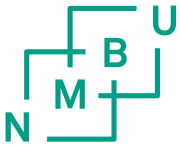
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Abstract

During a 97-day winter period, six duplicated groups of 81 Atlantic salmon (*Salmo salar* L.) were fed to satiation 2, 3, 4, 5, 6 or every day (D2, D3, D4, D5, D6 and D7(control)) throughout the working week with the purpose of investigating production efficiency, health and quality parameters. The body weights showed a linear increase corresponding with the number of feeding days until day 5. The significantly highest TGC was observed for D5 and D6 (2.1-2.2) and lowest for D2 and D3 (0.7-0.8). The FCR was significantly lower for D2 and D3 (1.6-1.8) compared with D4, D5, D6 and D7 (1.0-1.2). The frequency of the feeding produced two significantly different fillet weight groups, analogous to increasing feeding days per week up to day 4. Registration of production efficiency and health parameters took place at the sampling, while quality measurements followed 6 days after. Weekdays of feeding had no significant impact on fillet fat content, post-rigor pH or slaughter yield. Furthermore, somatic-indexes expressed no significant influence for the feeding regime treatment, which applies also for the melanin in abdominal wall and gut health parameters.

In summary, this study suggests a restricted feeding regime as an effective tool in obtaining a healthy Atlantic salmon of good quality and desirable growth during the winter season.

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1. Introduction

With the world population, exceeding 7 billion (*Worldmeters - real time world statistics* 2014) meeting the nutritional needs of a growing population with limited available resources for food production has become an urgent issue of concern. Aquaculture continues to be the fastest growing animal food-producing sector and at the present, aquaculture is considered to outpace population growth (FAO 2012).

In the world of aquaculture production Atlantic salmon (*Salmo salar* L.*)* is one of the most dominating carnivorous fish species. Season independent availability of high quality fish is an important factor that has contributed to the successful growth of the salmon production. Four leading farmed Atlantic salmon producing countries, Norway, Chile, United Kingdom and Canada, provide over 85% of the world's production today with Norway being the world's largest producer and exporter (Liu & Sumaila 2008). During the first three months of the year 2014, the value of Atlantic salmon exports from Norway amounted to 10.7 Billion NOK. This represents a growth of 32 percent, or 2.6 billion NOK from last year 2013 (NSC 2014).

The conversion of dietary feed energy to edible food is high for salmon compared with land-living domesticated animals of today, but a further improvement of the dietary utilization is preferred and focused. The ability of the farmers to deliver a sustainable product of acceptable price and quality to the consumer while being economical viable is the basis of a successful aquaculture production.

Optimizing the feeding frequency and time of feeding is important to find the best possible solution for a cost and quality effective aquaculture production. A decrease in feed amount may diminish product yield and change the quality parameters. Some of the changes in feeding routines may lead to a better quality result, as an increase in slaughter-yield, but not on filet yield, has been recorded upon starvation of Atlantic salmon prior slaughter (Lie & Huse 1992). Decreasing the feed ration over a longer period can cause reduced or negative

fish growth, lower the total fat content and alter the fatty acid composition in Atlantic salmon (Hillestad et al. 1998; Storebakken & Austreng 1987). Short-term starvation is a standard practice prior to slaughter (Fiskeridirektoratet 1996) and it improves freshness quality (Aksnes 1995). On the other hand, a 110-day restricted feeding trial showed that feeding to satiation (ration level at 100%) gave no clear benefit to feed efficiency when compared to a slightly lower feeding ratio (ration level at 75%) (Einen et al. 1999).

Nevertheless, the industry has to consider customer preferences and their willingness to pay depending on the product quality (Alfnes et al. 2006). Important fillet quality traits for the acceptance of fish products include flavor, texture, gaping, nutritional value and appearance mostly dominated by color. The importance of any of these given attributes may differ upon the specific product and the intended market (Haard 1992). Gaping is an appearance of holes in fish fillets resulting from connective tissue failure to keep the muscle fibers in bond (Lavety et al. 1988). Consequently, appearance of gaping can negatively influence further processing of the fillets. Furthermore, fillet color is a variable that is susceptible to changes, dependent on accessibility of the pigments from the feed given to the cage-reared salmon, but and feeding routines can influence the final color of the product. Post-mortem pH of the fish muscle reflects the energy status of the fish (glycogen stores). This is a parameter that has impact on processing quality of the fish after slaughter, since high amount of lactic acid (low post-mortem pH) weakens the connective tissue and hence the muscle integrity (Love 1988).

Influencing the range of traits that are of commercial importance in the fish industry, trough control of timing and frequency of feeding is a method that requires detailed investigation. Using growth charts to estimate production progress is a common practice in the modern salmon farming. Growth charts mostly are based on calculations of the amount of feed required to achieve an expected growth of a given fish species, depending on fish size and water temperature. On the other hand, appetite feeding is focusing on minimizing the uneaten feed amount, thus lowering the feed expenses, but it has little to do with the actual synchronization with the feed utilization by the fish.

It is not surprising that the growth rate and the growth pattern differ significantly geographically having in mind the Norwegian coastline long stretch. The feed costs for Rogaland, Hordaland, Sogn og Fjordane, Møre og Romsdal, Sør-Trøndelag, Nord-Trøndelag, Nordland, Troms and Finnmark differ, but overall share roughly 42% of the total production costs (Tveteras 2002). On the other hand, the cost function determined only by feed, labor and capital unquestionably displays the possibility of weakly separating these cost inputs from the others in the production process (Berndt & Christensen 1973).

The goal of this study was to investigate the effects of different feeding regimes during the winter period (97 days) on cage-reared Atlantic salmon, with attention on growth and feed utilization, biometric and quality parameters, followed by internal organ and intestinal health evaluation of the salmon fed to satiation 2, 3, 4, 5, 6 or 7 days per week.

2. Theoretical background

A successful salmon production results from balanced aquaculture operations that lead to a product that is attractive to the consumer and at the same time has economic viability. Attributes that are important in achieving this goal are, cost efficient production methods with high production results, good fish health, stable and attractive quality of the final product. To achieve the desired quality of final product one could revise several methods. The quality of the final product depends on: genetic selection of the fish material; season of production; environmental control; feed formulation; the live material handling; feeding treatment; the slaughtering procedures; handling procedures after slaughter; transport techniques and storage methods (Johnsen 2006). The present study will focus on the production efficiency, health and quality parameters of the Atlantis salmon fish subjected to six different feeding regimes during the winter period in North Norway.

2.1. Production parameters

High production efficiency is essential in intensive aquaculture production and defining optimal feeding strategies is receiving considerable attention of the fish farmers. In experiments where the goal is to optimize feeding and subsequent growth in fish, the experimental design is following a more or less similar pattern. Primarily, dividing the fish in groups of individuals, where a group of fish (control group) is fed ad libitum rations during the entire period of the experiment, while other experimental groups (treated group) receive a restricted rations during the first period of the experiment followed by an ad libitum feeding ration in the second period of the experiment. In many of these experiments the restricted group upon re-feeding compensates the reduced growth and by the end of the experimental period achieves the same, or nearly the same, body size as the control group (Skalski et al. 2005). Compensatory growth, or “catch-up”, is a term that refers to the rapid growth, induced by increased appetite (hyperphagia), that can occur after a period of feeding restriction when animals are again in conditions of unlimited feed availability

(Johansen et al. 2001). There is limited knowledge on the growth response in fish subjected to cyclic feeding; i.e. when feeding is restricted within a week.

Production efficiency, health and quality of the fish can vary within the same production conditions depending on the genetic origin of the fish (Thodesen & Gjedrem 2006). Moreover, Atlantic salmon fish is genetically prone to a seasonally dependent production efficiency. Seasonal variation in growth is a characteristic present in immature salmon as growth is dependent on water temperature and day length (Boeuf & Le Bail 1999; Forsberg 1995; Smith et al. 1993). A study of farmed Atlantic salmon, transferred to sea water after 9 (0+ salmon) or 16 months (1+ salmon) in fresh water, revealed a seasonal variation in growth and feed utilization as well as quality parameters between (Mørkøre & Rørvik 2001). The water temperature and natural light intensity varies along the Norwegian coast. Manipulation of light conditions in the grow-out period in the production of salmon is one of the most effective methods contributing to the production efficiency of the modern fish farming industry. For example, in-door control of environmental parameters, for example in hatcheries, is making all-year round salmon egg production possible. Thereby, the production of out-of-season 0+ smolt, together with 1+ smolt, is a commercially used strategy that provides availability of marked-sized salmon throughout the year (Duncan et al. 1998).

Exposing Atlantic salmon to an artificial light source during winter (dark) part of the year results in growth expansion throughout the year and growth retardation and quality downgrading due to early sexual maturation (Endal et al. 2000; Hansen et al. 1992).

Modern salmon fish industry, has changed the focus from just maximizing the production volume to more qualitative goals, the turn mostly influenced by environmental impact of feed production, sustainability of marine fisheries and the need for alternative feed ingredients (Johnsen et al. 2013). When investigating a new feed ingredient, sustainable profile of the one in question should also include a favorable production result (Rosenlund et al. 2001)

In Norway, it is common that the commercial farmers frequently record higher growth rates than expected from the table values. The SGR value is a change in daily weight presented as

a percentage of the whole body weight increase, while the FCR is the amount of feed used to gain 1 kg of growth (Einen & Mørkøre 1996). The table values are calculated and refer to the SGR levels for Atlantic salmon of a certain size and water temperature (Austreng et al. 1987). On the other hand, by calculating the TGC farmers take in consideration the actual fish size, water temperature and time as a variable, unlike SGR, TGC makes it possible to compare growth at different farming sites and between season, hence being independent on the full thermal range (Jobling 2003).

Previous studies revised the interaction between feeding biology and feeding regimes in cage reared Atlantic salmon and the possibility of manipulating the feeding intensity in order to reduce costs of the production (Juell et al. 1994; Talbot 1993). Manipulation of the feeding regimes through controlled timing and frequency of feed delivering is a way of influencing a number of traits that are of commercial importance. At present farmers are using different feeding strategies, as results on optimal feeding regimes are inconsistent. For example, a study done on Atlantic salmon (initial weight of 2866 ± 494 grams) fed with high-energy diets (for 88 days) for either 1 or 22 hours per day, showed no influence on growth, feed utilization or body traits (Sveier & Lied 1998). On the other hand, Boujard et al. (1995) and coworkers recorded the best growth performance in rainbow trout (Oncorhynchus mykiss) fed a single meal (1.5% of their body weight) at dawn and lowest in the group fed at mid-night. Another previous study concluded that 1 – 3 meals per day is the best frequency corresponding feeding biology of the Atlantic salmon hence providing the best growth (Thomassen & Fjara 1996).

Feeding a restricted meal sizes can cause competition among fish upon re-feeding and may lead to increased variability in growth (Jobling et al. 1995). Feeding to satiation, on the other hand, could neutralize unwanted feeding inequality. In addition, expectation of a restriction feeding leading to compensatory growth, requires that the period is long enough to reduce the energy reserves in the restricted group, thus triggering compensatory growth response upon re-feeding (Johansen et al. 2001).

2.2. Biometric traits

Wild Atlantic salmon populations often face a challenge of not having a continuous availability of the same quantity and quality of food resources that may oscillate in space and time. In the modern aquaculture practice, feeding a restricted ration is a way to manage product quality likewise slaughter and fillet yields. The reduction in fillet yield after a long-term food deprivation results a lower proportion of muscle mass compared to bones, fins and head as indicated in a leaner body shape and a higher slaughter yield due to the reciprocal relationship between slaughter-yield and relative viscera weight (Einen et al. 1998; Lie & Huse 1992). Leaner body shape is described by the condition factor hence decreasing or increasing during times of low water temperatures and/or low food availability, increasing towards spawning, declining after spawning and a second increase after spawning (Froese 2006).

The value adding procedures of filleting and trimming implies removal of head, bones, fins and visible fat making the fish more adequate for packing and more appealing to consumer (Mørkøre et al. 2001). One of the feeding restriction methods for influencing the final product quality is also starvation prior slaughter. The highest weight loss occurs during the first 2-3 days of starvation in Atlantic salmon, and is due to the decrease in relative viscera weight with the last feed-residuals discharged (Handeland et al. 2008; Storebakken et al. 1999; Sveier et al. 1999). The current guidelines for slaughtering of Atlantic salmon are suggesting, based on a finding of no food-residuals in the gastrointestinal tract after 3 days of starvation, that 3-4 days of starvation at 6 °C prior slaughter is sufficient (Fiskeridirektoratet 1996).

2.3. Quality parameters

There are many physical and chemical properties that contribute to the total quality of the salmon flesh, based on a literature review the following quality parameters are of particular importance: fat content, color intensity, texture, gaping, flavor and odor (Sigurgisladottir S. et al. 1997). These quality parameters are oscillating in different extents depending on the feeding regime design as consequence of Atlantic salmon metabolism coping with the feed restriction.

2.3.1. Composition

Atlantic salmon stores excess energy as lipids in both the abdominal cavity and in the fillet. Additionally the glycogen reserves built up, although they contribute only to a small amount of the total energy in farmed salmon. The dietary fat content is significantly influencing the lipid deposition. However, the effect of the feed formulation is not only dependent on the individual percentages of the nutrients chosen, but also on their interaction during the digestion. After 9.5 months long study period, where salmon diets contained medium fat level of 32% or high fat level of 39%, it was found that fish fed high fat content had more total carcass lipid deposits that correlated positively with the pigment (astaxanthine) content in the flesh (Bjerkeng et al. 1997). The total amount of fat deposited in the flesh of farmed Atlantic salmon is among the most important quality attributes. Hence, the relative deposition of the fat in the different parts of the fillets is a phenomena that needs to be taken into consideration when determining the level of quality (Jobling & Johansen 2003). The fish body composition appears to be influenced by the feed ration levels and increasing fish size also results in enhanced adipose deposition (Rasmussen 2001). In compliance, a previous feeding regime study done by Einen et al. (1999), a duration of 110 days of starvation was required to reduce the average fat content by 2–3% units. In addition, the total fat content in the Atlantic salmon flesh varies depending on the season. In the study done by Mørkøre and Rørvik (2001) the fat content increased most substantially from July to November (12–13% units).

Carbohydrates are mostly stored in the liver as glycogen that represents an energy reserve used during the periods of low feeding frequency or starvation. In compliance, a feeding ration experiment done by Einen et al. (1999) the in vivo glycogen levels increased with the increasing feed ration levels. After the slaughter, the glycogen goes to the process of glycolysis that results in a drop of the pH levels. The lowering of end pH in muscle due to the accumulation of lactic acid is one of the most is a significant post mortem change of the muscle. In previous studies, the decrease in post mortem pH was connected with the incidence of softer tissue (Bjørnevik et al. 2004). Handling procedures stress before the slaughter accelerates glycogen degradation and hence causes a faster drop in pH post-mortem. A fast drop in pH and low final pH in the fish muscle has been associated with, faster rigor-mortis development, autolysis and bacterial spoilage. In a research done by Skjervold et al. (2001b), salmon that were crowded before slaughter had a higher muscle pH after 5 and 14 days of ice storage time, when compared with the un-crowded group, hence the relationship between handling stress and low pH can deviate from the general pattern. A higher pH 5 days post mortem can also reflect accelerated degradation processes. The common storage temperatures for slaughtered fish are 4 °C. Lower storage temperatures of Atlantic salmon fillets leads to prolongation of rigor-mortis period (Kiessling et al. 2006).

2.3.2. Appearance

The red muscle color of Atlantic salmon fish is an important quality criterion for consumer acceptance (Sigurgisladottir S. et al. 1997) and preference (Alfnes et al. 2006) . The pigments giving red color to the salmon flesh are carotenoids with astaxanthin as the predominat source in aquaculture. Carotenoids must be provided in the diet because the salmon is unable to biosynthesize them de novo. In addition, carotenoids are poorly utilized by the fish, and the muscle retention of astaxanthin in Atlantic salmon is usually less than 12% (Bjerkeng et al. 1999). A previous study by Torrissen et al. (1995), found that increasing the astaxanthin level above 60 mg kg dry feed^{-1} has no significant effect on astaxanthin deposition rate in the flesh of the fish. Depending on the feeding ration level (0.6% or 1.2% of body weight per day), the apparent digestibility of astaxanthin changes from as low as 14.5% to considerably higher 38% (Rørvik et al. 2010). A previous study demonstrated that both feed intake and temperature affect digestibility of astaxanthin in salmon, being

reduced at lower temperatures and with higher feed intake and growth (Ytrestøyl et al. 2005). In addition, the mean flesh pigment levels are significantly different depending on the dietary pigment source. In a seasonal variation study, done by Roth et al. (2005), two groups of size sorted 0+ smolt and 1+ smolt showed only minor differences in quality when slaughtered in January and June ($P>0.15$), but a harder texture, higher fat content and redder color compared with fish slaughtered in October. Another study performed by Erikson and Misimi (2008), showed significantly altered color of salmon being exercised to exhaustion before slaughter.

2.3.3. Texture and gaping

Texture and gaping are important quality characteristics for the fish processing industry and for the consumer perception. Texture is a sensory attribute that is determined by touching the product or when taken in the mouth.

The fish flesh consists of numerous muscle segments bound together with the help of the connective tissues. The phenomena of gaping is referring to the holes appearing in the fish fillets. This occurs when the connective tissues fail to hold the muscle segments together. The modern Atlantic salmon industry requires a fillet of good strength and resilience, while the soft texture and gaping leads to reduced yield and downgrading. The major problem with severe gaping is that fillets are not suited for further processing and the final products are unattractive to the consumer. Causes of abnormal texture and gaping relates to the genetic material of the fish, season of the harvest, handling after slaughter etc. In addition, crowding stress can increase the problem with gaping and soft texture (Sigholt et al. 1997). On the other hand, good control of the water conditions during fish transport, quick and efficient final handling and efficient stunning prior slaughter, and proper post-mortem handling can prevent the adverse effect on fish quality after slaughter (Erikson et al. 1997). The time of filleting also influences the texture and gaping.

In an experiment done by Skjervold et al. (2001c), the incidence of gaping was significantly reduced in the fish group filleted 2 hours after slaughter (pre-rigor), then in groups filleted 1 and 2 days (in-rigor) or 5 days (post-rigor) after slaughter. In addition, another study done by

Skjervold et al. (2001a) show that pre-rigor fillets, in addition to having lower gaping score, had a significantly firmer texture and improved colour characteristics when compared to post-rigor fillets.

2.4. Fish health

In the present study the aim was to record a potential relationship between the different feeding regime and incidence of floating feces and impaired intestinal health. Knowledge of the causes of impaired intestinal health and incidence of floating feces is important for the salmon industry, both in terms of how this may affect salmon production intensity, and intestinal health of the fish and welfare. The intestinal health of the fish is a key part of the immune system. The mucous membranes of the intestines are a barrier to pathogenic organisms, and if weakened, the salmon becomes more susceptible to diseases and impaired growth, indirectly compromising the end quality of the fish fillets. It is also likely that the floating feces can lead to impaired feed efficiency, because a higher proportion of fat excreted through floating feces compared with normal feces. The negative economic effect may follow the floating feces and intestinal abnormalities as they peak during summer and fall when production is at its highest (Hillestad et al. 2013).

Generally, exposing the fish to long periods without food is not a practice used in the modern salmon farming. A short-term starvation practice before slaughter is a method used to empty the gut for feed residuals (hygienic reasons) and improve fish physiologically for slaughtering procedure. However, a long-term fasting practice is a controversial matter in the industrial fish farming, even dough fasting is a condition that many wild animals are facing throughout a year cycles. Long-term fasting is causing a decrease in tissue mass and activity of digestive enzymes inside the gastrointestinal tract. In order to induce compensatory growth, the farmer must know a threshold, in which enzymes involved in metabolic activity remain intact and are able to restore their preferred activity upon re-feeding (Bélanger et al. 2002). Upon re-feeding, metabolic enzyme activity increases rapidly and the fish re-gain body mass. In a research done by Krogdahl and Bakke-McKellep (2005), it was concluded that re-feeding should start with less than maximal meal amount (25% of

estimated feed intake) for the first 3 days and thereafter increase the meals gradually the first week. In addition, a previous study showed that the intestinal bacterial population in the fish is sensitive to dietary change, and the intestinal bacterial community structure differs between healthy and inflamed intestines in Atlantic salmon, which may play a role in occurrence of enteritis (Reveco et al. 2014).

The fish welfare and health are depending on the handling procedures prior to implementing restricted feeding, for example, vaccination procedures. In a research done by Poppe and Koppang (2014) it was concluded that the acute side-effects of the vaccination can be divided into those resulting from poor handling, anesthesia, contamination of the vaccine, and genuine side-effects caused by the vaccine itself. Currently, the side-effect profile of the vaccine determines the choice of the vaccine. The vaccine side-effects are mostly being scored based on the rough ordinal scales for vaccine-induced abdominal lesions (adhesions) (Midtlyng et al. 1996). Earlier studies of injection of oil-adjuvant vaccine side effects have shown that they lead to appearance of adhesions between internal organs, that are persistent throughout the production cycles and dependent on time of vaccination and temperature (Berg et al. 2007; Grini et al. 2011).

Furthermore, vaccination may be associated with abnormal pigmentation in the fish tissues and organs (Koppang et al. 2005). Such pigments may either be of exogenous or endogenous origin. Melanin is an endogenous pigment found in fish flesh that manifests as dark spots if abnormal pigmentation. The abnormal pigmentation of melanin probably arises as a result of chronic inflammations and scar tissue formation caused by disease or injury. When present in larger amounts it leads to downgrading of salmon fillets and loss in production. In a research done by Larsen et al. (2014), results showed that the proportion of fish with intramuscular melanin deposits was not significantly different between vaccinated and unvaccinated fish, regardless of fish being diploid or triploid. Hence, factors other than vaccine seem to be involved in melanin deposition.

The status of some vital internal organs, for example liver indirectly indicate the overall health condition of farmed Atlantic salmon. Abnormalities in the liver size and color are indicators of a possible health disorders (Mørkøre et al. 2013). In addition, , it is not

preferable that salmon uses the feed energy for accumulating large quantities of intestinal fat and excessive fat deposition may also be associated with general health problems.

3. Materials and Methods

3.1. Experiment design

A total number of 972 Atlantic salmon (*Salmo salar L.)* (V-11G), with an average weight of 1931 ± 65 grams (mean ± S.D.), were randomly distributed into 12 research net-pens (81 fish each) at Helgeland Havbruksstasjon AS in Dønna, Helgeland region in Nordland, Norway. Starting from 28th of January 2012 until 9th of May 2012 the fish were fed to satiation two days per week (D2), three days per week (D3), four days per week (D4), five days per week (D5), six days per week (D6), and every day of the week (D7); two net-pens per regime.

The experimental design excluded fish with abnormal exterior appearance from the experiment. The experimental fish were sexually immature and provided with artificial light during the course of the restricted feeding trial. Each net-pen was equipped with a Lift-up system for registering feed consumption and collecting the uneaten feed. MLSIQ-net system registered water temperature, salinity and oxygen every 15 minutes (Storvik Aqua AS). According to the established routines, Helgeland Havbruksstasjon AS done the registration of weekly amount of feed consumption for each research net-pen. Feeding done was two times per day with temperature above 8˚C and once a day with temperature below 8˚C. The same feed type used throughout the experimental period was a standard commercial high-energy diet CPK 2000 (Combined Protein Knowhow) produced by BioMar. CPK 2000 consisted of ≈35.2 % protein, ≈35.5 % fat and 20-40 mg/kg of astaxanthine (*Biomar* 2014).

The fish was bulk weighted on 19th of April 2012.

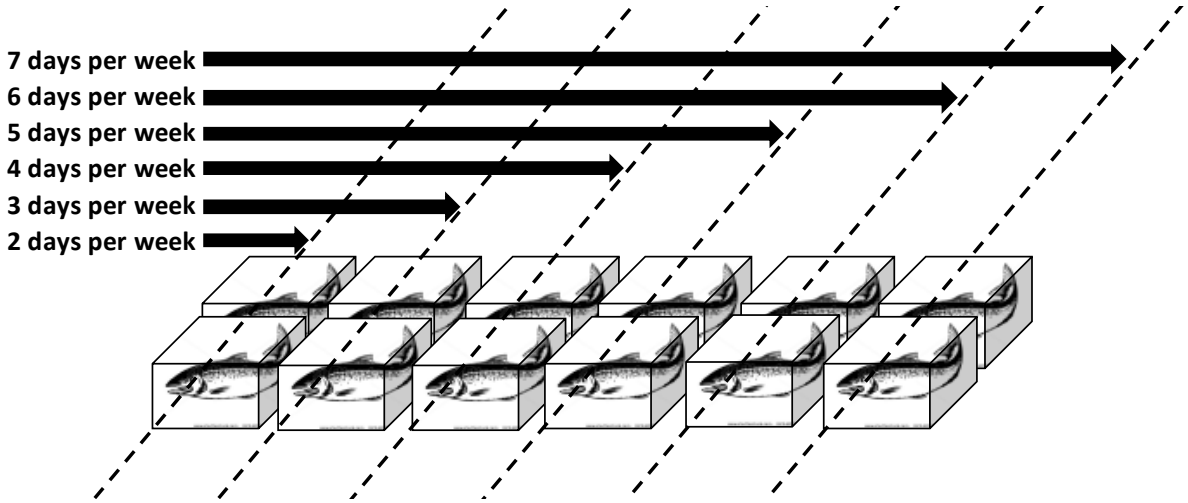

Fig. 1. The picture illustrates the experimental design with feeding regimes represented with the arrows and the "fish" representing each of the research net-pens.

3.2. Fish sampling

The fish sampling for experimental analysis was on 10th of May 2012. Eight fish from each net-pen were killed with a hit to the head and bled in sea-water for 15 minutes after cutting the gill arches. Thereafter, measurements started with round body weight, gutted weight, fillet weight and fork length. Internal organ adhesions were examined thereafter, through a standardized scoring system for vaccine-induced lesions, and modified by using a visual analogue scale, with scoring 0 as the lowest and 6 as the highest (Aunsmo et al. 2008). Degree of melanin was classified by macroscopic observation of the abdominal organs (visceral peritoneum) and abdominal wall (parietal peritoneum), and scored on separate (0–3) scales. Amount of visceral fat was evaluated according to a scale panel that scored from 1 to 5 based on visibility of the pyloric caeca. Simultaneously, the fish gender registration took place. The weight of the livers was recorded and liver color was evaluated according to scale from 1 – 5 where score 1 is light, 2 is light-brown and 3 is brown, 4 is dark-brown and 5 is dark (Mørkøre et al. 2013). Registration of heart weights took place after removing hearts bulbous and atrium. An experienced worker did the filleting of the gutted fish. Therefrom, the left fillets were packed in sealed plastic bags, preserved on ice, and transported to fish laboratory at Nofima, Ås, for fillet quality analysis six days after slaughter.

3.3 Image analysis

The fat and color were analyzed of the whole fillets using the equipment provided by PhotoFish AS. The system consists of a closed box with standardized light and color conditions, a digital camera, and a computer for the image and software for analyses (Folkestad et al. 2008). The results presented color as total amount of pigment (mg/kg), while the fat in percentage of the whole tissue.

3.4. pH measurements

The pH was measured in the dorsal fillet part, with assistance of professional personnel, with a pH-meter 330i SET (Wissenschaftlich-Technische Werkstatten Gmbh & Co.KG, WTW, Weilheim, Germany) with a pH muscle electrode (Schott pH-electrode, Blueline 21 pH, WTW, Weilheim, Germany). Simultaneously, temperature in the fillets was measured with a temperature probe (TFK325, WTW, Weilheim, Germany). The electrodes were, for obtaining consistency in the results, kept clean and frequently calibrated in buffers during the measurements.

3.5. Gaping measurements

The fillet gaping “A” (Andersen’s test) registration was performed using a scale ranging from 0-5, where score 0 represents no gaping and score 5 represents maximal gaping score (Andersen et al. 1994). This method applies minimal handling of the fillets, hence gaping score reflects the inherent muscle integrity (strength of connective tissue between the muscle segments, myocommata).

The fillet gaping “I” (Industry test) representing a gaping score test done on a fillet after applying a certain amount of force on the fillet. The force was applied from the skin side of the fillet, with bending the dorsal and ventral fillet side closely together, starting from the

neck and sliding towards the tail. This was done in order to simulate the ability of the fillets to withstand rough handling through, for example, an industrial processing machine. The registration was in scale from 0-5, where the score 0 represents no gaping and score 5 represents the maximal gaping score (Erikson 2009).

3.6. Texture analysis

The mechanical properties of the sampled fillets were measured instrumentally on the dorsal muscle of the fillet and on the Norwegian Quality Cut (NQC) (anterior and posterior to the dorsal fin) with a use of Texture Analyzer TA-XT2 (Stable Micro System, Surrey, England). A flat-ended cylinder (Ø 12.5 mm) was pressed into the fillet at 1mm s^{-1} until it reached 90% of the fillet height. The parameter (total work) used from the time-force graphs, was the total area under the graphs (N*s). This parameter has previously shown a good correlation with sensory perceived firmness (Mørkøre & Einen 2003).

3.7. Intestinal health analysis

An examination with a focus on intestinal changes conducted after the organ adhesion and melanin scoring. The state of the entire gastrointestinal tract, thereby a detailed examination of the tissue condition along with consistency of feces present. In addition, a prevalence of floating feces registration followed (Hillestad et al. 2013). Helgeland Havbruksstasjon AS provided the professional personnel that did the scoring and inputting the data in a scoring form (Appendix 1).

3.8. Calculations

The specific growth rates: SGR= 100 x ($\ln W_1 - \ln W_0$) / t

Feed conversion ratio:

FCR= (kg feed DM fed) x (kg final biomass– kg initial biomass + kg dead fish)$^{-1}$

Thermal growth coefficient: TGC= $[(W_1)^{1/3} - (W_0)^{1/3}]$ x (days x ^{o}C)$^{-1}$ x 1000

Condition factor: CF= W (g) x (fork length, cm)$^{-3}$ x 100

Weight gain: WG= W_1 (g) – W_0 (g)

Visceral somatic index: VSI=Viscera weights/ Body weight (g) x 100

Cardio- somatic index: CSI= Hearth weight/ Body weight (g) x 1000

Hepato-somatic index: HSI= Liver weight/ Body weight (g) x 100

Slaughter yield: SY= Gutted weight (g) / Body weight (g)

Fillet Yield: FY= 2 x Fillet weight (g) / Body weight (g)

Where:

DM: dry matter

W: the body weight of the sampled fish in grams

W_0: the initial mean body weights of the fish in grams

W_1: the final mean body weight of the fish in grams

3.9. Statistical analysis

The results were analyzed using one-way and two-way analysis of variance (ANOVA) in the Statistic Analysis Software (SAS) edition 9.3 for Windows (SAS Institute Inc. Cary, NC, U.S.A.). This software is a collection of statistical models that can establish the differences between group means and to study correlation among the variables, where the user is able to determine the model of preference.

The one-way analysis of variance ($ANOVA^1$) was done for production parameters BW, FCR, SGR and TGC with the days of feeding being the class variable. The results corrected for start weight differences. The analysis was on the data gathered after 79 days of treatment. Net pens were the experimental unit in this statistical model.

The one-way analysis of variance ($ANOVA^2$) done for production, biometric, quality and fish health parameters was on the data from sampling after 97 days of the experiment. Bonferroni correction model additionally inspected data for significant differences between non-parametrical parameters. Statistical analysis revealed significant differences between the distribution of female and male salmon fish inside the treatments. Because the imbalanced female and male distribution across the feeding treatments, gender was used as a covariate in the statistical model and adjustment for body weight differences when relevant. The individual fish was the experimental unit in this analysis.

Microsoft Excel 2013 contributed to the graphical presentation of polynomial or linear regression between two variables.

Pearson's correlation coefficient is the measure used to investigate dependence between the variables. The level of significance, alpha, was set was set at $P < 0.05$. R^2 is displaying the portion of the total variation explained by the linear or polynomial regression model.

4. Results

4.1. Production parameters

The body weight (BW, kg) of the fish measured after 79 days of treatment varied significantly between the different regimes (P=0.03). Body weight was significantly the lowest for regimes D2 and D3, and significantly highest for regime D5, D6 and D7. The body weight was highest for regime D6 (2.37kg) to lowest in regime D2 (2.1kg) (Table 1).

Gain varied significantly between the regimes (P =0.0003). The highest weight gain was recorded for the fish from regime D5 (427g), D6 (431g) and D7 (391g). No significant differences were found between these feeding regimes, but the weight gain of D5 (P=0.11) and D6 (P=0.1) tended to be higher when compared with D7. The weight gain of D4 was intermediate (331g), whereas D2 (157g) and D3 (141g) had significantly lowest gain (Table1).

The Specific Growth Rate (SGR) varied significantly between the regimes (P<.0001). It was significantly lowest in regimes D2 (0.1) and D3 (0.08) whereas the significantly highest SGR was for D5 (0.25) and D6 (0.25). SGR for regimes D7 and D4 were intermediate with D7 (0.23) being significantly higher than D4 (0.21) (Table 1).

The feed conversion ratio (FCR) varied significantly between the regimes (P<.0001). The significantly lowest FCR was for the fish from regimes D4 (1.09), D5 (1.02), D6 (1.12) and D7 (1.17), and significantly highest for the fish from regimes D2 (1.83) and D3 (1.63) (Table 1).

Calculated thermal growth coefficients (TGC) varied significantly between the regimes (P<.0001). The highest TGC was observed for the fish from regimes D5 (2.13) and D6 (2.16), whereas D7 (1.97) was intermediate. The significantly lowest TGC fish group was from regimes D2 (0.79) and D3 (0.7) while the fish from regime D4 (1.73) was intermediate (Table 1).

Table 1

Body weight (BW, kg), Specific growth rate (SGR), Feed conversion ratio (FCR) and Thermal growth rate (TGC) of the Atlantic salmon (*Salmo salar* L.) confined to different feeding regimes during 79 days.

	Days of feeding						ANOVA[1]		
	2	3	4	5	6	7	Pooled SE	P value	R^2
BW (kg)	2.1^{c}	2.08^{c}	2.27^{b}	2.37^{a}	2.37^{a}	2.33^{a}	0.11	0.03	0.93
SGR	0.1^{d}	0.08^{d}	0.21^{c}	0.25^{a}	0.25^{a}	0.23^{b}	0.04	<.0001	0.99
FCR	1.63^{a}	1.83^{a}	1.09^{b}	1.02^{b}	1.12^{b}	1.17^{b}	0.005	<.0001	0.92
TGC	0.79^{d}	0.7^{d}	1.73^{c}	2.13^{a}	2.16^{a}	1.97^{b}	0.01	<.0001	0.99

The values are calculated based on the bulk weight of the fish and the average sea water temperature (≈5.1°C) during 79 days of the experiment (Chapter 3.8).

[1]Results from one-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

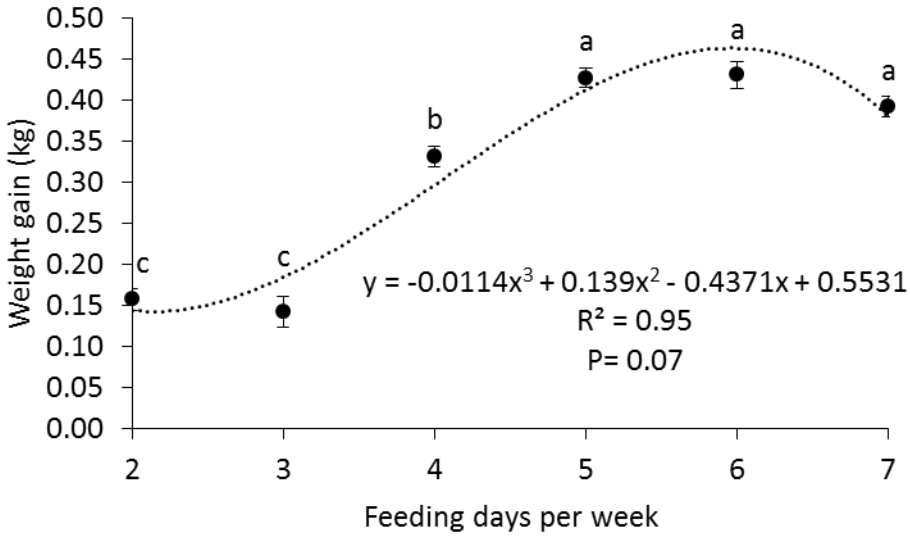

Fig. 2. Weight gain (LSmean ± standard error) after 79 days of the feeding treatments. The mean values found to be significantly different are marked with different letters over the value points. The dotted line represents the polynomial regression (R^2= 0.95) and P is the level of significance.

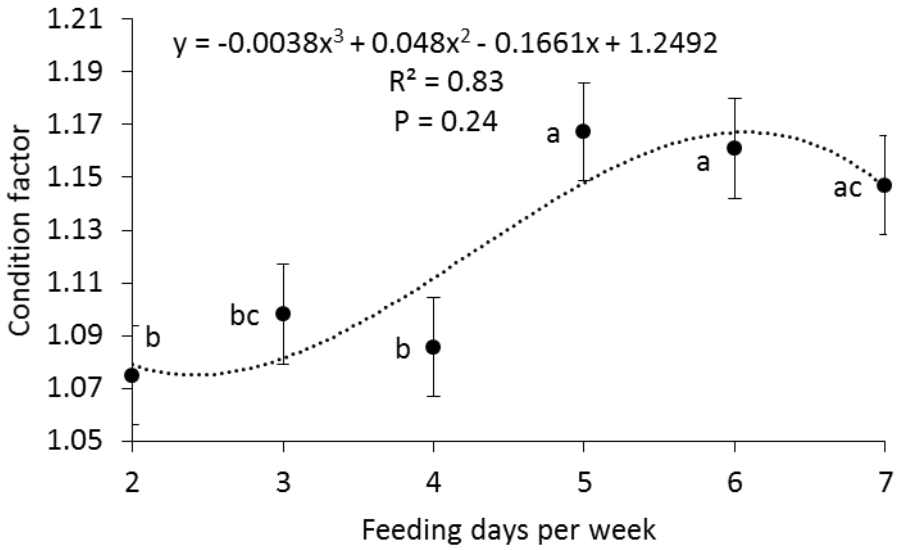

Fig. 3. Condition factor (LSmeans ± standard error) calculated after 97 days of the feeding regime plotted against the feeding days. The mean values found to be significantly different are marked with different letters over of the value points. The dotted line represent the polynomial regression (R^2 =0.83) and P is the level of significance.

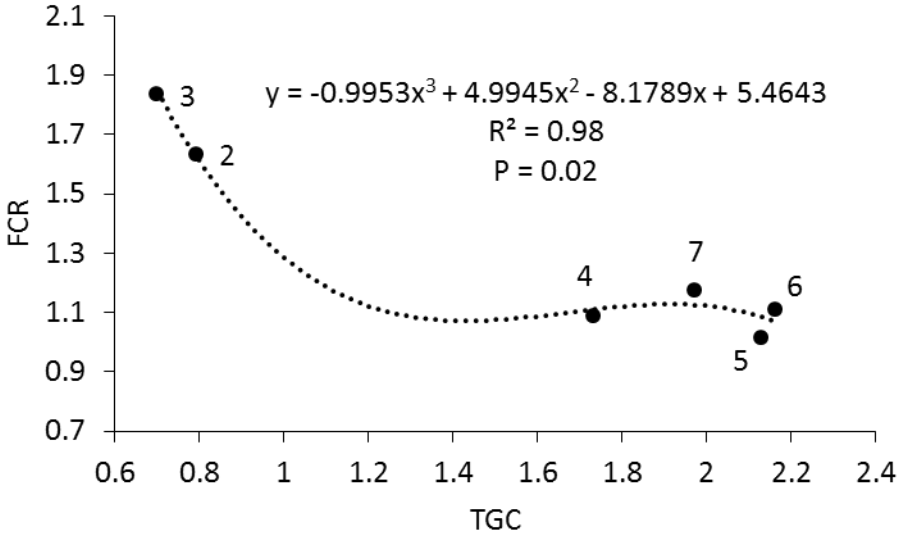

Fig. 4. Thermal growth rate coefficients (TGC) plotted against the feed conversion ratios (FCR) calculated after 79 days of the feeding regime. The dotted line represent the polynomial regression (R^2=0.98) and P is the level of significance. The numerated points represent different feeding regimes.

Table 2

Overall correlations between the production parameters corrected after 79 days of feeding. The production parameters studied include Body weight (BW), Specific growth rate (SGR), Feed conversion ratio (FCR) and Thermal growth rate (TGC) of the Atlantic salmon (*Salmo salar* L.)

Production parameter		SGR	TGC	BW_{start}	BW_{end}	Gain
FCR	*r*	-0.92	-0.92	-0.39	-0.80	-0.89
	P	<.0001	<.0001	0.230	0.003	0.000
SGR	*r*	1.00	0.48	0.91	0.99	
	P	<.0001	0.132	0.000	<.0001	
TGC	*r*		0.53	0.93	0.99	
	P		0.962	<.0001	<.0001	
BW_{start}	*r*			0.80	0.59	
	P			0.003	0.055	
BW_{end}	*r*				0.96	
	P				<.0001	

The values in the table are referring to the correlation coefficients (*r*) between the productions parameters with the level of significance (P) set to 5 %.

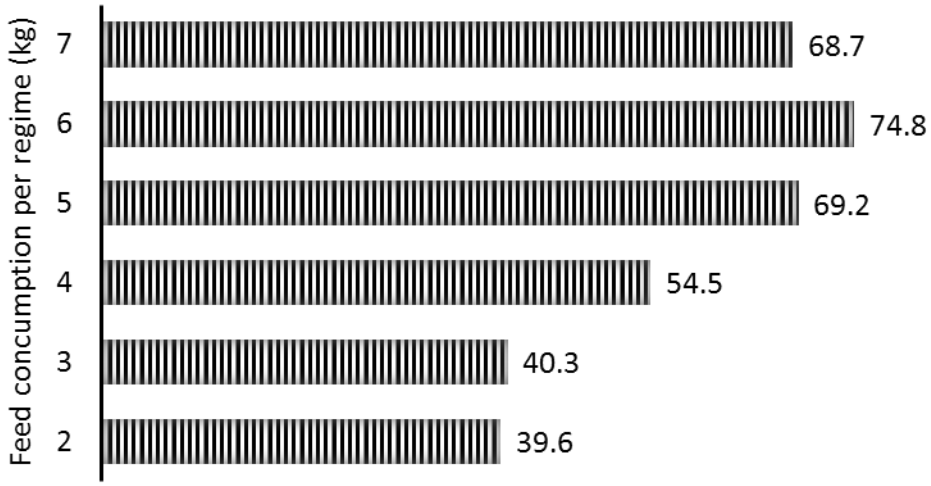

Fig. 5. The feed consumption during 79 days of the experiment. The calculation is weight gain x FCR x number of fish per regime.

4.2. Biometric traits

The body weights (BW) of the fish sampled for analysis varied significantly between the regimes after 97 days of the trial (P=0.005). The average body weights were significantly the lowest for the fish from regimes D2, D3 and D4, and significantly highest for regimes D5, D6 and D7. The weights was highest for fish from regime D6 (2.61kg) to the lowest for regime D2 (2.18kg) (Table 3).

The gutted weight (GW) varied significantly between the treatments (P=0.006), being significantly lowest for the regimes D2, D3 and D4 and significantly highest for the regimes D5, D6 and D7. There was no significant difference between regime D4 and D5 (Table 3).

The fillet weight (FW) varied significantly between the feeding regimes (P=0.002). The average FW was significantly lowest for the fish from regimes D2, D3 and D4, and significantly highest for regimes D5, D6 and D7 (Table 3).

The fork length (FL) varied significantly between the feeding regimes (P=0.008). The average FL was significantly the lowest for the fish from regimes D2, D3 and D4, and significantly highest for regimes D5, D6 and D7. The total range in FW was from 60.7cm (D6) to 58.5 (D3) (Table 3).

The calculated Condition factor (CF) varied significantly between the feeding regimes (P=0.001). The average CF was significantly the lowest for the fish from regimes D2, D3 and D4, while significantly highest for regimes D5, D6 and D7. The total range of the CF values was from 1.17 (D5) to 1.07 (D2) (Table 3).

The relative viscera weight (VW) varied significantly between the feeding regimes (P=0.02). The average VW was significantly lowest for the fish from regimes D2, D3 and D4, while significantly highest for regimes D5, D6 and D7. The total range in VW was from 331g (D6) to 256g (D2) (Table 3).

The overall hearth weight (HW) varied significantly between the regimes (P=0.02). The average HW was significantly lowest for the fish from regimes D2, D3 and D4, while

significantly highest for regimes D5, D6 and D7. The total range in HW was from 2.54g (D6) to 1.99g (D2) (Table 3).

The overall liver weight (LW) varied significantly between the regimes (P=0.014). The average LW was significantly lowest for the fish from regimes D2, D3 and D4, while significantly highest in regimes D5, D6 and D7. The total range of LW values was from 28.6g to 22.4g (D2) (Table 3).

The overall average slaughter yields (SY) did not vary significantly between the different feeding regimes after 97 days of the trial (P=0.46). (Table 4).

The calculated fillet yields (FY) varied significantly between the regimes after 97 days of the experiment (P=0.001). The average FY was significantly lowest for the fish from regimes D3 and D4, while significantly highest for regimes D2, D5, D6 and D7. The total range of FY values was 61.9 % (D2) to 58.8% (D3) (Table 4).

The calculated viscero-somatic index (VSI) did not significantly vary between the different feeding regimes after 97 days of the trial (P=0.46) (Table 4).

The calculated cardio-somatic index (CSI) did not vary significantly between the different feeding regimes after 97 days of the trial (P=0.62) (Table 4).

The calculated hepato-somatic index (HSI) did not vary significantly vary between the different feeding regimes after 97 days of the trial (P=0.58) (Table 4).

Table 3
The body weight, gutted weight, fillet weight, fork length, the condition factor, intestine weight, hearth weight and liver weight of the Atlantic salmon fish (Salmo salar L.) exposed to different feeding regimes during 97 days of the experiment.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
Values presented in (kg)											
Body weight	2.18^{b}	2.22^{b}	2.24^{b}	2.51^{a}	2.61^{a}	2.52^{a}	0.10	0.001	0.005	0.000	0.231
Gutted weight	1.92^{c}	1.94^{c}	1.96^{bc}	2.2^{ab}	2.3^{a}	2.22^{a}	0.08	0.001	0.006	0.001	0.217
Fillet weight	0.67^{b}	0.65^{b}	0.66^{b}	0.76^{a}	0.80^{a}	0.78^{a}	0.03	0.001	0.002	0.001	0.227
Value presented in (cm)											
Fork length	58.5^{b}	58.5^{b}	59^{ab}	59.7^{ab}	60.7^{a}	60.3^{ab}	0.7	0.008	0.149	0.000	0.173
Calculated value											
CF	1.07^{b}	1.1^{bc}	1.09^{b}	1.17^{a}	1.16^{a}	1.15^{ac}	0.02	0.002	0.001	0.027	0.207
Values presented in (g)											
Viscera weight	256^{c}	281^{bc}	280^{bc}	321^{ab}	331^{a}	300^{abc}	17	0.001	0.023	0.000	0.222
Hearth weight	1.99^{c}	2.2^{abc}	2.1^{bc}	2.49^{a}	2.54^{a}	2.36^{ab}	0.13	0.001	0.023	0.001	0.212
Liver weight	22.4^{b}	23^{bc}	23.1^{bc}	26.4^{ac}	28.6^{a}	25.7^{abc}	1.36	0.002	0.014	0.000	0.210

The data represents LSmeans values of 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen. Condition factor (CF) calculated according to the formula in chapter 3.8.

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P value is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

Table 4

Slaughter yield (SY) and fillet yield (FY) of the Atlantic salmon (*Salmo salar* L.) subjected to different feeding regimes during a 97 days experimental period.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
Slaughter yield %	88.3	87.5	87.5	87.3	87.4	88.1	0.004	0.17	0.46	0.06	0.09
Fillet yield %	61.9^{a}	58.9^{c}	59.3^{bc}	60.8^{ab}	61.2^{a}	61.7^{a}	0.006	0.001	0.001	0.75	0.22

The results represents calculations based on 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen (Chapter 3.8).

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P value is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

Table 5

Viscero-somatic index (VSI), cardio-somatic (CSI) and hepato-somatic (HSI) of the Atlantic salmon (*Salmo salar* L.) treated with different regime feedings during 97 days of the experiment.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
VSI (%)	11.66	12.52	12.52	12.72	12.62	11.95	0.44	0.17	0.46	0.06	0.09
CSI (%)	0.92	1.00	0.94	1.00	0.96	0.93	0.04	0.54	0.62	0.36	0.05
HSI (%)	1.02	1.02	1.03	1.05	1.09	1.02	0.03	0.45	0.58	0.09	0.06

The data represents calculations based on data from 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen (Chapter 3.8).

[2]Results from the two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance.

4.3. Quality parameters

The fillet fat content (%) did not significantly vary between the different feeding regimes (P=0.42) (Table 6).

The measured fillet firmness (N*s) showed a significant difference between the different feeding regimes (P=0.01). The significantly highest average fillet firmness was for the fish from regimes D5, D6 and D7, while significantly lowest in feeding regimes D2, D3 and D4. The overall range was from 168.6 N*s to 145.4 N*s (D4) (Table 6).

The gaping scores (Gaping A) showed a significant difference between the different feeding regimes (P=0.02). The significantly highest average gaping A score was for the fish from regime D4, while significantly lowest in feeding regimes D2, D3 and D5. Feeding regimes D6 and D7 had an intermediate gaping A scores. The overall range was from 1.1 (D4) to 0.3 (D2) (Table 6).

The industry gaping scores (Gaping I) did not differ significantly between the different feeding regimes (P=0.14). The gaping I score values varied between being the highest for the fish from regime D4 (1.1) to the lowest in regime D5 (0.9) (Table 6).

The pH measurements showed no significant differences between the different feeding regimes (P=0.68). The numerically highest recorded pH was for the fish fillets from regime D3 (6.13), while the lowest pH recorded was for fish from regime D7 (6.08) (Table 6).

The total pigment content (mg/kg) measured in the sampled fillets differed significantly between the different feeding regimes (P=0.07). The significantly highest average pigment content was for the fish from regime D2, while significantly lowest in feeding regimes D5 and D6. Feeding regimes D3, D4 and D7 had an intermediate fillet pigment content. The average range of total pigment contents was 6.8 mg/kg (D2) to 5.9 mg/kg (D6) (Table 6).

Table 6

Percentage of fat in the fillets, firmness, gaping A, gaping I, pH-measurements and total pigment (mg/kg) of the Atlantic salmon fish (*Salmo salar* L.) managed with different feeding regimes during 97 days of the experiment.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
Fat in the fillets %	14.2	14.2	14.3	14.8	14.9	14.6	0.3	0.14	0.42	0.01	0.10
Firmness (N*s)	152.5^{b}	149^{b}	145.4^{b}	167.4^{a}	157.3^{ab}	168.6^{a}	5.23	0.001	0.01	0.00	0.22
Gaping A	0.3^{b}	0.4^{b}	1.1^{a}	0.3^{b}	0.6^{ab}	0.6^{ab}	0.19	0.03	0.02	0.43	0.15
Gaping I	1.6^{ab}	1.5^{ab}	1.8^{a}	0.9^{b}	2^{a}	1.2^{ab}	0.3	0.20	0.14	0.85	0.90
pH	6.1	6.13	6.12	6.09	6.09	6.08	0.02	0.76	0.68	0.28	0.08
Pigment	6.8^{a}	6.5^{ab}	6.4^{ab}	6.2^{b}	5.9^{b}	6.5^{ab}	0.19	0.06	0.07	0.39	0.13

The data represents LSmeans values of 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen.

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

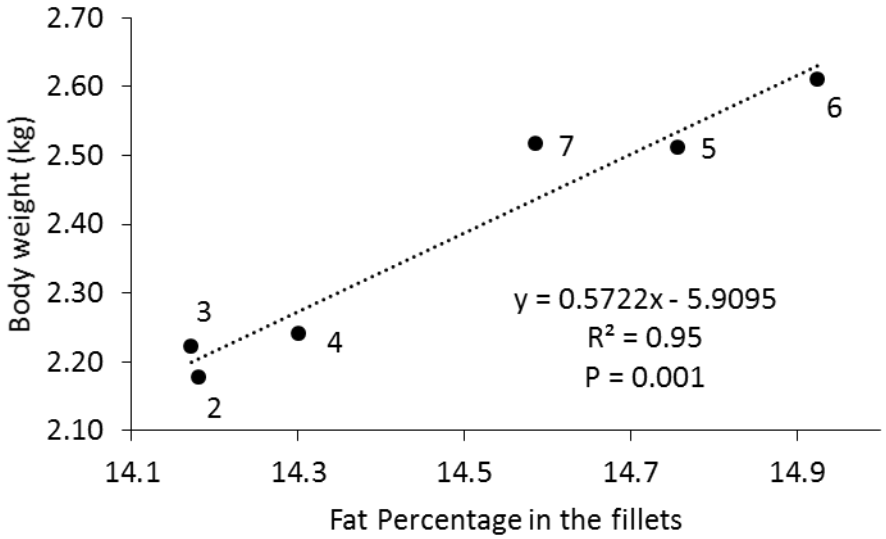

Fig. 6 Body weight of the fish sampled after 97 days of the regime plotted against the instrumentally measured fat percentage in the fillets. The dotted line represents a linear regression (R^2=0.95). The numerated points represent every feeding regime.

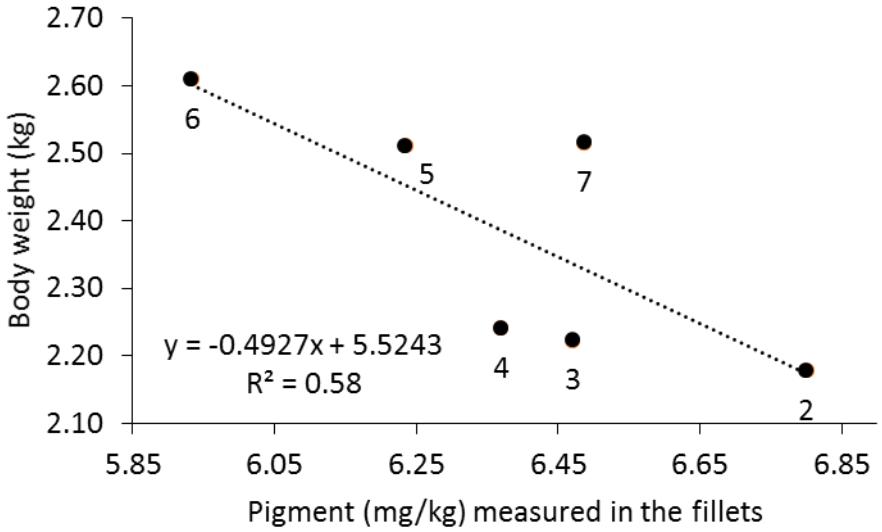

Fig. 7. Body weight of the sampled after 97 days of the feeding regime plotted against the instrumentally measured pigment content in the fillets. The dotted line represents a linear regression (R^2=0.58). The numerated points represent every feeding regime corresponding statistically processed data.

4.4. Fish health

4.4.1. Organ health evaluation

The overall statistical analysis showed that the internal organ adhesions (IOA) had no significant differences between the different feeding regimes (P=0.071) (Table 7).

Melanin in the organs scores (MIO) differed significantly between the treatments (P=0.011). The significantly highest melanin in organs score was for the fish from regime D7 (1.05), while the significantly lowest score recorded was in fish from treatment D2 (0.25) (Table 7).

Melanin in the abdominal wall scores (MIAW) showed no significant difference between the regimes (P=0.61). The highest score for melanin in the abdominal wall recorded was in fish from treatment D3 (1.82), while the lowest melanin in the abdominal wall score recorded was in fish from treatment D2 (1.31) (Table 7).

The visceral fat score (VF) evaluation showed a significant difference between the feeding regimes (P=0.16). The significantly highest visceral fat score recorded was for the fish from regime D7 (1.9), while the significantly lowest fat score recorded was for regime D4 (1.3) (Table 8).

The liver color score (LC) showed a significant difference between the treatments (P=0.006). The significantly highest liver color score recorded was in fish from treatment D2 (4.6), while the significantly lowest fat score recorded was in fish from treatment D6 (3.6) (Table 8).

Table 7

Internal organ adhesions (IOA), melanin spots in the organs (MIO) and melanin spots in the abdominal wall (MIAW) of the Atlantic salmon fish (*Salmo salar* L.) subjected to different feeding regimes during 97 days of the experiment.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
IOA	0.00^{b}	0.03^{bc}	0.23^{ac}	0.3^{a}	0.16^{ab}	0.08^{ab}	0.08	0.029	0.071	0.047	0.144
MIO	0.25^{c}	0.39^{bc}	0.57^{bc}	0.69^{ab}	0.73^{ab}	1.05^{a}	0.16	0.011	0.011	0.557	0.167
MIAW	1.3	1.8	1.7	1.5	1.5	1.4	0.21	0.043	0.612	0.001	0.133

The data represent LSmeans values of 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen.

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

Table 8

Visceral fat score and liver color of the Atlantic salmon fish (*Salmo salar* L.) subjected to different feeding regimes during 97 days of the experiment.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	diet	sex	R^2
Visceral fat score	1.5^{bc}	1.7^{ab}	1.3^{cd}	1.4^{bd}	1.7^{ab}	1.9^{a}	0.11	0.006	0.016	0.136	0.182
Liver color	4.6^{a}	4.2^{ab}	4.4^{ab}	4^{bc}	3.6^{c}	4^{bc}	0.18	0.008	0.006	0.892	0.176

The data represent LSmeans values of 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen.

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance. Different superscripts within the same row indicate significant differences between the feeding regimes ($P < 0.05$).

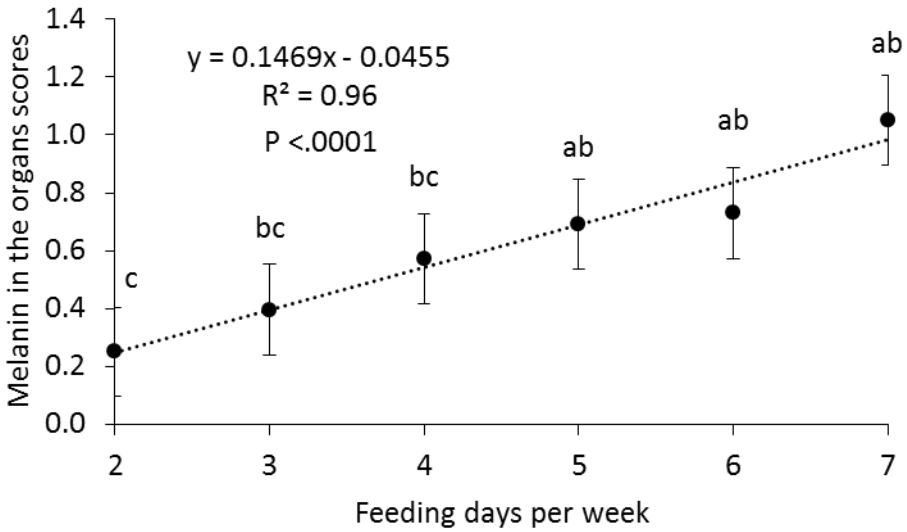

Fig. 8. Melanin in the organs (scores 0-3) after 97 days of the treatment plotted against the different feeding regimes. The dotted line represents a linear regression (R^2=0.95), P is the level of significance. The mean values found to be significantly different are marked with different letters over the value points. The vertical bar through the value points is the standard error.

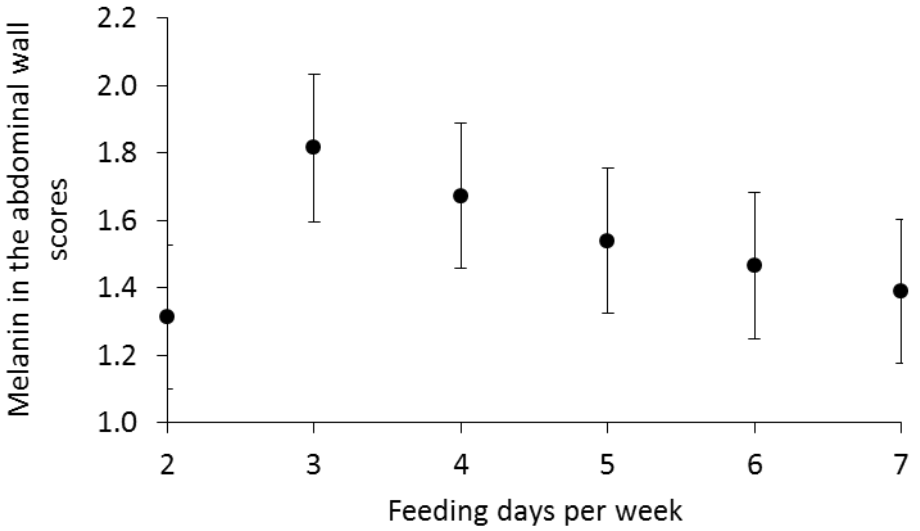

Fig. 9. Melanin in the abdominal wall scores after 97 days of the treatment plotted against the corresponding feeding regimes. The dots represent MIAW scores corresponding statistically processed data and the bar placed over and under the value points is the standard error.

4.4.2. Intestinal health evaluation

The total pylorus inflammation scores (TPINFL) showed no significant difference between the feeding regimes (P=0.63). The numerically lowest TPINFL score was recorded for the fish from regime D2 (8.7), while the highest was for the fish from regime D4 (9.63) (Table 9).

The total mid-intestine inflammation scores (TMIINFL) showed no significant difference between the treatments (P=0.49). The numerically lowest TMIINFL score was for the fish from regime D5 (1.12), while the highest was for the fish from regime D4 (2.07) (Table 9).

The total intestinal scores (TINT) showed no significant difference between the different feeding regimes (P=0.12). The numerically lowest TINT score was for the fish from the regime D5 (TINT=9.97), while the highest TINT score was for the fish from regime D4 (11.71) (Table 9).

The total inflammation scores (TINFL) showed significant difference between the regimes (P=0.07). The numerically lowest TINFL score was for the in fish from regime D2 (1.46), while the highest TINFL score for the fish regime D4 (3.02) (Table 9).

The total floating feces scores (TFF) showed no significant difference between the feeding regimes (P=0.63). The numerically lowest TFF score was for the fish from regime D3 (TFF =1.64), while the highest TFF score was for the fish from regime D6 (TFF=2.86) (Table 9).

Table 9

Total pylorus inflammation score (TPINFL), Total mid-intestine inflammation score (TMIINFL), Total intestinal score (TINT), Total inflammation score (TINFL) and Total floating feces score (TFF) of the Atlantic salmon fish (*Salmo salar* L.) fed different feeding regimes during 97 days.

	Days of feeding						ANOVA[2]				
								P value			
	2	3	4	5	6	7	Pooled SE	model	feeding	sex	R^2
Total pylorus inflamation score	8.7	9.4	9.6	8.8	9.1	9.3	0.44	0.33	0.63	0.17	0.12
Total mid-intestine inflamation score	1.7	1.9	2.1	1.1	1.6	1.7	0.21	0.07	0.49	0.20	0.19
Total intestinal score	10.4	11.3	11.7	10	10.7	11	0.46	0.10	0.12	0.45	0.17
Total inflamation score	1.5	1.9	3	2	2	1.9	0.35	0.11	0.07	0.83	0.17
Total floating feces score	2.8	1.6	2.8	2.3	2.9	2.8	0.58	0.22	0.63	0.12	0.14

The data represent LSmeans values of 96 fish sampled from six different regimes, 2 net-pens per regime with 8 fish per net-pen.

[2]Results from two-way analyses of variance (ANOVA) where S.E. is the pooled standard error and P is the level of significance.

Table 10

A table of marginal values used for determination of intestinal health of the Atlantic salmon fish (*Salmo salar* L.) subjected to different feeding regimes during 97.

	Normal/mild	**Moderat**	**Bad**
TINFL score	0-3	4-6	7-9
TMIINFL score	0-1	2	3
TINT score	0-6	7-13	14-18
TPINFL score	0-5	6-10	11-15
TFF score	0-3	4-6	7-9

5. Discussion

5.1. Production parameters

The present experiment clearly demonstrated that the fish from the restricted feeding group managed to compensate growth when compared to the control group. This compensation in growth occurred upon a feeding restriction in a small scale coincides to other restricted feeding experimental designs (Hornick et al. 2000; Johansen et al. 2001).
The present study recorded growth for all feeding regimes after 79 days resembling a sinusoid curve for body weight increase (Fig. 2.). The weight gain correlated negatively with the overall FCR (r = -0.89), but FCR showed no significant difference between D4, D5, D6 and D7. A*s* expected the feed consumption increased with increasing ration, but it is interesting that there was no significant difference in growth rate between D5, D6 and D7. Hence feeding fish 5 days a week gave similar results as feeding fish 7 days a week during a winter period with relative cold-water temperatures. The results indicate that Atlantic salmon most efficiently metabolized feed at the feeding frequency of regime D5. This ability could be something that fish developed during the experiment, or naturally timed phenomena due to an occurrence of digestible enzyme/stomach capacity synchronization. A previous restricted feeding study found that there was no clear benefit towards feed conversion and growth from feeding to saturation compared to a 75 % ration during the winter period (Einen et al. 1999).

The significantly highest specific growth rate was recorded for fish from regime D5 and D6 (SGR=0.25), while D7 (SGR=0.23) had a significantly lower result (Table 1). These calculated SGR values are complying to the expected table values of fish size > 2000grams and low water temperature (≈5.1 °C) (Austreng et al. 1987). On the other hand, regimes D2 (SGR=0.1) and D3 (SGR=0.08) were below the expected table values. The overall SRG correlated negatively with the overall FCR (r=-0.9; P=<.0001) (Table 2), which coincide with a seasonal variation study during late autumn and winter for both 1+ and 0+ smolt (Mørkøre & Rørvik 2001). Even though the amount of feed used for D5 was ca. 1% higher compared with D7 and ca. 9 % lower compared with D6, FCR showed no significant difference between D5, D6

and D7 (Table 1). Nevertheless, the results in this case are promoting the significantly lowest FCR group, including regime D4, D5 and D6, as satisfactory. According to a previous study by Einen et al. (1995), a well performing farmed salmon has a TGC approximating 3.3. Highest TGC recorded in the present study was 2.16. The overall TGC correlated positively with the overall SGR (r=1.00; P=<.0001) (Table 2). This high correlation could be an indicator of the seasonal variation of SGR in Atlantic salmon (Nordgarden et al. 2003).

5.2. Biometric traits

The sampling reviled that the body weight distribution between the regimes established itself already after 79 days of the experiment, judging according to the significant differences. With an exception of regime D4, which after 97 days fall into the significantly lower body weight group (Table 1, 3). The body weights and the gutted weights were increasing with increasing ration level with an exception for the fish from regime D7 (Table 3). Gutted weights of the fish from regime D4 and D5 were not significantly different at the end of the experiment (Table3). Even though, measured gutted weights displayed a significant difference between the treatments the end sampling revealed no significant difference between the calculated slaughter-yields (Table 3, 4). In a previous restricted feeding study, done by Einen et al. (1999), a 2% increase in SY after 110 days of starvation when compared with the control group. The feeding regimes D5, D6 and D7 had, significantly highest fillet yields, with more than 10% higher FY when compared with the low FY group (D4, D3 and D2) (Table 3). In a previous study done by Herbinger and Friars (1991), it was concluded that a significant positive correlation between the condition factor and total fat content can be a good, non-lethal indicator of energy reserve status in Atlantic salmon. The condition factor was ranging from 1.07 (D2) to 1.17 (D5) (Table 3). This CF result is under the acceptable value of 1.2 (Barnham & Baxter 2003). Exposing Atlantic salmon fish to restricted feeding gives a slimmer body shape indicated by a lower condition factor (Lie & Huse 1992). On the other hand, a seasonal variation study done by Mørkøre and Rørvik (2001), disclosed that CF for both 0+ and 1+ smolt increased from 1.1 in July to 1.3 in September, whereas CF in 0+ continued to increase until November.

VSI, CSI and HIS are percentages of viscera, hearth and liver of the whole body weight used as an assessment of health condition. Even though there was a significant difference in viscera weights, hearth weights and liver weights between the regimes and a high dependency on gender (P <.0001), the two-way ANOVA[2] showed no significant effects and interactions of sex distribution and feeding regime for VSI, CSI and HSI (Table 3, 5). A previous study by Einen et al. (1999) found no significant differences in VSI between the regimes after 45 days of the experiment, but HSI was significantly different increased with increased ration level. Research done by Røra et al. (2001) concluded that an average Atlantic salmon embody VSI in a range 6% to 12%. On the other hand, in a high/low fat digestion study by Bendiksen et al. (2003), VSI tended to be higher in the fish given the high fat feeds, and were also higher at 2 °C than at 8 ° C. In this study, VSI values averaged between 11.66 % and 12.72 % (Table 5). The CSI values averaged between 0.92 and 1, whereas HSI values averaged between 1.02 and 1.09 (Table 5). These CSI and HSI values are both in a range of an average healthy Atlantic salmon (Einen & Roem 1997; Karalazos et al. 2007; Solberg 2004).

5.3. Quality parameters

This restricted feeding study registered a high positive correlation between body weight and fillet fat content (*r*= 0.97, P=0.001), but made no significant impact on the fillet fat content (Table 6). Previous studies have revealed a positive correlation between the fillet fat content and body weight of the sampled fish. This could be a consequence of seasonally changing fat metabolism in Atlantic salmon fish (Mørkøre & Rørvik 2001; Nordgarden et al. 2003; Roth et al. 2005). In addition, this result complies with the previous restricted feeding studies where the fillet content showed no significant difference between the regimes after the results were corrected for weight imbalance (Johnsen 2006; Young et al. 2005). On the other hand, a starvation study performed by Mørkøre et al. (2008), where fish were fed to satiety or starved for 35 days, no significant difference in the fat content was established between fed and starved fish. Even with occurrence of significant difference in fillet fat content after a long-term starvation procedure, this method would never be lucrative as a tool for reducing

fillet fat content in Atlantic salmon fish production (Einen et al. 1999). Nevertheless, the results from this study are opposing previous studies where restrictive feeding regime reduced fillet fat content in Atlantic salmon (Einen et al. 1999; Johansen & Jobling 1998). In a previous restricted feeding regime study performed by Young et al. (2005), the sampled Atlantic salmon (weight 6.5 – 7.6 kg) showed no connection between the fat in the fillets and fillet firmness. The firmness results in this study are opposing the earlier studies that found that restrictive feeding and starvation provoke a harder texture in the fillets (Einen & Thomassen 1998; Einen et al. 1999). The significantly hardest fillets (167.4 N*s) were sampled from the fish subjected to the feeding regime D5 making this regime preferable (Table 6). A previous Atlantic salmon texture study, done by Mørkøre et al. (2009), revealed that the area under the time-force graph was significantly highest for the fillets that were characterized with small fibers (≤12.500 μm^2), when analyzed using a cylindrical probe. The higher body weight of the sampled fish correlated positively (r =0.76, P =0.08) with harder firmness.

In a previous restricted feeding study, the incidence of gaping A in the fillets was significantly increased with increasing feeding ratio level (Einen et al. 1999). Statistical analyses of the gaping scores in this study pointed out a significantly highest, both gaping I and gaping A score, in fish from the regime D4 (Table 6). On the other hand the significantly lowest, both gaping A and gaping I score, were recorded in fish fillets from regime D5 making this regime texturally superior (Table 6). A study of connective tissues in fish resulted a negative correlation (r=-0.78) between gaping and muscle pH (Lavety et al. 1988). Post rigor pH levels expressed no significant differences between the feeding regimes in the presented study (Table 6). On the other hand, a previously conducted feeding ratio study have shown that greater feed rations give a lower post rigor pH in the Atlantic salmon flesh (Einen et al. 1999).

The industrial gaping procedure gave in average 310% greater gaping score when compared with gaping scores from the procedure according to Andersen. The fish in treatment D2 had significantly highest gaping I score (1.8), displaying an increase of 500% in gaping score when compared to gaping A score (Table 6). Consequently, a rough industrial handling would therefore eliminate the previous advantage that fish from regime D2 had in gaping scores. On the other hand, the industrial gaping scores in fish fillets from treatment D4 (1.8) got the

lowest increase in gaping 140% due to rough handling, nevertheless they still remaining significantly the highest in gaping score (Table 6).

The non-invasive pigment and fat content measurement used in this study is providing sufficient accuracy for the salmon industry and the market requirements (Folkestad et al. 2008). A previous starvation study had concluded that there was no clear advantages or disadvantages in coloration of feeding restriction up to 86 days (Einen & Thomassen 1998). A previous restricted feeding study by Einen et al. (1999) showed that as the feeding ratio increased, the pigment levels were decreasing. The present study showed significantly highest pigment content was for the fish fillets from regime D2 (6.8 mg/kg) (Table 2). Nevertheless, there was no significant difference in the pigment content between the control and restricted feeding groups (Table 6).

5.4. Fish health

5.4.1. Organ health evaluation

The present study recorded that internal organ adhesions scores differed significantly between the feeding treatments indicating the most preferable IOA score in fish from regime D2 (Table 7). Ranging between 0 and 0.3, IOA scores indicated a good intestinal health condition (Aunsmo et al. 2008).

The cause of melanin spots is not certain while presence of melanin spots in the farmed Atlantic salmon industry represents a substantial fillet quality problem. Melanin evaluation in this study revealed a significant difference in MIO, but not in MIAW (Fig. 8, 9) (Table 7). The MIO scores showed a high positive correlation with increasing feeding days (r=0.98, P<.0001) with the significantly highest MIO recorded for the fish from D7 (Fig. 8).

Visceral fat scoring in this study revealed significantly highest score for feeding regimes D3, D6 and D7 (Table 8). D7 scored highest (1.9) hence having the highest feed access during the experiment (Rasmussen 2001).

A starvation study performed by (Einen et al. 1998) revealed a steady decrease in liver fat content with increasing starvation time, indicating that the liver fat reserve is one of the important metabolic energy reserves. A paler or a discolored liver is not preferable as it points out to a metabolic disorder (Mørkøre et al. 2013). The livers of all sampled fish were in the borderlines of brown to dark-brown coloration (Table 8).

5.4.2. Intestinal health evaluation

TPIINFL scores were in moderate levels (6-10) for the fish in all treatments. TMIINFL scores were in the range of normal (0-5), with an exception of fish from treatment D4 (TMIINFL=2.07) that was in average scored moderate (6-10). TINT scores for all the treatments were in moderate levels (7-13). TINFL scores for all the treatments were not exceeding the normal values (0-3). In addition, all of the TFF scores were in the normal scoring boundaries (0-3) (Table 10).

The two-way statistical analyses of variance (ANOVA^2) indicated no statistically significant difference between the intestinal traits scores. After a Bonferroni's correction for non-parametrical parameters, the intestinal health data statistical results fluctuated and pointed out a possible statistically significant difference between the treatments in TPI scores (P=0.03) and TFF scores (P=0.02). Unfortunately, the distribution of the significant differences was not possible to establish.

6. Conclusion

The presented study demonstrated preeminence of restricted feeding over continuous feeding of Atlantic salmon during the winter period. Therefore, a wisely adjusted restricted feeding regime can reduce the labor and feed use in a significant degree without a negative outcome on weight gain or quality, having also in mind that the fish can be unsupervised and deprived of feeding for a considerable number of the weekdays during winter period.

The results indicate that a restrictive feeding regime during the winter period in Northen Norway is an effective way of producing a healthy Atlantic salmon fish with a higher gain and lower feed conversion ratios compared to salmon fed throughout the whole week. In addition, a restricted feeding design can be implemented with no negative effects on the quality parameters such as fillet fat content, post-rigor pH or the slaughter yield. With more than 2 weekdays of feed restriction it can lower the firmness of the fillets, while elevate the pigment content.

In addition, all health examinations done during this experiment indicate that this restricted feeding design does not impair Atlantic salmon health in any way possible to detect by the procedures used in this study.

Presented restricted feeding design can synchronize both quality and quantity during the winter period, hence optimize feed ration for more cost effective Atlantic salmon production. The question of how would this winter regime provoke compensation growth with continuous feeding during upcoming spring stays unanswered.

In summary, a reduction of feeding frequency to 5 days per week during the winter period in Northern seem applicable, but commercial scale verification is warranted. Having in mind the advantages of this production method have no counteract one could only conclude that a continuation of this research is bound to take place in the near future.

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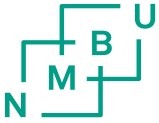
8. Appendix 1

Time last feeding									Time sampling start								
Pen: ____ **Merd**			Liver	Stomach (ST)			Pylorus (PI)/Pylorus ceccae (PC)										
Species: _____							**FF= floating feces**										
Fish #	Weight, g	Weight gutted	Length, cm	Liver score	ST content score	Infl. score	PI content score	PI FF score	White PI	Blooming PI	Swollen PC	Blooming PC	White content from PC				
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
Aver.																	

Mid intestine (MI)								Distal intestine (DI)						
										UDP				
MI content score	MI FF score	MI UDP #	MI UDP size	MI UDP score	MI UDP value	White MI	Infl. score	DI content score	DI FF score	*DI UDP#*	*DI UDP Size*	*DI-UDP score*	*DI UDP value*	Infl. score

Max score					Vekt		
9	15	3	18	9			
Total infl score	Total PI tarm score	Total MI tarm score	Total tarmscore	Total FF score	Hjerte	Lever	Comments



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