

The main conclusions from the fist part of this project, was that the feed intake was higher in rainbow trout fed the diet with low water stability (Feed B) compared to those fed harder and more water stable pellets. Furthermore, the feed intake was reduced when the fish was kept at fluctuating environment. In fish with the highest feed intake, severe water an oil separation occurred in the stomachs. Although fat belching was not observed, the separation of lipids in the stomach could potentially lead to fat belching. No mortality was observed during the experiment, and the fish appeared to be at good health (Terjesen et al., 2008). The growth parameters did not show significant differences among the treatment groups, however, the data indicate that the growth in the groups fed Feed B, and kept at stable environment may have been higher if the experiment was prolonged for a longer period. Accordingly, Sørensen et al. (2007) also showed greater growth rate in rainbow trout fed softer pellets. The results from the previously performed trial are discussed in more detail by Terjesen et al. (2008).

The measurements of physical properties of the feeds in the present part of the project showed unambiguously that high water stability of the feed was associated with harder pellets, less dust formation and less broken pellets compared to the feed with low water stability.

From the calculations of relative disappearance (%) of nutrients from the stomach, it appears that there were substantial absorption of nutrients relative to yttrium in the stomach, and, for many nutrients, significantly more from Feed A than from Feed B. Absorption from the stomach in salmon has been reported earlier (Austreng, 1978, Denstadli et al., 2004), although the pyloric cecae and the intestine are main sites for absorption (Bakke-McKellep et al., 2000, Denstadli et al., Ruyter, 2004). However, the relative disappearance of nutrients is calculated from the nutrient:yttrium ratio. Thus, the apparent stomach “absorption” figures may be a result of selective transit of nutrients from stomach to intestine, resulting in inhomogenous distribution of yttrium in the stomach content and intestinal content. This may explain the negative relative disappearance of lipid, energy and DM (and ash) in trout fed Feed B at constant environment, where severe oil separation was found, suggesting that the separated oil does not contain the same yttrium concentration as the rest of the stomach content. The negative relative disappearance values found for e.g. starch and Trp in all treatment groups, is another indication of differences in the rates at which yttrium and nutrients are removed from the stomach, because these nutrients can not be produced in the stomach. It should be noted that both for relative disappearance of minerals from the stomach and the ADC of minerals are invalidated by the fish drinking water containing minerals.

Contrary to what one might expect, the ADC (%) of dietary amino acids was highest from Feed A, which had the highest pellet hardness and water stability. A possible explanation may be that high feed intake reduces transit time through the gastrointestinal tract, thus reducing the absorption (%) in the intestine. Baeverfjord et al. (2006) did not find any significant differences in ADC of protein, lipid or starch from diets with high or low water stability fed to rainbow trout in a similar trial. In that study however, there were no significant differences in feed intake and growth between the treatment groups.

The interaction between diet and environment shown for ADC of several amino acids may also be related to the feed intake. In general, the difference in ADC between the two diets was smaller in trout kept at variable environment, which had lower feed intake, compared to those kept at constant environment.

Due to the large differences in feed intake, the total absorbed amount (feed intake x feed concentration x ADC) of most nutrients reflected the feed intake (data not shown). Despite the fact that ADC was in general lower from Feed B compared to Feed A, the absorbed amount of lipids, N, the sum of amino acids, all individual amino acids, dry matter and energy was highest (significantly, $P<0.05$, or with a trend, $P<0.1$) in fish fed Feed B, and in fish kept at constant environment. (Starch was an exception, due to large differences in the feed concentration.) Accordingly, the growth followed the same pattern, although most growth parameters did not differ significantly among treatment groups.

The plasma concentration of sodium and chloride ions was highest in fish kept at constant environment, and there was no effect of feed. This is in contrast to other studies, where water-filled stomachs are related to elevated plasma ion levels (Rorvik et al., 2000, Staurnes et al., 1990). The fish drink water when fed extruded feed, and rainbow trout has a greedy eating pattern. Consequently, the high feed intake observed for the trout kept at constant temperature in the present trial may have forced the fish to drink large amounts of salt water, leading to increased plasma ion levels. Fish kept at fluctuating environment had a lower feed intake and consequently drank less. Lower drinking rate and the fact that some of the water was of lower salinity may explain the lower plasma level of sodium and chloride.

The effect of feed quality and environment on feed intake was apparent in the present experiment, and many of the treatment effects found may have been related to the feed intake. The data are in contrast to the data from a similar study (also with rainbow trout, fed feeds with high or low water stability, and kept at stable or fluctuating environment), where no significant differences in feed intake, growth or nutrient digestibility were found (Baeverfjord et al., 2006). In that study however, only salinity fluctuated in the environmental treatment group, and no physical properties of the feeds were measured, except for water stability. Since the results from the present trial and that of Baeverfjord et al. (2006) are somewhat conflicting, we can not draw any conclusions about how the nutritional value of a feed is affected by its physical properties, only that it is affected. It may seem though, that feed intake is a key parameter, dependent on physical feed properties.

A fish farmer will normally require feed with a high pellet durability to avoid loss in the form of dust and broken pellets. However, the data from the present trial suggest that pellets with lower hardness, durability and water stability may be economically favourable, although the gain in terms of feed intake and growth may be difficult to register and quantify. The data also clearly demonstrated that utilisation of the two feeds with different quality was affected by the environment, possibly due to reduced feed intake at suboptimal environmental conditions. Although more research is needed, the data from the present study clearly demonstrated that the nutritional value of a feed is related to its physical properties.

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