Norwegian University of Life Sciences
Department of Animal and Aquacultural Sciences (IHA)
Faculty of Biosciences

Philosophiae Doctor (PhD)
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Dynamic dietary salmon farming in the Faroe Islands with regards to production, quality and economic improvements

Ernæringsmessig dynamisk lakseoppdrett på Færøyene med hensyn til produksjon, kvalitet og økonomiske forbedringer

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Tórshavn, November 2018

Rúni Weihe

CONTENTS

ABBREVIATIONS

AA	Amino acid
ANF	Antinutritional factor
BW	Body weight
CW	Carcass weight
EBIT	Earnings before interests and tax
FCR_{BW}	Biological feed conversion ratio based on whole body weight
FCR_{CW}	Biological feed conversion ratio based on carcass weight
FCR_{E}	Economic feed conversion ratio based on whole body weight
HAM	Hypaxial anterior muscle
HOG	Head-on gutted
HP	High protein-to-lipid ratio
LP	Low protein-to-lipid ratio
MAG	Monoacylglycerol
MFAT	Muscle fat
SFR	Specific feeding rate
TAG	Triacylglycerol
TGC	Thermal growth coefficient
VSI	Viscerosomatic index
WC	Weight class
S0	Under year-old smolt
S1	Year-old smolt

SUMMARY

The development of feeds for Atlantic salmon has been an ongoing process since the industry started in the 1970s. In the early years, salmon feeds had higher share of proteins than lipids, but after the introduction of extrusion and vacuum coating technology in the feed manufacturing process, the dietary protein-to-lipid ratio was reduced below 1 during the mid 1990s. Correspondingly, the development in dietary lipids has increased overall dietary energy in feeds for salmon. In addition to the oils having increasingly replaced proteins, the traditional marine proteins and oils have also been substituted with plant alternatives which now represent the main ingredients in salmon feed. The combination of reduced dietary protein and use of plant ingredients results in a lower feed price compared to feed prices based on both higher protein content and high marine ingredient inclusions.

In this thesis, the preferred dietary strategy using high-fat feeds in the salmon industry has been tested and compared with an isoenergetic protein denser dietary strategy. Thus, the thesis presents the main results of dietary induced differences in two isoenergetic diets with a high protein-to-lipid ratio (HP) and low protein-to-lipid ratio (LP) with regards to biometric production performance (Paper I, II), morphometric quality attributes (Paper I, III) and economic performance (Paper IV). The presented results are based on experiments conducted in both large-scale commercial facilities (Paper II, III) as well as in small-scale research environment (Paper I, II, III). Results from the large-scale trials were based on the whole production cycle, whereas within the two small-scale experiments, the results were based on three separate feeding periods. Experimental fish in the second small-scale trial was based on restocked fish fed HP diets in the first small-scale trial.

Overall differences in feed intake were not observed in the large-scale trials (Paper II). However, differences in periodic feed intake were observed in the small-scale trials (Paper I, II) during the latter part of the year from July to December. The significantly higher feed intake in the HP group compared to the LP group during the July-September period, was considered to be initiated by the lower fat content development in the HP group during the June-July period prior to the autumn (Paper I). In the subsequent late autumn period from September to December the LP group had significantly higher feed intake. Taken together, the feed intake during the latter part of the year seems to be influenced by both fat content in the fish prior to the initiating autumn as well as the dietary protein/lipid balance.

Based on all trials, the HP feed was significantly better converted into carcass growth (Paper I, II, III). This was reflected with significantly lower feed conversion ratios based on carcass weight

(FCR_{CW}) in the HP group (Paper I, II) together with significantly improved carcass-to-body weight yields (Paper I, II, III). These observations coincided with thicker hypaxial anterior muscles in HP groups produced in large-scale, whereas both higher muscle fat content and higher viscerosomatic index (VSI) was registered in the LP groups (Paper III). Thus, the HP diets seemed to provide a mix of substrates which induce more efficient weight gain based on muscle development compared to the LP diets which produced a fish with higher fat content in both visceral and muscle tissue. Combined with improved condition factor in the fish fed HP diets, these morphometric attributes are considered positive for improved yields during primary processing compared to the preferred LP feed strategy in the industry (Paper III). Thus, an improved feed to carcass weight conversion is considered highly beneficial with regards to both production performance as well in technological quality assessment.

The improved feed conversion led to significantly better growth performance in the HP groups (Paper I, II). This enables farmers to either harvest fish at an earlier stage and consequently reduce associated risks of having the fish in the sea. On the other hand, this also raises the opportunity of producing larger fish which typically generate a higher price (Paper IV). Nonetheless, the results also highlighted that the dietary LP strategy performed equally well as the HP strategy during the energy demanding periods in the spring-summer season for the post smolt (Paper I) and in the cold winter period (Paper II). This entails that an overall improvement in farmed salmon performance demands a dietary dynamic approach in salmon farming where lipid denser diets are preferred during the winter period. However, caution is advised with regards to increasing the lipid content during the spring-summer period for the post-smolt, as this might increase fat content in the fish to an undesirable high level with a subsequent consequence of reducing growth performance in the following autumn.

Despite a general higher price level for a dietary HP strategy, the improved conversion process of feed to carcass was modeled to result in lower total feed cost (Paper IV). However, the dietary LP strategy performed economically better during the winter period, which emphasizes the need for a dynamic approach to the choice of dietary strategy with regards to improve the production cycle as a whole. In line with increased costs of salmon production, the value of a reduced production cycle has increased as well. This is especially momentous with high salmon prices. Thus, in the model production time represents an opportunity cost in which there was a positive trade-off in using a HP feed strategy (Paper IV). The combination of increased production costs as well as significant price increase of salmon, the opportunity cost has increased significantly from 2009 to 2016, and the positive trade-off value has grown correspondingly faster than the general increase in feed prices.

In conclusion, the findings in this thesis demonstrate that a dietary dynamic feeding strategy, predominantly based on a HP feed, may significantly improve overall feed to carcass conversion, growth rate as well as induce morphometric attributes beneficial for primary processing in farmed salmon. Although feed prices for a HP strategy are higher compared with a LP feed strategy, improved feed conversion, faster growth rate and higher yield of tradeable product generate an overall improved economic performance in a dietary HP strategy. Depending on that the feed industry has sufficient protein concentrated ingredients to use in the feed formulation, an HP based feed strategy can relatively easily be implemented in salmon production.

SAMMENDRAG

Utvikling av fôr til atlantisk laks har vært en kontinuerlig prosess siden industrien startet på 1970-tallet. I de første årene hadde laksefôret en høyere andel av proteiner enn lipider, men etter innføring av ekstrudering- og vakuumerings-teknologi i fôrproduksjonen ble protein-til-lipid-forholdet redusert til under 1 i midten av 1990-tallet. Tilsvarende har utviklingen i lipid-innhold økt den totale energien i fôr til laks. I tillegg til at oljer har erstattet proteiner i økende grad, har de tradisjonelle marine proteiner og oljer også blitt erstattet med plantealternativer som nå representerer hovedbestanddelene i laksefôr. Kombinasjonen av redusert protein-innhold og bruk av plantebestanddeler resulterer i lavere fôr-pris sammenlignet med priser av fôr basert på høyere proteininnhold og/eller mer marine ingredienser.

I dette arbeidet har lakseindustriens foretrukne ernæringsmessige strategi, med bruk av høy-fett-fôr, blitt testet og sammenlignet med en iso-energetisk men mer proteinrik strategi. De viktigste resultatene som ble funnet ved bruk av to iso-energetiske dietter med et høyt protein-til-lipidforhold (HP) og et lavt protein-til-lipidforhold (LP) med hensyn til biometriske produksjons-egenskaper (Artikkel I, II), morfometriske egenskaper (Artikkel I, III) og økonomisk ytelse (Artikkel IV) er beskrevet. Resultatene er basert på eksperimenter utført både i store kommersielle anlegg (Artikkel II, III) og i småskala forsøk (Artikkel I, II, III). De store forsøkene var basert på hele produksjons-syklusen, mens resultatene i de to småforsøkene var basert på tre separate fôringsperioder. Fisk som ble benyttet i den andre småskala-perioden kom fra fisk gitt HP-dietter i det første småskala forsøket.

Forskjeller i fôrinntak ble ikke observert i storskala-forsøkene (Artikkel II). Imidlertid ble forskjeller i periodisk fôr-inntak observert i små-skala-forsøkene (Artikkel I, II) i løpet av siste del av året fra juli til desember. Det betydelig høyere fôr-inntaket i HP-gruppen sammenlignet med LP-gruppen i juli-september-perioden ble antatt å skyldes den utviklingen av lavere fettinnhold som skjedde i HP-gruppen i juni-juli-perioden (Artikkel I). I den etterfølgende perioden, fra september til desember, hadde LP-gruppen betydelig høyere fôr-inntak. Samlet sett synes fôr-inntaket i løpet av den siste delen av året å være påvirket av både fettinnholdet i fisken før oppstart om høsten og protein /lipidbalansen i fôret.

Alle forsøkene viste at HP-fôret ble signifikant bedre omdannet til vekst av den sløyde laksen (Artikkel I, II, III). Dette fremkom gjennom signifikant lavere fôr-faktor basert på sløydvekt (FCR_{CW}) i HP-gruppen (Artikkel I, II) og betydelig forbedret utbytte i forhold til rundvekt (Artikkel I, II, III). Samtidig ble det observert bedre muskel-tykkelse i den fremre (hypaxial) del av buk-lappen

i HP-laks produsert i stor skala, mens både høyere fettinnhold i muskelen og høyere relativ innvollsvekt (VSI) ble registrert i LP-gruppene (Artikkel III). HP-diettene så derfor ut til å indusere mer effektiv vektøkning basert på muskelutvikling sammenlignet med LP-diettene, som produserte en fisk med høyere fettinnhold både rundt innvoller og i muskelvev. Kombinert med forbedret kondisjons-faktor i fisk som fikk HP-dietter, anses disse morfometriske egenskapene som positive for forbedret utbytte sammenlignet med dagens foretrukne LP-fôr-strategi i næringen (Artikkel III). En bedret fôr- til kropps-vekt konvertering kan anses som høyst fordelaktig med hensyn til både produksjonsytelse og teknologisk kvalitetsvurdering.

Den forbedrede fôr-konverteringen medførte betydelig bedre vekst i HP-gruppene (Artikkel I, II). Dette gjør det mulig for oppdrettere å høste fisk på et tidligere stadium og dermed redusere risikoen forbundet med å ha fisken i sjøen. På den annen side øker dette også muligheten til å produsere større fisk som vanligvis genererer en høyere pris (Artikkel IV). Likevel viste resultatene også at LP-strategien var like god som HP-strategien i de energikrevende periodene i vår/sommer-sesongen for postsmolt (Artikkel I) og i den kalde vinterperioden (Artikkel II). Dette innebærer at en samlet forbedring av oppdrettslaks-ytelsen krever en ernæringsmessig dynamisk tilnærming i lakseoppdrett hvor lipid-tettere dietter foretrekkes i vinterperioden. Med hensyn til å øke lipidinnholdet i løpet av vår/sommer for post-smolt må man imidlertid være noe mer forsiktig, da dette kan øke fettinnholdet i fisken til et uønsket høyt nivå gjennom sommeren, med den konsekvens at vekstytelsen i den etterfølgende høst reduseres.

Til tross for et generelt høyere prisnivå for en HP-strategi ble den forbedrede fôr-faktoren vist gjennom modellering å resultere i lavere total fôrkostnad (Artikkel IV). Imidlertid var LP-strategien økonomisk bedre i vinterperioden, noe som understreker behovet for en dynamisk tilnærming til valg av fôrstrategi med tanke på å forbedre produksjonssyklusen som helhet. I tråd med økte kostnader for lakseproduksjon har også verdien av en redusert produksjonsperiode økt. Dette er spesielt viktig ved høye laksepriser. Dermed representerer produksjonstid i modellen en mulighet for positivt utbytte ved å bruke en HP-strategi (Artikkel IV). Kombinasjonen av økte produksjons-omkostninger samt betydelig prisøkning på laks har mulighetene for bedret utbytte økt betydelig fra 2009 til 2016, og den positive «trade-off» verdien har vokst tilsvarende raskere enn den generelle økningen i fôr-prisene.

Som konklusjon viser funnene i denne oppgaven at en ernæringsmessig dynamisk fôringsstrategi, hovedsakelig basert på et HP-fôr, kan forbedre den generelle fôrfaktor, veksthastighet og også gi morfometriske gunstige egenskaper hos oppdrettslaks. Selv om fôr-prisene for en HP-strategi er høyere sammenlignet med en LP-fôringsstrategi, gir forbedret fôrkonvertering, raskere vekstrate og høyere avkastning på salgbart produkt en generell forbedret økonomisk ytelse gjennom bruk av en HP-strategi. Avhengig av at fôrindustrien har tilstrekkelige ingredienser med høyt proteininnhold til bruk i fôr-formuleringen, kan en HP-basert fôringsstrategi relativt enkelt bli implementert i lakse-industrien.

LIST OF PUBLICATIONS

The thesis is based on the articles listed below. Throughout the thesis, the text refers to these articles by their roman numerals.

Paper I

Dessen, J.-E., Weihe, R., Hatlen, B., Thomassen, M. S., Rørvik, K.-A. (2017) Different growth performance, lipid deposition, and nutrient utilization in in-season (S1) Atlantic salmon post-smolt fed isoenergetic diets differing in protein-to-lipid ratio. Aquaculture 473, 345-354.

Paper II

Weihe, R., Dessen, J.-E., Arge, R., Thomassen, M. S., Hatlen, B., Rørvik, K.-A. (2018) Improving production efficiency of farmed Atlantic salmon (Salmo salar L.) by isoenergetic diets with increased dietary protein-to-lipid ratio. Aquaculture Research 49, 1441-1453.

Paper III

Weihe, R., Dessen, J.-E., Arge, R., Thomassen, M. S., Hatlen, B., Rørvik, K.-A. (2018) Increased protein-to-lipid ratio in energy dense diets improves slaughter yields and muscle thickness of different weight classes of farmed Atlantic salmon (Salmo salar L.). Aquaculture Reports (accepted).

Paper IV

Weihe, R., Rørvik, K.-A., Thomassen, M. S., Asche, F. (2018) A model system to evaluate the economic performance of two dietary different feeding strategies in farmed Atlantic salmon (Salmo salar L.). Aquaculture (submitted).

1. INTRODUCTION

1.1 Atlantic salmon aquaculture

The global production of Atlantic salmon was 2.2 million tonnes in 2016, representing approximately 2 % of the global aquaculture production of plants and animals (FAO, 2018). Production of salmon started originally in the 19th century in United Kingdom as stocking of immature fish in freshwater to enhance the return of fully grown salmon for recreational fishing (Jones, 2004). Modern salmon farming was initiated in the 1970s and has developed into an efficient food production industry. During the last four decades the production process in the industry has improved rapidly. Pathogenic challenges have led to the development of vaccines together with continuous improvements in brood stock management, and challenges at the production facilities have led to improved technological solutions (Torrisen et al., 2011). Such challenges have functioned as drivers for improved production management, increased capabilities with regards to biological knowledge, evolvement and implementation of new production technologies and market development (Asche, 2008). Overall, these improvements have led to faster innovation rate and productivity growth for the salmon industry compared with the whole aquaculture sector (Asche and Bjørndal, 2011). With all respect to other aquaculture industries, salmon has been regarded as the global leading species (Asche, 2008).

Norway has been the industrial leader with the biggest salmon production, representing more than half of the global supply (FAO, 2018). Much of the industrial improvements with regards to biological knowledge, production technology and market development within the industry has been developed in Norway. Chile, Canada and United Kingdom (Scotland) are the next biggest producers after Norway, with just over a third of the global production of Atlantic salmon (FAO, 2018). The production in these four countries represents over 90 % of the total supply of farmed Atlantic salmon.

Fish are poikilotherms (ectotherms) which entails that their physiology, metabolism and feed intake is highly influenced by temperature (Jobling, 1997). Temperature tolerance in Atlantic salmon is influenced by fish size (Handeland et al., 2008) and there have been different propositions for what is the optimal temperature range for growth, from 6°C (Jones, 2004) up to 18°C (Johansson et al., 2009). According to Marine Harvest (2018), the world largest producer of Atlantic salmon, the optimal temperature for salmon is in the range of 8-14°C. Elliot and Elliot (2010) reported the critical temperatures for survival of Atlantic salmon as -0.8°C and 30-33°C. However, it is recommended to avoid temperatures below 6-7°C as this reduces growth and increases risk of winter ulcers, whereas temperatures above 17-18°C reduce appetite, growth performance, overall welfare and increase mortality (Noble et al., 2018). Thus, rearing salmon in conditions over 18°C is not a rational solution since feed intake is the precursor for converting feed to tood. Although temperature is the most pervasive environmental factor influencing salmon performance (Jobling, 1997), the overall production and welfare of salmon is influenced by a range of environmental factors (Oppedal et al., 2011; Noble et al. 2018). As a result of complex interactions between all these factors, there are relatively few feasible coastlines for traditional sea-based farming on a global scale (Marine Harvest, 2018). Thus, future growth in the industry seems to be dependent on better utilization of currently used areas unless new technology allows farming in more exposed conditions. However, an alternative approach in the industry is to invest in larger freshwater facilities on land (Jacobsen and Nielsen, 2016) to prolong the salmon production in freshwater and shorten the seawater phase which enables a higher productivity per site. During the last couple of years, the industry has seen an increase of smolt weights (Marthinussen, 2017).

1.2 Salmon culture in the Faroe Islands

Following the development in the salmon aquaculture industry, the production of salmon in the Faroe Islands started in the 1980s. Today, salmon farming in the Faroe Islands represents nearly 4 % of the global salmon production (FAO, 2018). The Faroese archipelago has a very limited coastline of 1,117 km, and the country stretches 75 km from east to west and 110 km from north to south. In comparison, the coastlines of Norway, Chile and Scotland are 25,000 km, 6,435 km and 9,910 km, respectively. The temperature in the Faroese fjords is primarily influenced by the flow of water coming from the North Atlantic Current which gives a relative stable sea temperature on the Faroe Shelf between ~5.5°C and ~11°C throughout the year (Hansen, 2000).

Based on the traditional farming with on-growing sites in sea, it is obvious that the Faroese salmon industry is very limited with regards to the overall production potential compared with the competing salmon producing countries. Nevertheless, in relative terms, the salmon industry in the Faroe Islands probably has the greatest influence on the national economics compared to all other salmon producing countries. Historically, the Faroese economy has relied heavily on the fishing industry for foreign trade, and wild caught fish has represented the majority of the Faroese export value. The introduction of salmon farming added a new tradeable product in the Faroese economy. Together, the combination of wild caught fish products and aquaculture products represents approximately 95 % of the current Faroese export value (Statistics Faroe Islands, 2018). The economic importance of aquaculture has grown steadily throughout the millennium, and salmon products represent nearly half of the overall export value of fish in 2017 (Figure 1). Thus, an optimal production in the Faroese salmon industry is not only important for the salmon producers, it is also crucial for the Faroese economy as a whole.

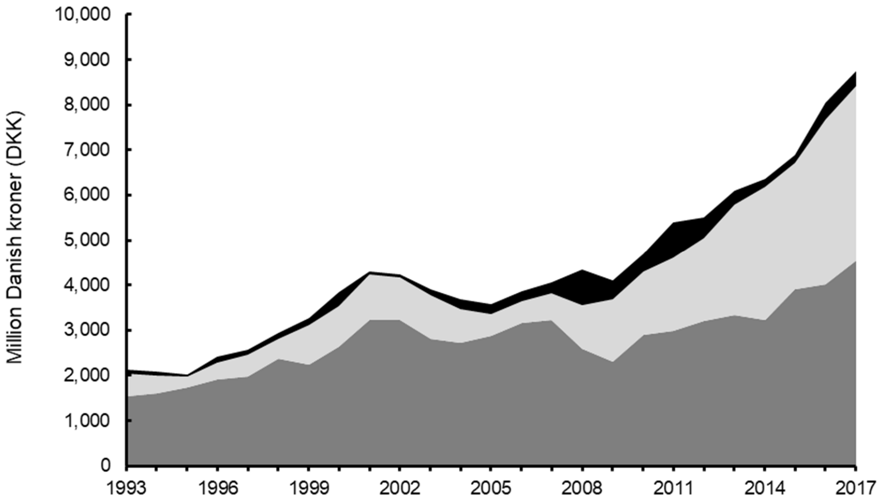

Figure 1 Export value in the Faroe Islands divided into three main groups of products/services: Fisheries (dark grey), Aquaculture (light grey) and all others (black). Values are given in Danish currency (million DKK).

1.3 Development in salmon feeds

Atlantic salmon is a carnivores species which entails that salmon has a demand for a balanced protein and lipid diet (National Research Council, 2011). This reflects wild salmon in their natural habitat where they predominately feed on crustaceans and small pelagic fish during the marine phase of their life (Huntingford et al., 2012). Consequently, ingredients rich in protein and/or lipid have been the main nutrients in commercial salmon feeds.

Fish consume proteins which are digested and hydrolyzed to free amino acids (AA) that are distributed to the various body tissues (Wilson, 2002). Twenty AAs serve as the building blocks in protein and are called protein AAs (Wu, 2013). Fish can synthesize ten protein AAs, whereas the remaining ten protein AAs cannot be synthesized (Wilson, 2002; National Research Council, 2011). Thus, these essential protein AAs need to be supplied in the diet. The net amount of body protein (protein deposition) is the result of a continuous process between protein synthesis (anabolism) and protein degradation (catabolism) (Dabrowski and Guderley, 2002; Webster and Lim, 2002). Proteins are essential components for all types of cells in the body and since salmon continue growing throughout their lives (Kiessling et al., 2006), they have a dietary protein requirement which exceeds the amount needed for maintenance only, to ensure good growth and subsequently an overall good production performance for the salmon farmer.

Lipids are a diverse group of molecules with a wide variety in structure and biological functions, but all share the common property of hydrophobicity. One of their primary roles in cellular function is to form the lipid bilayer permeability barrier of both cells and organelles (Dowhan et al., 2008). These lipids are mainly polar with a non-lipid head group (Bell and Koppe, 2010), typically in the structure of phospholipids (Tocher et al., 2008). Salmon need sufficient amount of polar lipids to ensure well-functioning cells, however, the major lipids in salmon feeds are neutral lipids in the form of triacylglycerides (TAGs). When consumed, TAGs are digested in salmon and mainly broken down into monoacylglyceridols (MAGs), free FAs (FFAs) and glycerol. Once inside the cell, the FAs are reesterified with glycerol to form TAGs.

FAs consist of carbon atoms with various chain lengths. In feed oils, virtually all the lipids are neutral whereof more than 70 % are TAGs (Tocher et al., 2008). The majority of fatty acids in typical feed oils (Turchini et al., 2011) contain more than 14 carbons (Dubois et al., 2007; National Research Council, 2011). Oils are 67 % more efficient energy carriers in salmon feed compared to protein, as the gross energy of these macronutrients is 39.5 MJ kg^{-1} and 23.6 MJ kg^{-1}, respectively.

A retrospective of the feed development in the Norwegian industry (Figure 2) depicts how the dietary energy has continuously increased since salmon culture started. In the beginning, the farming pioneers mainly fed salmon with raw fish. Subsequently through the 1980s, semi-moist and/or pelleted feed were predominantly used. The protein- and lipid ranges in these early diets were approximately 40-50 % and 10-20 %, respectively, with an energy content below 20 MJ kg^{-1}. Feed extrusion and vacuum coating technologies were introduced in the 1980s and 1990s. This technology is still used today, and it has enabled feed manufacturers to produce high-fat diets. Since the implementation of feed extrusion and vacuum coating, feeds for salmon have continuously become energy denser with an increased content of lipid accompanied with a subsequent reduction in protein. Earlier feed experiments have displayed successful growth performance with the use of high-fat energy dense diets (Hillestad et al., 1994; Hillestad et al., 1998; Karalazos et al., 2007; Karalazos et al., 2011), and these diets have become the preferred feeds in commercial salmon farming.

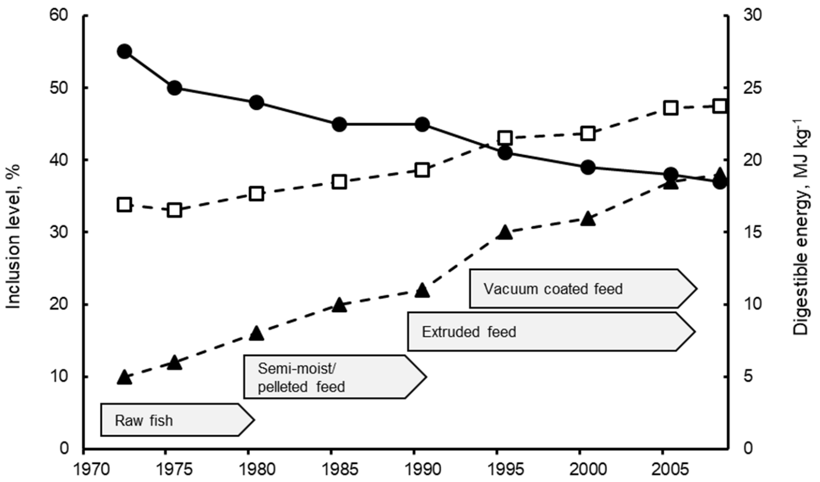

Figure 2 Historical feed development in the Norwegian salmon industry with regards to the proportion of protein (closed circles, y-axis) and lipids (closed triangles, y-axis) in the feed, and changes in digestible energy (open squares, z-axis). Milestones in feed technology are highlighted with arrow boxes (adapted from Tacon and Metian, 2009 and Torrisen et al., 2011).

As a whole, the aquaculture sector has been a growing consumer of fishmeal and fish oil, especially feeds for salmonids have relied heavily on the use of fishmeal and fish oil (Shepherd and Jackson, 2013). In the 1990s, the salmon industry was more dependent on fishmeal rather than fish oil (Tacon and Metian, 2008). This is a period where the grower diets for salmon relied more on proteins compared to the modern diets (Figure 2). However, this marine raw material dependency represented a growth restriction in the industry since the global aquaculture continuously grew (FAO, 2016) whereas the production of fishmeal and -oil production did not (Shepherd and Jackson, 2013). Thus, in the 1990s the feed manufacturers started to partially replace fishmeal proteins with plant protein alternatives, whereas substitution of fish oil started after 2000. Aas et al. (2018) depicts how fishmeal and fish oil in the Norwegian feed industry gradually has been replaced with plant proteins and -oils. In 2016, fishmeal and fish oil represented 14.5 % and 10.4 %, respectively, of the overall raw material inclusion (Aas et al., 2018). Nonetheless, the overall fishmeal inclusion in the Norwegian industry increased in 2017 coinciding with a reduction in fishmeal prices, the lowest since 2010 (Tarlebø, 2018). Thus, the use of fishmeal seems to be highly influenced by price, and not only availability.

In the Norwegian industry, soy protein concentrate is the main protein replacer of fishmeal followed by wheat gluten. Thereafter, sunflower, pea protein concentrate, corn gluten and faba beans have also been used as protein sources (Ytrestøyl et al., 2014; Ytrestøyl et al., 2015). Crude protein content in fishmeal ranges from 62-72 %, depending on the fish source (National Research Council, 2011). In the Nordic fishmeal producing countries which utilize species such as Blue whiting (*Micromesistius poutassou*), Sand eels (*Ammodytes spp*.) and European sprat (*Sprattus sprattus*) (EU Fishmeal, 2018), Norse-LT fishmeal containing ~71 % protein is generally considered the highest quality product and consequently highest priced (Holtermann, 2018; Nordsildmel, 2018). Except for wheat gluten which contains ~80 % protein (National Research Council, 2011), the plant proteins are not as protein dense as fishmeal. In addition, most plant proteins are generally deficient in either lysine or methionine or both (Gatlin et al., 2007). Thus, there seems not to be a "one-to-one" replacement of fishmeal with plant alternatives, and the diets need to be supplemented with crystalline AAs to adjust the protein AA balance. Salmon are evolutionary not adapted to a diet containing plant sources and are easily influenced by anti-nutritional factors (ANF) in plants (Cheeke, 1998; Francis et al., 2001; Jobling et al., 2001). These are chemical compounds that act as the plants' defense mechanisms from being consumed by other organisms. In addition, plants are generally rich in non-starch polysaccharides (NSP) that are indigestible for salmon and the plant energy cannot be utilized for growth and maintenance (Sinha et al., 2011). Also, the undesirable ANFs might potentially cause palatability problems and consequently reduce feed intake (Jobling et al., 2001). Nonetheless, increased treatments of plant proteins with for example heat and extraction methods have led to plant protein concentrates and isolates which excludes some of the ANFs as well as increasing protein concentration and protein digestibility. Soy-protein-concentrate and wheat gluten are examples of such plant products, and these represent the most frequent used plant proteins in salmon feeds (Ytrestøyl et al., 2014; Ytrestøyl et al., 2015).

Rapeseed oil is the most frequently used oil in salmon diets (Ytrestøyl et al., 2014; Ytrestøyl et al., 2015; Marine Harvest, 2018). Rapeseed oil has been frequently tested in various feeding experiments without detrimental effects on growth or feed conversion, the very first already in 1989 (Thomassen and Rosjø, 1989); in fact, some results report of improved biometric performance (Turchini and Mailer, 2011; Glencross and Turchini, 2011). As a result of several successful oil substitution trials, fish oil has been frequently replaced with rapeseed oil in the Norwegian industry. In 2010, the overall weighted oil inclusion in salmon feed in the Norwegian industry was ~29 % whereof ~43 % originated from plants. These numbers increased to ~31 % and ~66 %, respectively, in 2016 Aas et al., (2018).

Compared with plant oils, marine oils are rich in long-chain omega-3 polyunsaturated fatty acids (LC PUFAs), *eicosapentaeonic* acid (EPA, 20:5 n-3) and *docosahexaeonic* acid (DHA, 22:6 n-3). These omega-3 fatty acids are known to have positive effect on human health as they may reduce the risk of cardiovascular disease (CVD) (Kris-Etherton et al., 2002). Based on the convincing inverse relationship between consumption of EPA+DHA and decreased risk of CVD, both national and international bodies have established recommendations of daily EPA+DHA intake (GOED, 2018). Although the recommendations are not uniform and based on different criteria, The World Health Organization recommends the general population that 1-2 % of the daily energy intake should come from LC PUFAs (WHO, 2003), which is equivalent to ~500 mg per day and in line with GOED (2018) recommendations. The fatty acid profile in salmon reflects that of the feed (Thomassen and Rosjø, 1989; Torstensen et al., 2000; Bell et al., 2001; Bell et al., 2002; Bell et al., 2003; Bell et al., 2004; Torstensen et al., 2005; Stubhaug et al., 2007). Thus, the fatty acid profile in modern farmed salmon resembles the fatty acid profile in a feed oil blend which contains 70 % rapeseed oil and 30 % fish. To improve the nutritional product quality in salmon fed feed rich in plant oil, LC PUFAs may partially be restored by providing the salmon a finishing diet rich in fish oil during a period prior to harvest (Bell et al., 2003; Bell et al., 2004). However, this is generally not practiced within the industry, and despite the reduction of LC PUFAs in salmon fillet due the use of plant oils, salmon is still considered a healthy food alternative and can contribute to achieve the recommendation of daily EPA+DHA intake (Jensen et al., 2012). Thus, the substitution of marine oils with plant oils has not been a hinder of increased salmon production. On the contrary, the relative high price increase of salmon since 2012 (FishPool, 2018) may indicate that the industry has not been sufficiently able to meet the overall market demand for salmon.

1.4 Characteristics of the salmon farming competition

Initially, and throughout the 1980s, farmed salmon was supplied to high-end markets as a luxury product. However, increased productivity onwards to the millennium, led to more efficient production, growth with increased supply and consequently a reduction in price (Asche, 2008; Kumar and Eagle, 2016). This development characterizes an industry which has a focus on increasing production volume to achieve scale advantages (Asche and Bjørndal, 2011). Such an industrial competition typically results with a standard commodity where increased margins are achieved through cost reductions (Porter, 1980). Thus, the main product in the industry has been fresh head-on gutted salmon (HOG). Feed represents approximately 50 % of the total cost of production (Asche and

Bjørndal, 2011), and replacing dietary protein with higher oil inclusions has allowed the industry to attain cheaper high energy diets. Reduction in production costs together with increased supply lead to cheaper products and repositioned salmon to become affordable for more market segments as a competitive source of protein compared to other animal proteins (Tveteras et al., 2012).

Although salmon farming only is ~40 years old, the industry has evolved quickly and developed into a modern intensive food production system (Asche et al., 2018a). The intensive production of salmon in a controlled production process has allowed systematic knowledge gathering and improvements within several factors that influence the overall productivity (Asche, 2008; Asche and Bjørndal, 2011) such as enhanced biological knowledge and technological adaptations. In addition, improved cost performance has also been achieved in the industry due to increased consolidations through mergers and acquisitions (Asche et al., 2013). Thus, there are fewer but larger salmon companies competing in the industry. In a supplier-customer relationship, the consolidation of companies alters the bargaining powers from supplier to customer (Porter, 1980), in this case, the bargaining power shifts from feed suppliers to salmon farmers. With a historical focus on cost, increased bargaining power from salmon farmers has probably been a driver behind the feed development, which has seen the shift of increased fat content associated with the reduction of proteins combined with a shift towards cheaper proteins and lipids instead of the traditional fishmeal and fish oil. Consequently, this development reduced feed prices based on the price per unit of dietary energy, with the perception that this protein-sparing effect will lead to overall improved economic performance.

2. OBJECTIVES

The objectives of this thesis was to investigate the influence of isoenergetic diets differing in protein-to-lipid ratio on farmed Atlantic salmon with focus on:

- biometric production performance,
- quality attributes considered valuable for further processing yields and,
- an economic evaluation of the dietary feeding strategies,

in both large-scale production environment as well as in controlled small-scale facilities to ensure commercial relevance.

Based on the aims, the hypothesis were:

- Isoenergetic high protein-to-lipid diets contribute with substrates for more efficient feed conversion and improves growth compared to low protein-to-lipid diets (Paper I, II)
- Isoenergetic low protein-to-lipid diets increase fat content in the fish compared to high protein-to-lipid diets (Paper I, III)
- Isoenergetic high protein-to-lipid diets compared to low protein-to-lipid diets increase production yield and inflict body shape characteristics which are beneficial for further product processing (Paper III)
- Despite dietary proteins being higher priced than dietary lipids, the dietary low protein strategy with the lowest feed price does necessarily not lead to the best overall economical performance (Paper IV)

3. EXPERIMENTAL OVERVIEW AND METHODOLOGICAL CONSIDERATIONS

Results from four feed trials are included in this thesis, two large-scale commercial trials conducted in the Faroe Islands and two small-scale trials conducted on the west coast of Norway. Figure 3 gives an overview of the trial locations, experimental fish and rearing facilities, number of replicates per dietary treatment, seawater temperatures, feed management and trial duration. The overview also depicts in which papers the data from the trials has been used. Although the sea temperature in Norway vary greatly from the north to the south (Barentswatch, 2018), the temperature regime in mid Norway is most similar to the regime in Faroe Islands. Thus, comparing experimental data from the locations in the Faroe Islands with the data in Norway was regarded as highly applicable.

3.1 Measurement of feed intake

The present results are based on experiments conducted in both commercial large-scale and small-scale facilities (Figure 3). In small-scale feed experiments, we are able to overfeed the fish followed up with a subsequent feed collection and quantify feed spoilage to precisely calculate feed intake (Helland et al., 1996; Einen et al., 1999). This method is not applied in commercial scale, and therefore we need to assume that the daily quantities of feed supplied to the experimental net-pens are all consumed. This entails that there are risks of both over-feeding as well as under-feeding of the fish, however, this risk is equal for both dietary treatments, and there is no reason to consider the net-pens being treated differently. Since feed represents nearly half of the overall cost of commercial salmon production (Asche and Bjørndal, 2011), over-feeding is highly avoided, instead, feeding routines are managed thoroughly.

3.2 Number of replicates

In the small-scale trials, three (Paper II, III) and four (Paper I) replicates per dietary treatment were used to investigate the influence on biological responses (Figure 3). Three replicates are typically used in studies of feed responses, and some scientific journals do not accept manuscripts based on results from less than three replicates. Basing triplicate cages per dietary treatment will improve the statistical reliability. However, demanding triplicates to be used in large-scale trials will likely reduce the number of feeding experiment being conducted in large commercial conditions.

Thus, due to availability, practical and economical concerns two replicates were used in the large-scale trials.

3.3 Environmental differences in research facilities

In the large-scale trials, fish cages with a circumference of 128 m were used, whereas 5 m x 5 m x 5 m research cages were used in the small-scale trial (Figure 3). Such fundamental differences in rearing facilities are considered to generate different behaviors (Huntingford et al., 2012). However, the repetition of dietary induced differences with regards to feed conversion and growth, demonstrates that such results are reproducible and transferable between commercial scale-trials and small-scale research experiments. This also supports the use of duplicates in large-scale. Nevertheless, there were relative great morphometric differences in the experimental fish between the large-scale and small-scale trials, and the higher condition factors and fat content in the small-scale experimental fish is likely due to smaller rearing conditions which limits the swimming area for the fish in combination with the daily overfeeding. Because of such differences in production environment between small-scale and large-scale condition, this work underlines the importance of conducting feed experiments in large-scale and not only in small-scale so that the results can be become applicable for the industry.

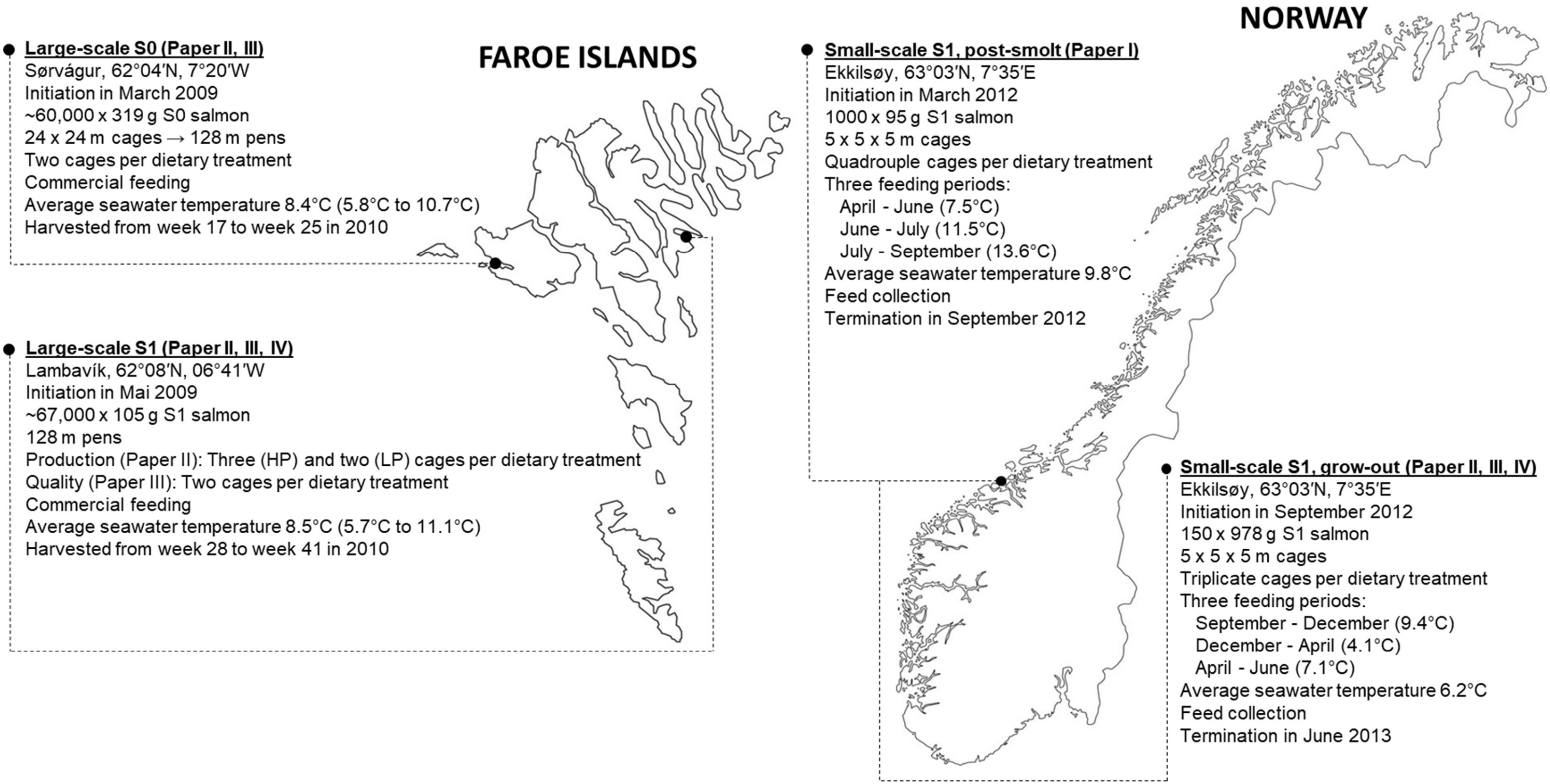

Figure 3 Maps of the Faroe Islands and Norway which specifies the locations of feed trials and corresponding information about experimental fish, trial duration, rearing conditions and feeding at the respective sites.

4. MAIN RESULTS AND DISCUSSION

4.1 Production – Key performance indicators

Studies investigating dietary balance of protein and lipids on feed utilization and growth performance in farmed Atlantic salmon have to a certain degree been replaced by research investigating the dietary effects of using alternative protein and lipid sources to fishmeal and fish oil (se for example Storebakken et al., 1998; Carter and Hauler, 2000; Espe et al., 2006; Torstensen et al., 2008; Øverland et al., 2009; Pratoomyot et al., 2010; Turchini et al., 2011). Since the earlier studies investigating dietary protein/lipid balances (Hillestad et al., 1994; Einen and Roem, 1997; Hillestad et al., 1998), breeding programs of salmon have been continuously ongoing (Gjedrem, 2010; Janssen et al., 2018). These programs have improved the salmon genetics whereof improved growth potential has been a key genetic marker. Therefore, the fish material used in recent experiments are based on improved genetic fish material compared to studies conducted up to twenty years ago.

Taken together in the commercial-scale trials, the HP group had virtually better feed utilization in converting feed to somatic body weight (FCR_{BW}: P = 0.06, Paper II). In addition, the HP feed was more efficiently converted in to carcass weight which resulted in significantly (P = 0.03) lower FCR_{CW}. The HP feed also induced significantly faster growth rate, both measured as whole-body weight (TGC_{BW}: P = 0.04) and carcass weight (TGC_{CW}: P = 0.02). As well as being the main building blocks in muscle tissue (Wu, 2013), amino acids may also function as appetite enhancers in several fish species (Li et al., 2009). However, because there were no differences in feed intake in the large-scale trials (Paper II), the improved production performance of the HP group was induced by better protein-to-lipid balance in the feed with a higher protein deposition and not higher appetite stimulation due to feed composition. Nevertheless, the large-scale trials were not broken down into shorter feeding periods, and it was therefore not possible to determine whether there may have been periodic feed intake differences. In the post-smolt trial (Paper I), differences in feed intake were not observed from March to July, where the fish grew from 95 g to ~280 g. Nor were differences observed in FCR and TGC in this period. The spring/early summer period is known as an energy demanding period for S1 salmon which is reflected in relative low relative retention of dietary fat (~20-30 %) and energy (~30-40 %) (Alne et al., 2011). This was also depicted in Paper I where both groups displayed a numerical drop in muscle fat from April to June (Figure 6). In the subsequent summer period from June to July, the groups maintained equal feed intake, feed conversion and TGC. The absolute retention of both lipid and energy was higher in the LP group in the JUN-JUL period which ultimately led to a significantly higher muscle fat content compared to the HP group (Figure 6). In the following early autumn period from July to September, the HP group had a significantly higher feed intake than the LP group. This result concurs with the hypothesis of a lipostatic regulation (Kennedy, 1953; Keesey and Corbett, 1984; Schwarts and Seeley, 1997; Jobling and Johannesen, 1999) which suggests that the amount of stored fat regulates the overall intake of energy to a certain homeostatic condition. The higher feed intake for the HP group led to significantly higher TGC and final body weight, and there was an overall linear relationship between the initial muscle fat status in July and final TGC in September ($P = 0.02$, $R^2 = 0.61$). This relationship corresponds with results reported for salmon by Rørvik et al. (2018) which demonstrated an inverse relationship between muscle fat status in the late summer and feed intake in the following autumn period. In the second small-scale trial, the LP group had a significantly higher feed intake than the HP group in the late autumn period from September to December (Paper II). Thus, based on the periodic differences in feed intake observed in the small-scale trials, it is therefore reasonable to assume that there might have been some differences in the large-scale trials although not depicted for the overall experimental period.

Results from the small-scale trials (Paper I, II) highlight that the latter part of the year with declining daylength (i.e. from summer to December), is a period of high feed intake and good growth. This corresponds with earlier studies (Alne et al., 2011; Oehme et al., 2010; Mørkøre and Rørvik, 2001), and the presented results of nutrient retention repeated that the second half of the year is a period with high retention of dietary fat (~70-80%) and -energy (~50-60 %), which seemed to remain relative stable within the dietary treatments throughout July to December (Paper I, II, Figure 4). However, in the late autumn (SEP-DEC), the HP group maintained a significantly higher relative lipid retention despite that the fish material was identical at trial initiation of the second-small scale trial (Paper II). Thus, the higher feed intake in the LP group in this period could not be a result of lipostatic response as indicated in the previous period (JUL-SEP) for the HP group. The increased feed intake in SEP-DEC did not induce a faster growth for the LP fed fish; instead the increased feed intake resulted in significantly higher FCR_{BW} (Paper II).

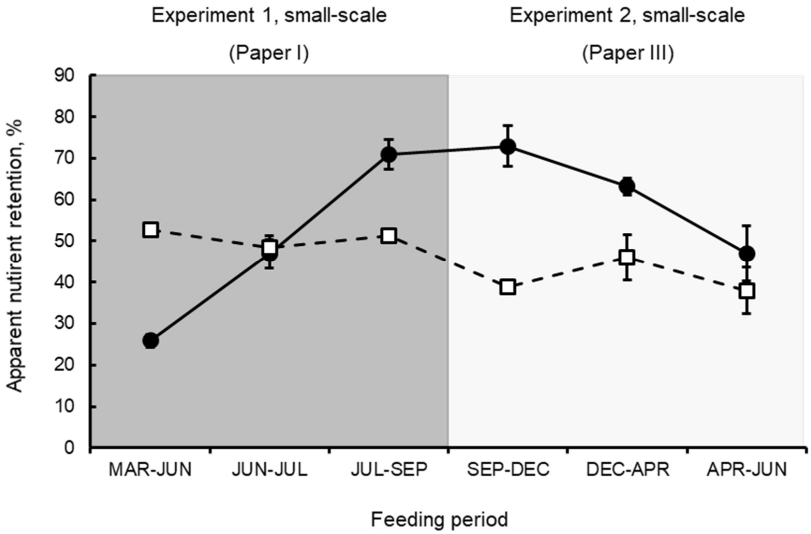

Figure 4 Mean apparent retention of protein (open squares) and lipid (closed circles) of all dietary treatments within feeding periods in the first (dark shade, Paper I) and second (light shade, Paper II) small-scale trials.

In contrast to the feed intake in the early autumn, increased feed intake in the LP group in the late autumn might be a response to insufficient protein content in the feed which consequently increases FCR_{BW} and impairs growth (Wilson, 2002). Nevertheless, common for both small-scale trials was that the LP group had significantly higher absolute retention of dietary lipids in the energy demanding periods: June-July in the post-smolt stage (Paper I) as well as in the cold winter period from December to April during the grow-out stage (Paper II). Apart from the autumn periods JUL-SEP and SEP-DEC, respectively, the dietary groups grew equally well with similar TGC (Paper I, II). However, these two autumn periods had the biggest influence on the overall growth performance in the small-scale trials when the overall weighted TGC was calculated based on periodic performances. Hence, an HP diet is most efficiently converted and utilized for growth during the second half of the year. Overall, the mean relative protein retention for both treatments in all feeding periods was much more stable compared to the periodic variation of lipid retention (Figure 4). Thus, the variation of periodic dietary energy retention in the treatments was explained by the differences in lipid retention. Figure 5 depicts that there was on overall positive linear relationship between absolute lipid retention and growth rates in the small-scale trials (Paper I, II).

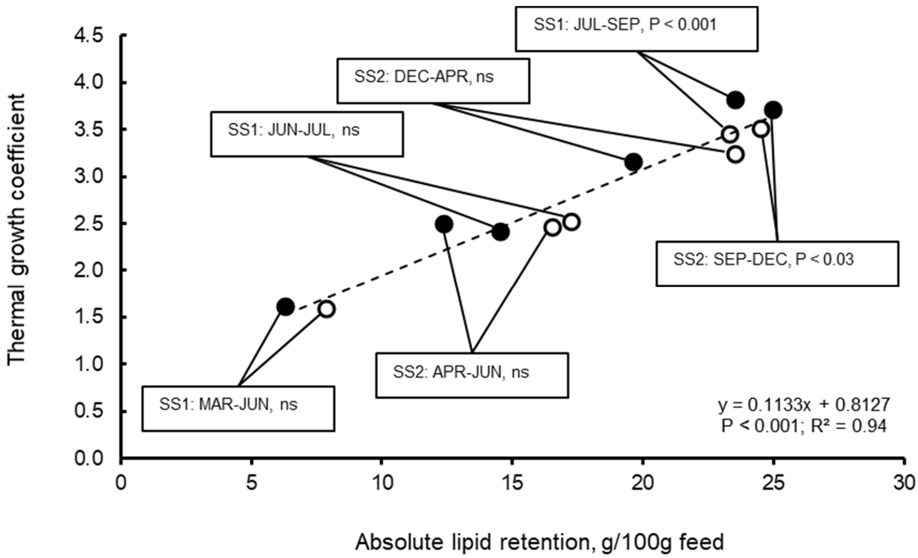

Figure 5 Relationship between thermal growth coefficient (TGC) in relation to the absolute lipid retention in salmon in both small-scale trials fed isoenergetic high dietary protein-to-lipid ratio (HP: closed circles) and low dietary protein-to-lipid ratio (LP: open circles). Differences in TGC within periods are highlighted with P-values, whereas non-significance is abbreviated as ns.

Based on the observations in the small-scale trials, by increasing dietary fat content in a dietary HP feeding strategy and therefore reducing the dietary protein-to-lipid ratio in in the cold winter period may further improve growth performance. However, increasing dietary fat content in the first spring period may not be equally beneficial, in case this increases body fat with the potential consequence of poorer feed intake and -growth response during the following autumn. It is therefore concluded, that a dietary feed strategy which prevents too high accumulation of muscle fat during the months before the autumn will induce a positive feed intake response in the autumn. Furthermore, in combination with an energy dense diet with high protein-to-lipid ratio, the high feed intake will increase protein deposition in carcass and improve both feed conversion and growth.

4.2 Quality

Quality is multifaceted which entails that quality preferences will differ in the "eye of the beholder". Nortvedt et al. (2007) arranged product quality into five categories: sensory, nutritional, microbiological, technological and ethical quality. How well salmon as raw material is fit for further processing is categorized as technological quality, and the present work has focused on the dietary influence on the intrinsic attributes of farmed salmon which are considered important for the fish farmer and fillet processing, i.e. the first two intermediates in the supply chain of salmon. First, the most predominant feature was the positive influence of the HP diets on slaughter yield, which in all four experiments was significantly higher in the HP fed fish compared with dietary LP group at trial terminations (Papers I, III). Thus, the HP diets induced higher carcass weights in relation to whole body weight. This is a positive quality trait (Rasmussen, 2001) which entails an increased tradeable raw material for the farmers. Improved carcass weight relative to body weight is also an important production parameter which is reflected in the improved FCRs when measured as FCR_{CW} instead of FCR_{BW} (Paper I, II).

In the small-scale trials, significant differences in slaughter yield were not observed until the end of the trials. However, the differences in slaughter yield developed immediately in the first feeding periods which continued throughout the trials (Figure 6, Paper I, III). This development coincided with increased VSI in the LP group (Figure 6, Paper I, III). High-fat diets have been used in salmon production with the risk of increased lipid deposition (Hillestad and Johnsen, 1994; Jobling, 2001; Refstie et al., 2001; Jobling et al., 2002), and this was clearly visualized in the small-scale trials where VSI in the LP group was consequently higher than in the HP group. The sum of body lipid and body moisture is typically 80 %, thus, increased amount of body lipid will consequently negatively correlate with reduced body moisture (Jobling, 2001). Muscle fat may be regarded as a positive quality attribute for the smoking process of salmon (Rørå et al., 1998; Mørkøre et al., 2001), however, increased levels of muscle fat may also increase the degree of fillet trimming (Rørå et al., 1998). The belly flap has the highest fat content of the whole fillet section (Einen et al., 1998) and this part of the salmon is cut/trimmed of during fillet processing (Norwegian Standard, 1996). Increased fillet fat overall might lead to bigger belly flaps and subsequently relative higher degree of trimming of this section, which may be a potential explanation for the observations of Rørå et al., (1998). Because the 2.4 kg weight class in the second small-scale trial (Paper III) had a very poor overall weight gain, likely caused by a poor feed intake, these fish were probably not representative for the feed effects in either of the dietary treatments. Consequently, when this weight class was excluded from the quality analysis, the dietary LP group displayed significantly higher muscle fat in all three trials at harvest (Paper III). Thus, an LP feed strategy increased lipid storage in both viscera and muscle which ultimately reduce slaughter yield and is considered to represent an increased risk of reduced fillet yield.

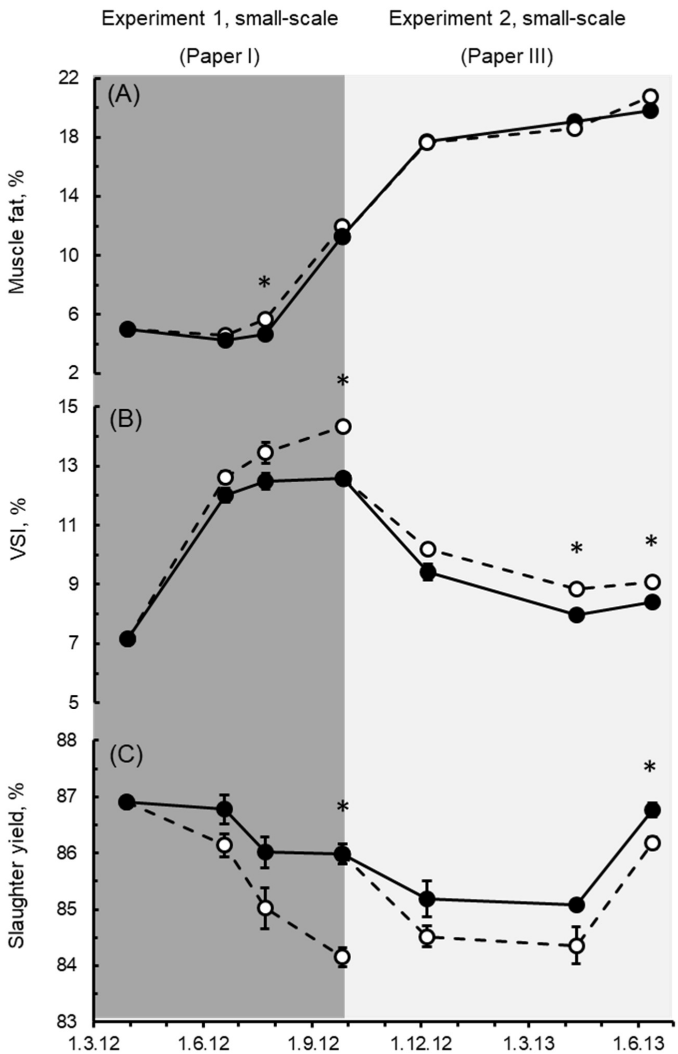

Figure 6 Development in muscle fat (A), viscerosomatic index (VSI, B) and slaughter yield (C) in salmon fed feed with high dietary protein-to-lipid (HP: closed circles, solid line) and low dietary protein-to-lipid ratio (LP: open circles, broken line) in the first (dark shade, Paper I) and second (light shade, Paper II) small-scale trials. Astertisks denote significant differences ($P < 0.05$) between dietary treatments within period.

4.3 Economic evaluation

Deriving dietary energy from protein sourced feed ingredients has generally been more expensive compared to dietary energy coming from oil sources. Thus, aiming to spare dietary protein energy in the conversion process from feed to salmon is therefore considered a rational economic solution. As described in the introduction, the dietary energy development in the industry depicts that this protein sparing effect has been achieved in the industry and high fat diets are generally preferred as grow-out diets for salmon. This solution is supported by studies of isoenergetic diets that have found an LP based feed strategy to be equally efficient as an HP based feed strategy with regards to fish performance (Karalazos et al., 2007; Karalazos et al., 2008). As expected, the protein denser HP diets in all present experiments resulted in higher feed prices compared to high-fat LP diets (Paper IV). However, the improved biological performance of the HP groups in all three trials using S1 smolts (Paper I, II) resulted in overall improved economic performance (Paper IV). The economic performance was possible to measure periodically in the small-scale trials as these were divided into shorter feeding periods. In every feeding period, it was clearly demonstrated that the HP diets were the more expensive than the LP diets (Paper IV, Figure 7), and the overall difference in weighted prices (FC_P) were USD 0.034 kg^{-1} and USD 0.111 kg^{-1} in the first and second small-scale trials, respectively (Paper IV). However, when including the feed conversion efficiency based on whole body weight ($FC_{P\ BW}$), the dietary induced HP improvements reduced the overall difference in feed cost. If the results from both small-scale trials are put together, the HP feed strategy was USD 0.111 kg^{-1} more expensive (FC_P) than the LP strategy, but economic performance was USD 0.03 kg^{-1} better when evaluated as $FC_{P\ BW}$ (Figure 7). Nevertheless, the LP feed strategy had better economic performance ($FC_{P\ BW}$) in the JUN-JUL and DEC-APR periods in the first and second trial, respectively. These periods have previously been depicted as energy demanding which concurred with periods of higher absolute lipid retention for the dietary LP group (Paper I, II). Thus, a dietary HP strategy is necessarily not always the best feed strategy for the economical performance, and the presented trade-off for the improved cost performance in the HP strategy might not be valid throughout a whole production cycle (Paper IV).

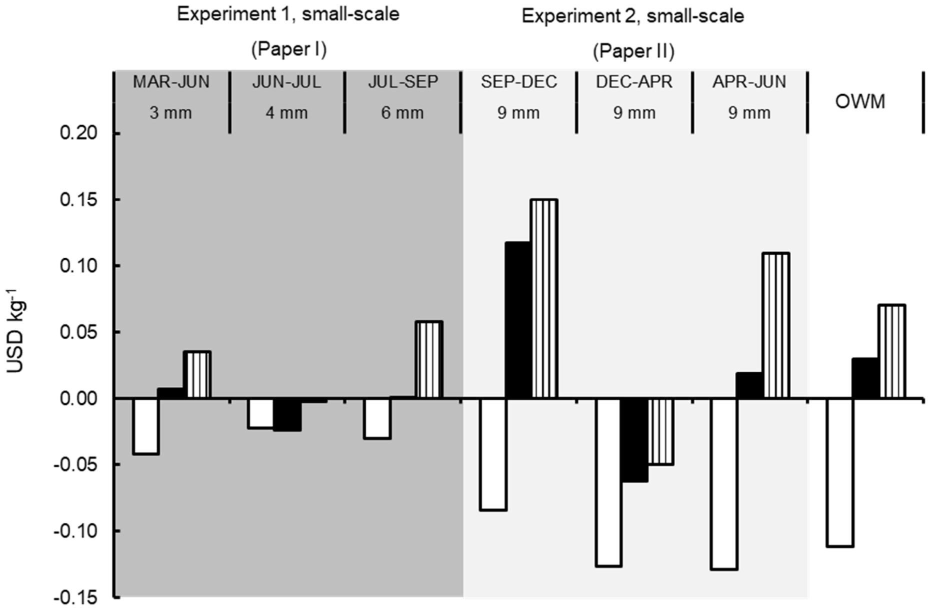

Figure 7 Differences in direct feed cost development in S1 salmon in the first (dark shade) and second (light shade) small-scale experiments, using a dietary high protein-to-lipid feed strategy (HP) and a low protein-to-lipid feed strategy (LP). Negative and positive numbers represent a higher cost and lower cost, respectively, for the HP feed strategy (Paper IV). Difference in feed price (FC_P: white bars), difference in feed cost assessed after including the whole-body weight-based feed conversion ratio ($FC_{P\ BW}$: black bars), difference in feed cost assessed after including the carcass weight (head-on-gutted, HOG) based feed conversion ratio ($FC_{P\ CW}$: vertical striped bars), OWM: overall weighted mean of both trials.

The model depicts the difference between two feeding strategies and not the exact cost of a certain strategy. This is because feed raw material prices can display great fluctuations (Dahl and Oglend, 2014), and reproducing a certain feed price and subsequent feed cost development is virtually not possible. Nevertheless, since the carcass weight represents the primary tradeable product and such the primary source for income, the model was modified to estimate differences in feed cost based on the feed to carcass conversion ($FC_{P\ CW}$). Because the HP strategy displayed more efficient feed to carcass conversion in all trials (Paper I, II, III) the differences in $FC_{P\ CW}$ were USD 0.039 kg^{-1} and USD 0.07 kg^{-1} better for the HP group in the first and second small-scale trial, respectively (Paper IV). This led to an overall weighted mean of improved $FC_{P\ CW}$ USD 0.07 kg^{-1} for the HP strategy in both small-scale trials (Figure 8).

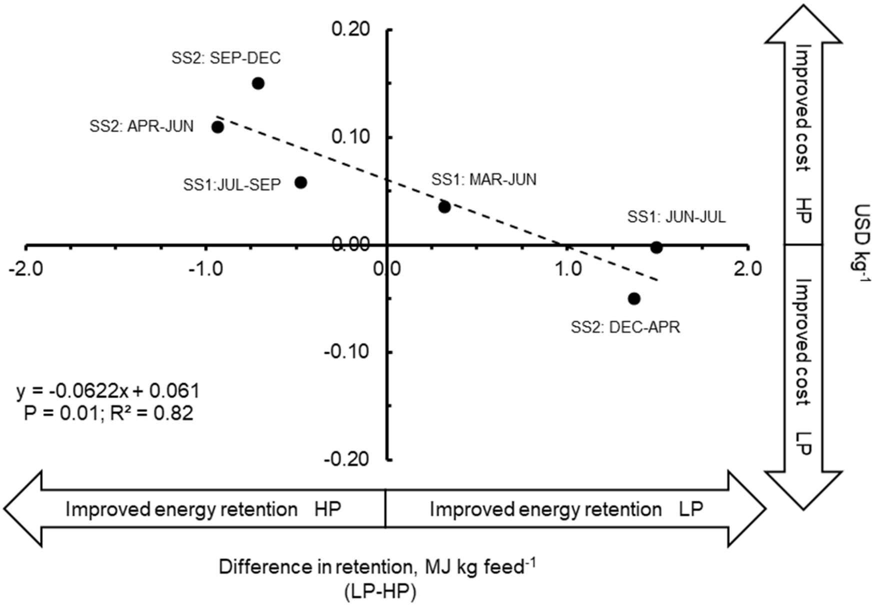

Figure 8 Overall relationship in both small-scale trials between the differences in the dietary treatments with regards to the periodic differences in absolute retention of dietary energy and periodic differences in feed cost performance assessed in carcass weight ($FC_{P\,CW}$). Improved energy retention and economic performance in the group fed dietary high protein-to-lipid (HP) is depicted on the negative x-axis and positive y-axis, respectively, whereas the improvements in the group fed dietary low protein-to-lipid (LP) is depicted on the positive x-axis and negative y-axis, respectively. The small-scale trial and corresponding feeding period is depicted for each observation. SS1: first small-scale trial, SS2: second small-scale trial.

Taken the economic evaluation of the small-scale trials as a whole, we found a significant linear relationship between the periodic differences in absolute retention of dietary energy and the corresponding differences in $FC_{P\,CW}$ (Figure 9). Most of the variation in the model is explained by the absolute retention of lipids, and Figure 9 displays that the improved lipid retention in the energy demanding periods for the LP group yields better or similar economic performance as HP, whereas numerically higher energy retention for HP group during the second half of the year as well as the second spring in sea yields a substantial better economic performance for this group. This is especially noteworthy since the differences in feed price between the feeds in the SEP-DEC and APR-JUN periods were amongst the highest (Figure 8).

Increased carcass weight represents an increased quantity of salmon to spread the total costs on. Irrespective if a producer wants to gain competitive advantage by following a cost leadership strategy where the focus is to reduce economic costs below the costs of competitors or wants to supply a superior product which differentiates from the others in the industry (Porter, 1980; Barney, 2007), keeping costs at a minimum within the strategically chosen operations will always be a focus point. As presented earlier, the industry has predominantly focused on cost efficiency by achieving scale advantages (Asche and Bjørndal, 2011). However, recent information highlights that larger salmon companies in Norway with several farming licenses and large biomass do necessarily not yield these scale advantages compared to smaller salmon producers (Kontali, 2018). Nevertheless, data from the Norwegian Directorate of Fisheries (2018) show that "other operational costs" in the industry have increased by nearly 200 % from 2009 to 2016 while the relative cost of feed per year within the period has been decreasing (Paper IV). Costs associated with fish health are amongst the operational costs. Due to its intensive production form where high animal density is kept in closed captivity, there are great economical risks associated with salmon farming. Mortality represents a huge risk for the industry and Marthinussen (2017) reported production losses from 20 % to 30 % in the Scottish, Chilean and Norwegian industry in the salmon generations stocked in 2009 to 2015, whereas in the Faroese industry the losses were 10 %. Naturally, these losses are typically caused be pathogenic diseases and accompanied treatments which are conducted in an effort to control the challenges (Costello, 2009; Aunsmo et al., 2010; Martinez-Rubio et al., 2012; Martinez-Rubio et al., 2013; Torrisen et al., 2013; Martinez-Rubio et al., 2014; Abolofia et al., 2017; Iversen et al., 2017). These challenges reflect the development in the economic feed conversion ratio (FCR_E) which has been increasing since 2012 (Iversen et al., 2017). Compared with the FCR_{BW}, the FCR_E incorporates the losses in the conversion equation, which entails that lost biomass has used feed which is not converted into harvestable salmon biomass – the higher the lost biomass during production, the higher the FCR_E becomes. Interestingly, Dessen et al. (2018) found that a dietary high protein-to-lipid feed, compared to a regular high-fat diet, reduced mortality during an outbreak of pancreas disease, the most serious viral disease in Norwegian salmon production (Hjeltnes et al., 2018). Reducing mortality leads to better economic performance as this yields a higher biomass to spread the costs on, as well as generating higher tradeable biomass for income. Thus, a reduced production cycle with increased survival of salmon and correspondingly reduced production risk represents a highly valuable cost opportunity (Paper IV).

The dietary HP feed strategy was found to improve growth performance in all experiments (Paper I, II) which subsequently represents a trade-off between cost and growth performance. The first economic model only evaluated the feed performance influence on direct cost, whereas the second economic model in Paper IV included the value of reduced production time. When having the choice between two feeding strategies which ultimately will generate different growth patterns, the saved production time due to difference in growth performance will represent the opportunity cost of the poorer performing feed strategy. In our work, the opportunity cost represents the sacrifice in growth performance by choosing a LP feed strategy instead of the faster growing fish induced by a HP strategy (Buchanan, 1991). To simplify the model, the opportunity cost was subtracted from the feed cost in the HP group to visualize the value of reduced production time that was defined as the total costs minus feed cost. Based on the S1 large-scale, the model depicted a significant value in improved growth performance during the experimental period in 2009-2010 (Paper IV), and an even bigger opportunity cost was identified when doing the same calculation on cost data from 2016 (Norwegian Directorate of Fisheries, 2018).

In addition to the evaluation of opportunity cost, the model incorporates the additional sales value of higher tradeable biomass, which was depicted with the improved feed to carcass weight conversion in the dietary HP strategy (Paper II, III, IV). As with the opportunity cost, the value of additional biomass and bigger harvest weight to trade has become more significant in 2016 compared to 2009 since the average price per kg salmon has more than doubled during this period (Paper IV). Thus, growing salmon to harvest and realizing the value of salmon is considered to be much more profitable than to focus only on feed prices. This is visualized in Figure 9 which depicts the break-even trade-off based on the average feed conversion ratio of 1.15 in the Norwegian industry (Marthinussen, 2017) and difference in growth performances (TGC). The comparison is based on the average costs and prices in 2009 and 2016 (FishPool, 2018; Norwegian Directorate of Fisheries, 2018) together with the production assumptions presented in table 1.

Table 1 Production assumptions used for scenario comparison of economic performane between 2009 and 2016.

Stocking weight, kg	0.1
Live weight at harvest, kg	6.0
Slaughter yield	0.85
Carcass weight, kg (HOG)	5.1
Average temperature, °C	8.5
Daydegrees	4413

To reach a break-even between income and costs in 2009 relied on a TGC of ~2,89 compared with a TGC of ~2,18 in 2016. With an average production temperature of 8.5°C, this growth difference is equivalent to ~180 production days. A TGC of 3.3 is regarded as a good growth performance according to Einen et al. (1995). Thus, the TGCs in the presented model are considered low. However, this also underlines the improved economic result that may be achieved with improved fish growth. Based on the two scenarios, in 2009 a TGC of 3.3 improved margin with NOK ~1.8 kg^{-1}, whereas in 2016 the improved margin with the same TGC was NOK ~12.4 kg^{-1}, i.e. a seven-fold improvement (Figure 9).

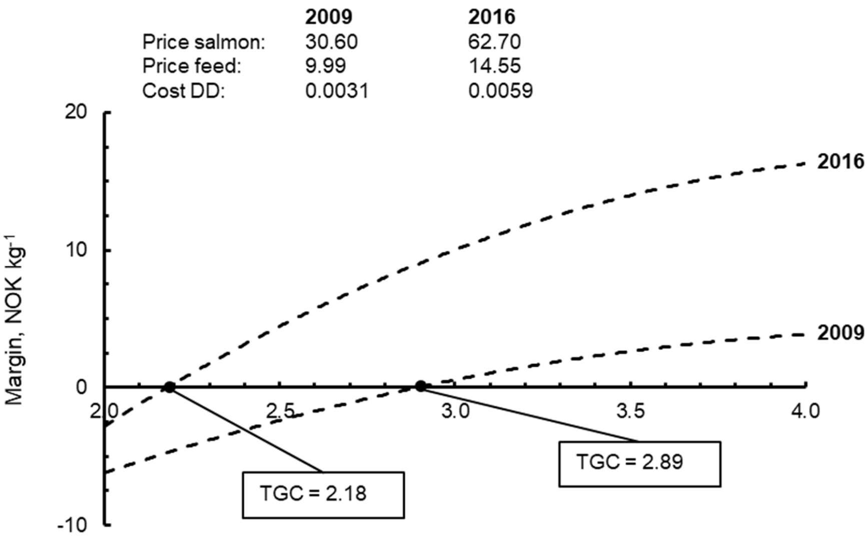

Figure 9 Margins in the salmon industry in 2009 and 2016 based on an FCR_{BW} of 1.15, price of salmon and feed and cost per day degree in the respective years, in relation to the production assumptions given in Table 1.

A TGC of 2.2 is very low and virtually unrealistic. Nonetheless, this also underlines that only a modest improvement in growth performance contributes greatly to the overall economic performance based on the last years increase in salmon prices (Paper IV). This evaluation corresponds with the development in margins in the Norwegian industry during the last couple of years, i.e. EBIT per kg (Kontali, 2018). Thus, taken together, we conclude that a dietary induced improved growth performance will contribute significantly to the overall economic performance in salmon production with current salmon prices, despite the general increase in production costs. Although production risks related to potential diseases and corresponding treatments are not included in the economic evaluation, we consider improved growth performance to have a positive influence on potential disease related costs as well. Therefore, the presented value of better growth performance is likely to be even higher.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The findings in this thesis demonstrate that there is a considerable potential of improving growth in farmed salmon and increasing the amount of tradeable yield by altering the dietary balance in the feed strategy that is more protein dense compared to the preferred high-fat diets in the industry. In addition, the presented work also demonstrates that this can be achieved with improved feed conversion. These improvements are especially momentous in the latter part of the year. The mix of substrates in a protein dense feed induces an improved feed to carcass conversion which results in improved slaughter yields. These yields are reflected in improved condition factor where the higher weights are based on thicker muscles and lower fat content in both viscera and muscle. Such quality attributes are considered beneficial for primary processing and are perceived as precursors for higher fillet yields (Einen, 1998; Rørå et al., 2001).

The results also depicted that a high-energy protein dense feed strategy does not improve performance in the cold winter period. Thus, during the winter period, a higher-fat based energy dense feeding strategy should be used instead of the protein dense feeding strategy, to optimize the performance for the overall production cycle. Although a high protein-to-lipid strategy does necessarily not improve growth and feed conversion in post-smolt S1 salmon compared to a low protein-to-lipid during the initial spring and summer period, it is not advised to use a cheaper higher lipid based feed strategy as this increases the risk of undesirable high fat content in the summer which subsequently may have a negative influence on growth performance during the latter half of the year (Rørvik et al., 2018). Based on the hypothesis that fat content in the fish influences feed intake and growth, future research should investigate how fat content in the fish can be kept at a minimum at the beginning of declining day length in the autumn without compromising feed utilization and growth performance. This should be done already in the freshwater stage, especially now as the industry in general is investing in recirculating aquaculture systems (RAS) to produce larger smolts. RAS systems enable farmers to have better control on environmental factors such as temperature (Kolarevic et al., 2014) which typically is kept a stable warm temperature. Increased temperature forms the basis for higher metabolism and consequently a higher feed intake compared to a traditional flow through system. Thus, it is a perceived risk of higher fat content in the RAS produced post-smolts when stocked into seawater which ultimately may lead to a poorer growth potential. This, together with an investigation of the potential of changing photoperiod on reduced accumulation of fat in closed RAS systems, needs to be addressed in future salmon research. Due to the risk of great influence on body fat and body shape development in salmon from small-scale facilities, such research needs to be conducted in controlled small-scale as well as in large-scale studies to ensure commercial relevance.

From a cost perspective, feed is the most important input factor in salmon production, but despite higher feed prices in a high protein-to-lipid strategy compared to the high-fat standard feeds in the industry, there is a positive trade-off using a protein based high-energy feed strategy. However, the results also highlight that the overall economic performance might be optimized, as seasonal variation influence how well salmon utilize the feed for growth. This is especially momentous in the winter period where a low protein-to-lipid strategy is a better economical solution. Therefore, a dynamic approach towards dietary feeding strategy is recommended with a shift between high protein and high fat diets.

Salmon farming is exposed to high risk and salmon producers seek to achieve high turnover rates of the production cycle. In line with increasing production costs, the value of a reduced production cycle has increased. Increase in production losses and corresponding increase in FCR_E in the industry (Iversen et al., 2017), demonstrates this risk of production. Combined with high salmon prices, the value of reducing the production cycle has a significant improved economical impact on the overall performance compared to the actual increase in feed prices. This is modulated with the protein dense diets which are somewhat higher priced but yield a better economic performance because of a combination of a lower feed conversion, shorter production cycle together with a higher tradeable weight per fish. In addition, the positive dietary influence of HP diets towards increased survival in commerical farming during outbreak of pancreas disease (Dessen et al., 2018) should be further investigated and include other diseases. Dietary influence on survival rate was not investigated in this thesis but should be included in future economic evaluations of feed experiments.

If there will be future limitations in production of salmon, either as governmental regulations and/or technological limitations within relevant coastline areas (Asche et al., 2018b, Misund and Nygård, 2018; Marine Harvest, 2018), it is highly likely that the opportunity costs in the industry will increase unless current challenges that cause the higher production cost are solved. Despite the results of an overall improved performance with protein dense grower diets, the industry has gradually been replacing fishmeal with plant protein alternatives. Plant proteins are generally not as protein dense as fishmeal and this might raise a challenge in manufacturing of protein dense grower feeds without compromising the total dietary energy by reducing oil inclusion. Thus, high protein-to-lipid dense diets for salmon in the future may not be commercially applicable unless plant alternatives reach protein concentrations that are close to or equally high as fishmeal protein. Therefore, future research should aim to formulate such dietary high protein-to-lipid dense diets based on alternative protein sources and investigate the dietary influence on production, quality and economic performance based on long term trials.

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Different growth performance, lipid deposition, and nutrient utilization in in-season (S1) Atlantic salmon post-smolt fed isoenergetic diets differing in protein-to-lipid ratio

Jens-Erik Dessen [a,d,*], Rúni Weihe [c,d], Bjarne Hatlen [b], Magny S. Thomassen [d], Kjell-Arne Rørvik [a,d]

[a] Nofima, NO-1432 Ås, Norway
[b] Nofima, NO-6600 Sunndalsøra, Norway
[c] Havsbrún P/F, Bakkavegur 48, FO-530, Fuglafjørður, Faroe Islands
[d] Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway

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ABSTRACT

The aim of the present study was to evaluate how isoenergetic diets with different protein-to-lipid ratio affects feed intake, growth performance, lipid deposition, feed and nutrient utilization in Atlantic salmon post-smolt. A 6-month's feeding trial was conducted with in-season (S1) Atlantic salmon post-smolt reared in the sea under natural conditions (May–September). Quadruple groups of salmon (initial weight 95 g) were fed two isoenergetic diet series formulated to contain a high (HP) and low (LP) protein-to-lipid ratio designed to resemble upper and lower levels of ratios used in commercial feeds. The group fed the HP diet had a significantly ($P \leq 0.05$) lower muscle fat content (HP = 4.7%, LP = 5.7%), whole body lipid (HP = 9.0%, LP = 9.6%) and energy content (HP = 7.7 MJ kg^{-1}, LP = 8.0 MJ kg^{-1}) than the group fed the LP diet after the period June–July. These differences were mainly due to significantly lower absolute apparent lipid retention in the summer period for post-smolt fed HP diet. In the subsequent experimental period (July–September), a significantly higher specific feed intake (HP = 1.38%, LP = 1.33%), thermal growth coefficient (HP = 3.82, LP = 3.46) and weight gain (HP = 658 g, LP = 552 g) were observed for fish fed the HP diet. The period from July–September was associated with high water temperatures and declining day length. The reduced feed intake in the LP group coincided with increased visceral mass and lipid deposition, indicating a possible involvement of lipostatic regulation. The retention efficiency of nutrients increased with the up-regulation in feed consumption. The HP fed fish had a significantly higher whole body lipid retention (HP = 74.4%, LP = 67.2%), but significantly reduced visceral mass compared to LP fed fish during the autumn. The overall improved growth, good protein utilization and reduced visceral adiposity among the HP fed fish resulted in significantly improved final condition factor (HP = 1.46, LP = 1.40), carcass yield (HP = 86.0%, LP = 84.1%), feed conversion based on gutted weight (HP = 0.98, LP = 0.93) and whole body protein (HP = 17.6%, LP = 16.9%). The present study reveals that low dietary protein-to-lipid ratios for salmon post-smolt may negatively affect production parameters, although digestible energy contents in the diets are similar.

Statement of relevance: The present study confirms the importance of balanced dietary lipid-to-protein ratios for optimal production efficiency and nutrient utilization, and the significant effects of dietary and seasonal interaction on lipid deposition and production related parameters. To our knowledge, few have investigated the effect of isoenergetic diets differing in protein-to-lipid ratio on growth performance and nutrient utilization of juvenile Atlantic salmon reared in seawater under natural conditions. The experiment used feed formulations, fish breed and rearing conditions relevant for current commercial salmon farming practices.

Considering the current increase in the cost of lipid sources, it would be beneficial for the aquaculture industry if dietary lipid content could be reduced without compromising growth and feed utilization of the fish. We believe our findings will provide useful and relevant information regarding dietary formulations and nutritional knowledge for the global fish feed industry and salmon producers.

1. Introduction

The majority of Atlantic salmon (*Salmo salar* L.) is farmed in open sea pens that are exposed to seasonal variations in environmental

* Corresponding author at: Nofima, NO-1432 Ås, Norway.
E-mail address: jens-erik.dessen@nofima.no (J.-E. Dessen).

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conditions. Important production parameters such as appetite, feed utilization and growth rate are modulated by temperature and photoperiod, and by a wide range of other internal and external factors such as genetics, health status, adiposity, water quality, fish size, dietary composition and feeding regime (Austreng et al., 1987; Bendiksen et al., 2003a; Bendiksen et al., 2003b; Einen and Roem, 1997; Gjedrem, 2000; Gjøen and Bentsen, 1997; Hillestad et al., 1998; Jobling and Johansen, 1999; Johansen and Jobling, 1998; Sveier and Lied, 1998; Thodesen et al., 1999; Thorarensen and Farrell, 2011). Farmed salmon in the mid-west part of Norway encounter periods with low feed intake, decreased growth rate, low lipid retention and the depletion of energy stores during their first spring in the sea (Alne et al., 2011). In contrast, the salmon experience high feed intake, rapid growth, and altered deposition and retention of lipids during the late summer and early autumn (Alne et al., 2011; Hemre and Sandnes, 2008; Mørkøre and Rørvik, 2001; Måsøval et al., 1994; Oehme et al., 2010). This phenomena seems to occur both for smolt transferred to the sea during the autumn and for those transferred during the spring (Alne et al., 2011), which suggests that salmon have a seasonal growth pattern that is triggered by external photoperiodic information. Thus, season-specific signals and internal factors induce metabolic changes in salmon that significantly affect the production efficiency in natural environments.

The minimum requirements of salmonids for protein, amino acids and energy have been partly established (NRC, 2011; Wilson, 2002). Juvenile salmonids undergoing rapid body growth require a higher portion of digestible protein than larger salmonids (Cho and Kaushik, 1990; Einen and Roem, 1997; Grisdale-Helland et al., 2013b), which use large amounts of the dietary energy for maintenance (Azevedo et al., 2004a, 2004b; Jobling, 1994). However, sufficient dietary energy is important to ensure optimal feed utilization (Hillestad and Johnsen, 1994; Hillestad et al., 1998). Several studies do not detect significant differences in growth performance between groups of salmon fed diets varying in protein/lipid ratio (Azevedo et al., 2004b; Hillestad and Johnsen, 1994; Hillestad et al., 1998; Karalazos et al., 2007; Karalazos et al., 2011). In particular, studies using isoenergetic grower diets identified no negative influence of low protein/lipid ratio on growth performance or feed utilization, but a favorable protein sparing effect (Karalazos et al., 2007; Karalazos et al., 2011). These observations imply that salmon have high ability to utilize large amounts of lipids in high-energy diets efficiently for growth. The above mentioned factors together with the fact that lipid has historically been a cheap source of energy compared to protein, have lead the industry to reduce the amount of protein and increase the lipid content in the diets (Torrissen et al., 2011). Consequently, the dietary protein/lipid ratio in modern diets is thus lower compared with the traditional diets for salmonids. However, high demand of lipids and competitive pressure from competing industries, including direct human consumption, has increased the cost of lipids. Nutritional knowledge, raw material availability and world markets are under constant change and development, and thus, cost-effective and sustainable salmon production requires optimal utilization of both protein and lipids.

Most studies examining different dietary protein-to-lipid concentrations for salmon use non-isoenergetic diets (Einen and Roem, 1997; Grisdale-Helland and Helland, 1997), although several adjusted the dietary ration level so that the diets tested were fed isonitrogenously or isoenergetically (Hillestad and Johnsen, 1994; Hillestad et al., 1998). In addition, some studies indicate that salmonids are able to adjust their feed consumption according to the dietary energy level (Bendiksen et al., 2002; Boujard and Medale, 1994). As a result, this may complicate the direct comparisons among studies. To our knowledge, few have investigated the effect of isoenergetic diets differing in protein-to-lipid ratio fed ad-libitum on growth performance of juvenile salmon (0.1–1 kg) reared in seawater under natural conditions. In-house laboratory studies with constant light and temperature or short-term experiments may disregard the vital impact of seasonal environmental variations that influence the growth pattern.

Salmon increase the deposition of muscle fat and visceral adiposity as the fat content in the feed increases (Bendiksen et al., 2003a, 2003b; Einen and Roem, 1997; Hillestad et al., 1998; Jobling et al., 2002a). The carcass yield consequently decreases (Hillestad et al., 1998). Increased amount of lipid deposition correlates with decreased feed intake in salmonids (Jobling and Johansen, 1999; Jobling et al., 2002b; Johansen et al., 2002; Johansen et al., 2003; Shearer et al., 1997a; Shearer et al., 1997b; Silverstein et al., 1999). This finding is consistent with the lipostatic regulatory hypothesis (Jobling and Johansen, 1999; Keesey and Corbett, 1984; Kennedy, 1953; Schwartz and Seeley, 1997), which suggests that the amount of stored fat is an important regulator of energy intake and the homeostasis of adiposity. The hypothesis suggests that adipose tissue exerts a negative feedback control on appetite and feed consumption in fish. There is, thus, a risk of impaired growth as lipid deposition becomes excessive.

In view of the above-mentioned studies, it can be assumed that a diet with a low lipid level but with sufficient energy content, (i.e. increasing the dietary protein/lipid ratio), is an effective approach to reduce the deposition of lipids and enhance feed intake. This may be especially prominent for S1 juvenile salmon the first autumn in sea, since this period is associated with rapid growth and, elevated deposition and retention of lipids (Alne et al., 2011; Hemre and Sandnes, 2008; Mørkøre and Rørvik, 2001; Måsøval et al., 1994). However, excessive dietary protein or lipids, may lead to increased catabolism of the dietary nutrients and reduce the retention efficiency of protein and lipids, respectively (Kaczanowski and Beamish, 1996; Refstie et al., 2001; Walton et al., 1984).

During a five month period after sea transfer, the present study test the hypothesis that increased dietary protein-to-lipid ratio improves the feed intake and growth of S1 Atlantic salmon, compared to lower dietary protein-to-lipid ratio (using commercially formulated ratios). The dietary and seasonal effects on lipid deposition, feed conversion, whole body composition, nutrient retention, body shape and carcass yield were assessed.

2. Materials and methods

2.1. Experimental diets

The diets used in the study were manufactured by Havsbrún (Fuglafjørður, Faroe Islands) by extrusion and vacuum coating with oil. Two diet series that differed in protein/lipid ratio, but were isoenergetic with respect to digestible energy (DE), were formulated. Diets were produced as 3, 4 and 6 mm pellets according to fish size. The ingredients used and the compositions of macronutrients in diets for pellets of each given size are shown in Table 1. The approximate chemical compositions of the diets are shown in Table 2. The high-protein diet series (HP) had a higher content of protein and a lower content of lipid than the low-protein diet series (LP). The formulations were

Table 1
Formulation (g kg^{-1}) of the experimental diets.

Pellet size	3 mm		4 mm		6 mm	
Diet code	LP	HP	LP	HP	LP	HP
Formulation, (g kg^{-1})						
Micro ingredients[a]	25	25	25	25	15	15
Wheat	119	105	138	100	140	125
Wheat gluten	20	58	20	63	28	69
Soy protein concentrate	38	26	15	61	56	45
Fish meal	492	531	520	511	387	425
Krill meal	55	55	15	15	-	-
Porcine blood meal	-	-	-	-	45	30
Fish oil	110	95	127	116	151	136
Rapeseed oil	110	95	127	116	151	136
Pigment[b] (mg kg^{-1})	50	50	50	50	50	50

[a] Vitamin and mineral premixes.
[b] Astaxanthin.

Table 2
Approximate chemical compositions (g kg^{-1}) of the diets.

Pellet size	3 mm		4 mm		6 mm	
Diet code	LP	HP	LP	HP	LP	HP
Chemical composition, (g kg^{-1})						
Crude protein ($N \times 6.25$)	444	483	413	452	390	441
Crude lipid	286	260	328	285	347	316
Ash	89	94	85	90	55	58
Water	71	73	64	79	62	62
Crude fiber	1.6	1.2	0.8	0.7	1.1	1.0
Total starch	73	73	77	69	101	88
NFE[a]	108.4	88.8	109	93	145	122
Gross energy, (MJ kg^{-1})	23.8	23.3	24.4	23.4	25.2	24.9
Crude protein/lipid ratio	1.55	1.86	1.26	1.59	1.12	1.40
Digestibility calculations[b]						
Calculated DP, (g kg^{-1})	382	415	355	389	335	379
Calculated DE, (MJ kg^{-1})	20.6	20.3	21.5	20.6	22.1	21.8
Estimated DP/DE ratio (g MJ kg^{-1})	18.5	20.5	16.5	18.9	15.2	17.4

[a] NFE = Nitrogen-free extracts = 1000 − (protein + lipids + ash + fiber + water).
[b] The amounts of digestible protein (DP) and digestible energy (DE) were estimated assuming 23.7, 39.5 and 17.2 MJ kg^{-1} as the gross energy content of protein, lipids and carbohydrates, respectively. The apparent digestibility coefficients (ADCs) for protein and lipids used were 0.86 and 0.94, respectively (Einen and Roem, 1997), whereas 0.50 was used for NFE (Arnesen and Krogdahl, 1993).

designed to resemble high and low protein-to-lipid ratios of commercial feeds used for salmon. The level of protein was decreased whereas the level of lipid was increased with the increase in pellet size, in accordance with commercial feed formulations. This upregulated the total energy level in order to account for the increase in fish weight. The difference in crude protein content (~40 g kg^{-1}) between the experimental diets was kept constant within all the pellet sizes, and the lipid level was adjusted to obtain equal levels of DE. The feed batches were stored in a refrigerated room (4 °C) and the amounts of feed corresponding to one-week consumption was taken out and kept in boxes at room temperature. Feed samples were taken on arrival from the manufacturer and stored frozen (−20 °C) until they were analyzed as described below. The diets were formulated to meet the NRC nutritional recommendations for salmonid fish (NRC, 2011).

2.2. Fish, rearing conditions and experimental design

On the 29 March 2012, 8000 S1 Atlantic salmon smolt from the Rauma strain (Rauma Broodstock AS, Sjøholt, Norway) were sorted out, weighed in bulk and distributed among eight tanks with 1000 fish in each, on a truck at the Straumsnes Settefisk AS hatchery at Tingvoll, Norway. The smolts were visually examined and individuals with similar size were selected and weighted in bulk. Fish with obvious signs of wounds, parr-marks or runts were removed. The fish were then transferred to Marine Harvest research station (former Nofima) at Ekkilsøy (63°03′N, 7°35′E) on the west coast of Norway during the same day. On arrival, fish from each tank on the truck were allocated to one of eight pens (5 × 5 × 5 m, 125 m^3 volume). The smoltification status was checked by conducting a seawater challenge test, followed by determination of plasma osmolality, chloride content and gill Na^+,K^+-ATPase activity (Clarke et al., 1996), before the fish were exposed to seawater. The mean initial body weight of the smolt was 95.1 ± 0.2 g (mean ± SD). Each pen was assigned to one of two dietary groups in a randomized block design of quadruple net pens.

The eight pens were initially illuminated by four submerged 400 W light sources, 24 h day^{-1}. This was done in order to promote schooling behavior and avoid physical contact with the net wall in the pens. The submerged lights were removed on 29 May, and the salmon were subsequently exposed to the natural photoperiod until the feeding trial ended on 25 September 2012 (Fig. 1A). Daylight hours were defined as the period from twilight in the morning until the center of the sun was 6° below the horizon in the evening, referred to as civil twilight (data obtained from the website; www.timeanddate.no). The experiment was divided into three periods: April–June (spring), June–July (summer) and July–September (autumn) (Table 2). The periods were adjusted to fit with the guidelines of the feed manufacturer with respect to pellet sizes, which have been determined according to the weight of fish (Table 3). The ambient seawater temperature and oxygen level were recorded daily at a depth of 3 m. The seawater temperature at transfer was 6 °C, and it increased to a maximum of 15 °C in late August. The average for the complete trial was 9.8 °C (Fig. 1A). The average temperatures for the three periods were: 7.5 °C in April–June, 11.5 °C in June–July and 13.6 °C in July–September. The oxygen level decreased with increasing water temperature, and ranged from 12.8 to 7.2 mg l^{-1}, with an average of 9.8 mg l^{-1} (Fig. 1B).

2.3. Feed-monitoring system and feed administration

The feed-monitoring system used in the trial was established by combining the methods described by Einen et al. (1999) and Helland et al. (1996). Feed was administered by automatic feeders (Betten Maskinstasjon AS, Vågland, Norway) and uneaten pellets were collected in a plastic funnel at the bottom of each net pen. The uneaten feed was pumped up into wire mesh sieves through a plastic pipe using pressurized air. The uneaten feed was collected after each meal and quantified each day, in order to determine the daily feed intake and feed conversion ratio accurately. The daily feed intake was calculated as described by Helland et al. (1996). All feeds were tested for the recovery of dry matter in empty net pens after the trial. The fish were fed to satiation (four times a day), and the feed ration was set such that they received approximately 10–20% more than the estimated daily feed intake. Adjustments of the feed ration were done according to the amount of uneaten feed collected.

2.4. Weighing and sampling procedures

All fish were counted and weighed in bulk at the end of each feeding period. The fish were collected from each experimental pen using a fish-landing net and anesthetized in batches with MS-222 (Metacaine 0.1 g l^{-1}; Alpharma, Animal Health Ltd., Hampshire, UK) in a 1000-l tank of fresh seawater. All fish with obvious signs of wounds, runts or sexual maturation were removed and killed during the weighing procedure (the weights and numbers of such fish were recorded). An initial sample of 30 fish (three pooled samples with 10 fish in each) was taken before sea transfer, and 10 fish from each pen were sampled (sampled fish presented a mean body weight corresponding to the mean weight of all fish in the net pen) at the end of each feeding period. Sampled fish were anesthetized in MS-222 and then killed by a blow to the head. The gill arches were cut and the fish were bled out in ice water. The fish were subsequently transported to the processing hall nearby, where individual weights and fork lengths were measured. The fish were then cut open, sex was determined by inspection of the gonads, and visceral fat was assessed visually on a score from 1 to 5 according to the visibility of the pyloric caeca (1 = clearly visible, 2 = visible, 3 = visible through cracks 4 = visible through the fat, 5 = not visible). The viscera (including the spleen) and the liver were dissected and weighed, in order to calculate the viscerosomatic index (VSI) and the hepatosomatic index (HSI). The heart and kidney were then removed before the fish was rinsed with water and the gutted weight recorded. Finally, muscle samples (Norwegian Quality Cut, NQC, NS 9401, 1994) were taken for analysis of lipid content. In addition, 30 fish (3 × 10) were taken at the start of the experiment, and 10 fish per pen on each sampling point, for the analysis of the whole-body proximate composition. These selected fish presented a mean body weight corresponding to the mean weight of all fish in the pen, then exposed to a lethal concentration of MS-222, before being frozen at −20 °C. The fish were not starved before the sampling occasions in June and July, so feed matter was removed from the esophagus, stomach and intestines of all fish taken for analysis at these samplings. At the final sampling in

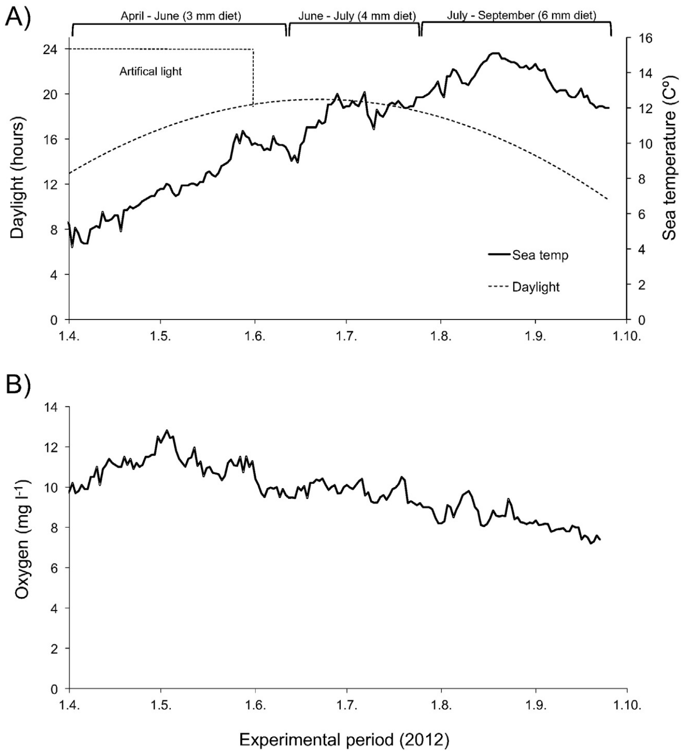

Fig. 1. A: Ambient sea temperature (°C) and hours of daylight during the trial. B: The measured oxygen level (mg l−1) during the trial. Diets used (3, 4 and 6 mm) and the duration of the feeding periods (months) are indicated in the top of the figure.

September, samples were taken 48 h after the last meal and no feed matter was observed in the gastrointestinal system.

The pens were checked for mortalities daily and all the dead fish, were collected and weighed. During period 1, 3 and 2 fish died in the HP and LP group, respectively. During period 2, the average morality rate was 1.0% for the HP group and 1.6% for the LP group. During period 3, the average morality rate was 1.4% for the HP group and 0.6% for the LP group. There were no significant differences in mortality.

2.5. Analysis

Feces and diets were analyzed gravimetrically for dry matter (DM) after drying at 105 °C until constant weight, and for ash by flame combustion and incineration at 550 °C. Nitrogen was analyzed using the semi-automated Kjeldahl method (Kjetec Auto System, Foss Tecator, Höganäs, Sweden) and crude protein calculated as N × 6.25. The amount of crude lipid after hydrolysis with hydrochloric acid (HCl) and petroleum ether extraction was determined using the Soxtec HT6 system and a Soxtec1047 hydrolyzing unit (Foss Tecator, Höganäs, Sweden). The gross energy content was determined by adiabatic bomb calorimetry (Parr 6400 oxygen bomb calorimeter, Parr Instrument Company, Moline, IL, USA). Starch was analyzed as glucose, after enzymatic hydrolysis using a Megazyme K-TSTA 05/06 total starch assay kit (Megazyme International Ltd., Wicklow, Ireland) according to the Association of Analytical Communities (AOAC) method, number 996.11. The amount of crude fiber was determined using a modified version of ISO 5498, by means of a Fibertec system (Foss Tecator, Höganäs, Sweden).

The amounts of crude protein and energy in homogenates of whole-fish body samples were determined as described for feeds. Whole-body fat was analyzed using a semi-automatic Soxhlet extractor (Soxtec Avanti 2055 apparatus, Foss Tecator, Höganäs, Sweden) with petroleum ether as the extracting solvent. The total fat content in muscle (NQC) was determined by extraction with ethyl acetate as described in NS 9402 (1994). The chemical analyses of muscle fat were conducted on pooled homogenized NQC samples from 10 fish per pen.

Table 3
The experimental periods with duration, dates, pellet size used and sampling date. The preferred fish weight intervals of the different pellet sizes are also given.

Feeding period	Duration	Dates	Pellet size used	Preferred fish weight (g)	Samplings
Apr - Jun	11 weeks	29 Mar. - 11 Jun.	3 mm	100 ~ 150	1: 11 Jun.
Jun - Jul	6 weeks	11 Jun. - 23 Jul.	4 mm	150 ~ 300	2: 23 Jul.
Jul - Sept	9 weeks	23 Jul. - 24 Sep.	6 mm	300 ~ 800	3: 24 Sep.

2.6. Calculations

The growth rates of the fish are presented as the thermal growth coefficients (TGC), calculated as described by Cho (1992).

$$TGC = \left({W_1}^{1/3} - {W_0}^{1/3}\right) \times (\Sigma T)^{-1} \times 1000$$

where W_0 and W_1 are the initial and final weights, respectively, and ΣT is the sum of day degrees during the period

$$(\text{feeding days} \times \text{average temperature}, °C).$$

The biological feed conversion ratio (FCRb) was calculated as:

$$\text{feed intake (kg)} \times (\text{biomass increase} + \text{biomass of dead fish (kg)})^{-1}.$$

The feed conversion ratio on gutted weight basis (FCRg) was calculated as:

$$\text{FCRg} = \text{FCRb} \times \text{carcass yield}^{-1}.$$

The specific feeding rate (SFR) was calculated as:

$$(\text{feed intake during the time period (kg)} \times \text{average biomass weight during the time period (kg)}) \times 100^{-1}.$$

The retention of nutrients were estimated on pen basis, using the values of cumulative feed intake, the chemical composition of the diets, and changes in the biomass and whole-body content of the nutrient: Relative nutrient retention (% of ingested) was calculated as:

$$100 \times (\text{final mass of nutrient in fish} - \text{initial mass of nutrient in fish})\,(\text{mass nutrient ingested})^{-1}.$$

Absolute amount of nutrient retained from the feed (g 100 g^{-1} feed) was calculated as:

$$\left((\text{nutrient in the diet} \times \text{percentage of nutrient retention}), \times, 100^{-1}\right).$$

For absolute nutrient retention of energy, MJ kg^{-1} feed was used.

The authors acknowledge that the relative and absolute lipid retention is apparent as the fish have the ability to synthesize this nutrient de novo. However, in the text the term apparent is not used.

The body weight (BW) of bled fish was estimated by adding 3% to the bled weight: (BW = bled weight × 1.03) (Einen et al., 1998).

Viscerosomatic index (VSI) and carcass yield were calculated as:

$$Y(\text{g}) \times \text{body weight(g)}^{-1} \times 100,$$

where Y is the weight of the measured visceral or carcass mass.

The condition factor (CF) was defined as:

$$100 \times \text{total body weight with blood (g)} \times \text{length}^{-3}.$$

The CF and carcass yield on gutted weight basis were calculated by applying the same formulas, but with gutted weight instead of the body weight.

2.7. Statistical analysis

The trial was conducted using a randomized block design and all data were analyzed using the GLM procedure in the SAS 9.3 computer software (SAS Institute Inc., Cary, NC, USA). Diet and block were used as class variables. If differences based on the block variable were not significant, the data were analyzed using diet as the only experimental factor. Net pen was used as the experimental unit. Percentage data were subjected to arcsine square root transformation before the statistical analysis. Homogeneity of variances was tested using Bartlett's test, and for data with heterogeneous variances, Welch's test for differences among groups was performed. Non-parametric data (visual score) were tested using the Kruskal-Wallis test. The Pearson product-moment correlation coefficient was used to describe the association between two variables. The level of significance was chosen at $P \leq 0.05$, and the results are presented as mean ± standard error of mean (SEM), unless stated otherwise.

3. Results

3.1. Feed intake, growth performance and feed utilization

The feed intake was low after sea transfer and throughout the first feeding period from April–June. It then increased gradually during the experiment. The feed intake did not differ between the dietary groups in April–June and June–July. The duration of daylight decreased in the period July–September and the water temperature was high (Fig. 1A and B). During this period, the fish fed the HP diet had significantly higher feed intake than those fed the LP diet (Table 4).

The growth rate reflected the feed intake, and TGC, FCRb and BW did not differ significantly between the dietary treatments in April–June or June–July (Fig. 3 and Table 4). The highest growth for both groups was observed during July–September (Fig. 3). In addition, during this period fish fed the HP diet presented a significantly higher TGC compared to fish fish fed the LP diet (HP = 3.82 ± 0.00, LP = 3.46 ± 0.03, $P < 0.001$). FCRb did not differ between the two groups (Table 4). Thus, the final body weight of fish in the HP group (945 ± 4 g) was significantly ($P < 0.0001$) higher than that of fish in the LP group (836 ± 11 g). Consequently, the weight gain (corrected for differences in start weight) for the HP group was 106 g higher (i.e. almost 20% higher weight gain) than the LP group. Fish given the HP diet had a significantly lower FCR on gutted weight basis (FCRg) than fish given the LP diet during the period July–September (Table 4).

3.2. Fat deposition, proportional visceral weight and body shape

The developments in muscle fat content and VSI for the two diets are shown in Fig. 2. The amount of muscle fat was the same in both dietary groups until the second sampling in July, when the group fed the HP diet had lower muscle fat content than the LP group (HP = 4.7 ± 0.3%, LP =

Table 4
Weight gain, feed intake and feed utilization (mean ± SEM, n = 4).

Dietary group	LP	HP	Dietary effect (P-value)
April – June, 3 mm diet			
Weight gain, g	66 ± 1	67 ± 2	0.533
SFR	0.55 ± 0.01	0.54 ± 0.00	0.205
FI, g^{-1} $fish^{-1}$	52 ± 1	51 ± 1	0.487
FCRb	0.79 ± 0.01	0.76 ± 0.02	0.277
FCRg	0.93 ± 0.02	0.88 ± 0.01	0.087
June – July, 4 mm diet			
Weight gain, g	123 ± 2	126 ± 2	0.383
SFR	1.03 ± 0.02	1.05 ± 0.02	0.536
FI, g^{-1} $fish^{-1}$	92 ± 1	95 ± 2	0.280
FCRb	0.74 ± 0.00	0.75 ± 0.01	0.210
FCRg	0.89 ± 0.01	0.88 ± 0.01	0.372
July – September, 6 mm diet			
Weight gain, g	552 ± 9.3	658 ± 2.3	<0.001
SFR	1.33 ± 0.02	1.38 ± 0.01	0.054
FI, g^{-1} $fish^{-1}$	452 ± 9	527 ± 2	<0.001
FCRb	0.81 ± 0.01	0.80 ± 0.00	0.126
FCRg	0.98 ± 0.01	0.93 ± 0.00	0.013

SFR, specific feed intake; FI, feed intake; FCRb, biological feed conversion ratio. FCRg, feed conversion based on gutted weight.

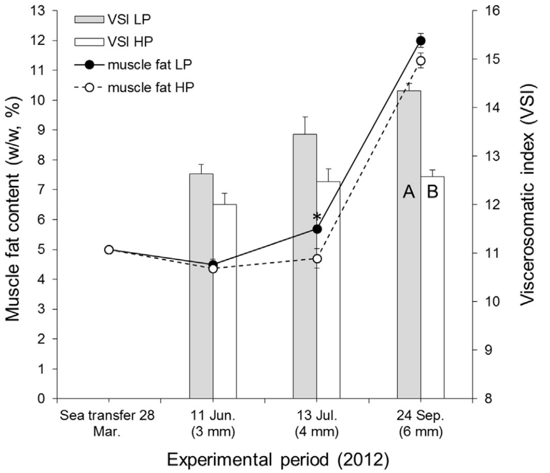

Fig. 2. Changes in muscle fat content, % w/w (lines) and viscero-somatic index, % (bars) for S1 Atlantic salmon fed isoenergetic diets with high (HP) or low (LP) protein/lipid ratio. Significant differences between dietary groups within each sampling (11 Jun, 13 Jul and 24 Sep) are indicated by * over the lines and different letters on bars. The diets used (3, 4 and 6 mm) before the samplings are shown in the parenthesis. Data are presented as means ± SEM, n = 4.

Table 5
Biometric parameters at each sampling point (mean ± SEM, n = 4).

Dietary group	LP	HP	Dietary effect (*P*-value)
11 June, Sampling 1, end of 3 mm diet			
Body weight, g	150 ± 3	151 ± 4	0.849
Gutted body weight, g	129 ± 3	131 ± 3	0.646
Body length (fork), cm	23.9 ± 0.1	24.0 ± 0.2	0.554
Condition factor (CF)	1.10 ± 0.01	1.09 ± 0.01	0.571
Condition factor gutted (CFg)	0.94 ± 0.01	0.94 ± 0.01	1.000
Carcass yield, %	86.1 ± 0.2	86.8 ± 0.3	0.102
Visceral score, 1-5	1.3 ± 0.1	1.5 ± 0.1	0.139
23 July, Sampling 2, end of 4 mm diet			
Body weight, g	274 ± 2	277 ± 2	0.393
Gutted body weight, g	233 ± 2	238 ± 2	0.087
Body length (fork), cm	29.0 ± 0.0	29.2 ± 0.2	0.234
Condition factor (CF)	1.12 ± 0.01	1.11 ± 0.02	0.526
Condition factor gutted (CFg)	0.95 ± 0.01	0.95 ± 0.01	1.000
Carcass yield, %	85.0 ± 0.4	86.0 ± 0.3	0.072
Visceral score, 1-5	1.4 ± 0.1	0.9 ± 0.1	0.017
24 September, Sampling 3, end of 6 mm diet			
Body weight, g	815 ± 20	926 ± 6	0.002
Gutted body weight, g	685 ± 16	796 ± 7	0.001
Body length (fork), cm	38.7 ± 0.3	39.9 ± 0.2	0.023
Condition factor (CF)	1.40 ± 0.01	1.46 ± 0.02	0.025
Condition factor gutted (CFg)	1.18 ± 0.01	1.25 ± 0.01	0.008
Carcass yield, %	84.1 ± 0.2	86.0 ± 0.2	<0.001
Visceral score, 1-5	2.7 ± 0.1	2.4 ± 0.1	0.106

Initial sampling before sea transfer, 29 March: body weight; 92.8 ± 0.3 g, length; 19.1 ± 0.0 cm, condition factor; 1.33 ± 0.01.

5.7 ± 0.1%, $P = 0.03$). Muscle fat content of both groups increased substantially ($P < 0.001$) from July to September (6.5%-units on average) and no significant differences in muscle fat content were detected between the two dietary groups in September (Fig. 3). The VSI of the group fed the LP diet increased steadily during the trial, whereas the VSI of the group fed the HP diet remained almost constant. At the final sampling in September (Fig. 3), the VSI of the HP group was lower than that of the LP group (HP = 12.6 ± 0.1, LP = 14.3 ± 0.2, $P < 0.001$), and thus the final carcass yield was significantly higher (Table 5). The CF and CFg followed a similar pattern throughout the trail as that from the lipid level: they did not increase during the two first periods, but then increased sharply in the period July–September. At the final sampling in September, the length, CF, CFg, and gutted weight were all significantly higher for salmon fed the HP diet compared to those fed the LP diet (Table 5).

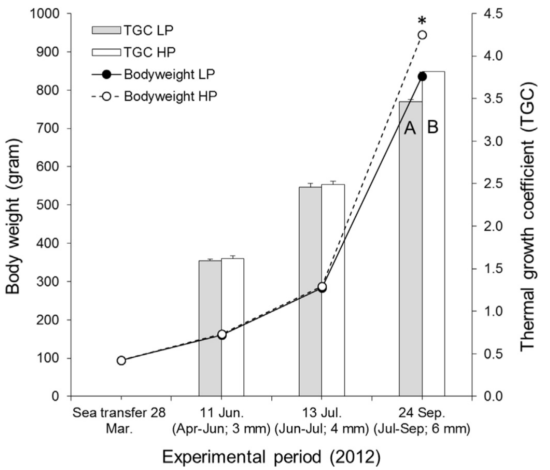

Fig. 3. Changes in body weight (lines) and thermal growth coefficient (bars) for S1 Atlantic salmon fed isoenergetic diets with high (HP) or low (LP) protein/lipid ratio. Significant differences between dietary groups within each sampling (11 Jun, 13, Jul and 24 Sep) or feeding period (Apr–Jun; 3 mm, Jun–Jul;4 mm and Jul–Sep; 6 mm) are indicated by * over the lines and different letters on bars. Data are presented as means ± SEM, n = 4.

3.3. Whole body analysis and nutrient retention

The fish fed the LP diet had significantly higher whole body lipid and energy content than the fish fed the HP diet at the sampling in July. The levels of whole body fat and energy were not different between the two groups at the final sampling in September. However, fish in the HP group had a significantly higher protein content than those in the LP group at the September sampling (Table 6). The relative retention of protein (% of ingested) did not differ between the dietary groups in

Table 6
Whole body composition of lipids, protein and energy at each sampling point (mean ± SEM, n = 4).

Dietary group	LP	HP	Dietary effect (*P*-value)
11 June, Sampling 1, end of 3 mm diet			
Lipids (%)	9.6 ± 0.3	9.0 ± 0.0	0.075
Protein (%)	17.9 ± 0.1	18.1 ± 0.2	0.287
Energy (MJ kg^{-1})	8.0 ± 0.1	7.7 ± 0.0	0.098
23 July, Sampling 2, end of 4 mm diet			
Lipids (%)	10.9 ± 0.1	9.2 ± 0.3	0.003
Protein (%)	17.1 ± 0.1	17.2 ± 0.1	0.357
Energy (MJ kg^{-1})	8.4 ± 0.1	7.8 ± 0.2	0.011
24 September, Sampling 3, end of 6 mm diet			
Lipids (%)	16.4 ± 0.1	16.0 ± 0.3	0.301
Protein (%)	16.9 ± 0.1	17.6 ± 0.1	0.004
Energy (MJ kg^{-1})	10.3 ± 0.1	10.3 ± 0.1	0.867

Initial sampling before sea transfer, 29 March: Lipids; 12.0 ± 0.2 %, Protein; 17.3 ± 0.1 %, Energy; 8.8 ± 0.0 MJ kg^{-1}.

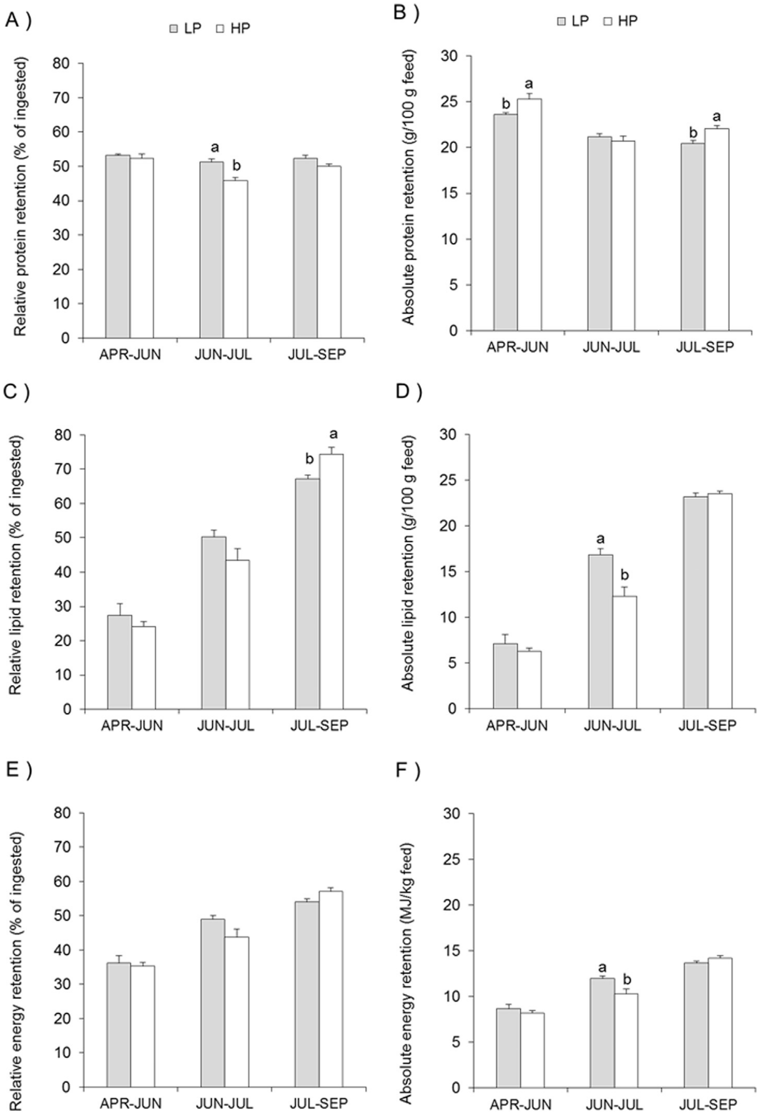

Fig. 4. Relative nutrient retention (% of ingested) of protein (A), lipid (C) and energy (E), and the absolute nutrient retention of protein (g 100 g−1 feed; B), lipid (g 100 g−1 feed; D) and energy (MJ kg−1 feed; F) for S1 Atlantic salmon fed isoenergetic diets with either a high (HP; white bars) or a low (LP; gray bars) protein/lipid ratio. Significant differences between dietary groups within each feeding period (Apr–Jun, Jun–Jul and Jul–Sep) are indicated by different letters over the bars. Data are presented as means ± SEM, n = 4.

the periods April–June or July–September. However, the absolute protein retention (g 100 g^{-1} feed) in the HP group was significantly higher than in the LP group during April–June (HP = 25.3 ± 0.6, LP = 23.6 ± 0.2, $P = 0.05$) and July–September (HP = 22.1 ± 0.3, LP = 20.4 ± 0.3, $P = 0.01$) (Fig. 4A and B). The relative protein retention differed significantly between the two diets only during June–July, when the retention of the protein was lower in the HP group than in the LP group HP = 45.8 ± 0.9%, LP = 51.2 ± 0.9%, $P = 0.01$ (Fig. 4A). No differences in absolute protein retention during this period were detected. In line with the whole body lipid in July, the LP group showed a trend towards higher relative lipid retention and significantly higher absolute lipid retention (HP = 12.4 ± 1.0, LP = 16.9 ± 0.6, $P = 0.01$) compared to the group fed the HP diet during the period June–July (Fig. 4C and D). In the period July–September, the HP group had a significantly higher relative lipid retention than the group fed the LP diet (HP = 74.4 ± 2.0%, LP = 67.2 ± 1.1%, $P = 0.02$, Fig. 4C), but no differences in absolute retention were observed (Fig. 4D). The relative retention of energy was not significantly different between the two dietary groups during the experiment (Fig. 4E). However, the absolute energy retention (MJ kg^{-1} feed) coincided with the absolute lipid retention with a significant difference between the groups in June–July (HP = 10.3 ± 0.5, LP = 11.9 ± 0.3, $P = 0.03$) (Fig. 4F).

3.4. Relationships between overall feed intake and other parameters

The overall daily feed intake was highly correlated with the temperature during the experiment ($r = 0.96$, $P < 0.001$). The relative lipid retention efficiency was positively correlated to the increase in feed intake ($r = 0.98$, $P < 0.001$). The SFR during the period July–September was negatively correlated with the level of muscle fat at the sampling in July ($r = -0.82$; $P = 0.01$).

4. Discussion

The feed intake and growth of salmon smolt are generally low during the first 4–8 weeks after seawater transfer (Alne et al., 2011; Jobling et al., 2002a; Oehme et al., 2010; Rørvik et al., 2007), and the manner by which feed intake and growth return to normal vary (Jobling et al., 2002a; Usher et al., 1991). After sea transfer, the fish need to adapt to new environmental conditions, a new feeding system and a new social hierarchy, and these are all factors that may influence feed intake and growth during the initial stages of a trial (Gilmour et al., 2005). In the present study, feed intake and growth improved as time progressed, and high SFRs (1.27–1.39) and TGCs (3.37–3.83) were observed during the latter stage of the trial in the period July–September. These corresponded to 120% of the growth predicted by Austreng et al. (1987) compared to only 40% during the April–June period. Condition factor, body lipids and energy all increased markedly during this period. These parameters often increase during the autumn (Alne et al., 2011; Mørkøre and Rørvik, 2001; Måsøval et al., 1994), which is a period when the duration of daylight declines rapidly and the water temperature is high. The changes by time in feed intake, growth, fat content and body shape are in line with those of previous studies of S1 smolt reared at the same site and under similar conditions (Alne et al., 2011; Mørkøre and Rørvik, 2001; Oehme et al., 2010). As in most poikilothermic species, feed intake was highest during the period July–September, when the average water temperature was 14 °C. This is in agreement with a study done by Handeland et al. (2008), showing that the feed intake of Atlantic salmon post-smolt is higher for those reared at 14 °C than for those reared at other temperatures (6, 10 and 18 °C).

Our results differ from those from Karalazos et al. (2007 and 2011), in which the dietary protein/lipid level did not affect growth when kept at a normal temperature (11 °C) or at low a temperature (4.2 °C). However, fish fed a diet with a low protein/lipid ratio tended to have lower final weights than fish fed other diets (Karalazos et al., 2011). Karalazos et al. (2007 and 2011) studied larger salmon (with initial weights of 1168 and 2053 g, respectively) and tested diets with a low inclusion of fishmeal and low protein/lipid ratios, ranging from 390/330 to 290/380 g kg^{-1}. Small salmonids require higher dietary proportions of digestible protein than larger salmonids (Cho and Kaushik, 1990; Einen and Roem, 1997), and this may explain why the results obtained in the previous studies differ from those presented here. Azevedo et al. (2004b) found no difference in weight gain or growth of rainbow trout or Atlantic salmon fed isoenergetic diets with different protein/lipid ratios. They used, however, a wild salmon strain, and both species were reared in freshwater with a constant temperature of 8 °C.

Salmonids seem to adjust their feed intake according to the dietary energy level (Bendiksen et al., 2002; Boujard and Medale, 1994), and this may be an influencing factor in trials in which feeds with different energy content are evaluated. Therefore, the use of isoenergetic diets eliminates this issue. Most studies that have investigated different protein/lipid levels for fish used diets with different total energy contents. Einen and Roem (1997) fed salmon reared from 1.0–2.9 kg in seawater diets that contained different protein/lipid levels and different energy levels. In this study, the TGC of a group fed a diet with a protein/lipid level of 480/308 g kg^{-1} (corresponding to a DP/DE ratio of 18.8 g MJ^{-1}) was significantly higher than that of a group fed a diet with a protein/lipid level of 425/364 g kg^{-1} (DP/DE of 16.4 g MJ^{-1}). The difference in growth observed in the latter study was only recorded during the last phase of the study, when the growth rates were high following a 60-day period with low appetite and growth. The results of Einen and Roem (1997) agree with those of the present study, and both indicate that a low ratio of dietary protein to lipids (below 16– 17 g MJ kg^{-1} DP/DE) reduces feed consumption in salmon. This in turn affects the intake of protein and other nutrients and reduces the availability of essential nutrients for optimal growth (Bendiksen et al., 2003b; Johansen et al., 2002; Shearer et al., 1997a; Shearer et al., 1997b; Silverstein et al., 1999). Our findings confirm this line of results using feed formulations, fish breed and rearing conditions commonly used in commercial farming of salmon.

The observed negative relation between muscle fat in July and the subsequent feed intake in the period July–September suggest that the significantly higher lipid deposition in the LP group may have suppressed appetite and reduced feed consumption. This, together with a leaner HP diet, may have contributed to a higher feed intake among HP fed fish in latter stages of our trial. The lower feed intake in the LP group than in the HP group is consistent with the theory of lipostatic regulation (Jobling and Johansen, 1999; Keesey and Corbett, 1984; Kennedy, 1953; Schwartz and Seeley, 1997). In accordance with this, the VSI of the group fed the LP diet increased continuously, indicating increased adiposity. However, the pure effect of body fat content on feed intake cannot be separated in the present trail. To be able to elucidate this, the two groups should have received the same feed in the period after achieving differences in lipid content.

The VSI of fish in the HP group did not increase during the experiment, whereas that of fish in the LP group increased gradually. Normally, an increase in VSI reflects a higher deposition of visceral fat (Bendiksen et al., 2003b; Hillestad et al., 1998; Jobling et al., 1998; Jobling et al., 2002a). The VSI correlated with both the visual assessment of visceral fat and the level of whole body lipids. This indicates that the HP group stored dietary lipids preferentially in the muscle, whereas the LP group stored lipids in both muscle and viscera. The muscle is the major site of fat deposition and storage in salmonids, accounting for 60–65% of the body mass (Aursand et al., 1994; Jobling et al., 2002a; Polvi and Ackman, 1992). The increase in VSI and consequent decrease in carcass yield of the LP group may suggest that dietary lipids were in excess, and the protein/lipid ratio unbalanced.

The increase in feed intake throughout the experiment (Table 4) correlated with the increased relative and absolute retention of energy and lipids (Fig. 4). Increased energy and lipid retention with increased feed intake are in accordance with the results obtained by Grisdale-Helland et al. (2013b). Our results are also consistent with the observation from Alne et al. (2011), who showed that S1 smolt had low relative lipid retention (~20%) during the spring and high relative lipid retention (~60%) during the autumn. The absolute lipid retention was identical between the two dietary groups during the autumn period, due to a significant up-regulated relative lipid retention for the HP group. This shift in relative lipid retention indicates that fat deposition and storage during this period are a high priority. However, it is noteworthy that although the absolute lipid retention was equal between the groups during autumn, the VSI of HP group was significantly lower than that in the LP group in September. The relative retention of protein was reasonably stable (at approximately 50%) and far less dynamic than the retention of lipid, as previously reported (Alne et al., 2011). The significantly higher absolute protein retention of the HP group compared with LP group during April–June and July–September, suggests that dietary protein was efficiently incorporated to body protein in the fish fed the HP diet during these periods. For the period Jul-Sep, the increased absolute protein retention coincided with the high CF, carcass yield and body protein content among the HP fed fish. These factors are again interrelated with the improved feed intake and growth in the fish fed the HP diet. The lower protein retention in the fish fed the HP diet compared to that in the fish fed LP diet in June–July is in accordance with several trials showing a protein sparing effect of reduced protein-to-lipid ratio within certain ranges (Einen and Roem, 1997; Grisdale-Helland and Helland, 1997; Grisdale-Helland et al., 2013a).

FCRb did not change significantly during the experiment. However, FCRg was significantly higher in fish fed the HP diet than it was in fish fed the LP diet during the period July–September (Table 4). This indicates that less of the dietary nutrients were used to increase the visceral mass, and more nutrients were used for carcass growth. This is consistent with the observed nutrient retention and is an important observation, as the carcass is the primary edible product for sale and holds the most value (often referred to as head on gutted, HOG, in relation to sale and price estimations).

5. Conclusion

Muscle fat content in fish fed high dietary protein-to-lipid ratio (HP) was significantly reduced compared to that in fish fed low dietary protein-to-lipid ratio (LP) prior to first autumn in sea, without any negative effects on growth and feed conversion. In the subsequent autumn period, fish fed the HP diet showed a significantly higher feed intake, growth rate and weight gain (almost 20%). During this period, HP fed fish presented a significantly higher absolute protein retention and reduced the visceral mass compared to LP fed fish, resulting in significantly higher whole body protein, condition factor, improved carcass yield and feed conversion based on gutted weight. The present study shows that it is possible to modulate lipid deposition and growth by seasonal and dietary interaction.

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Paper II

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ORIGINAL ARTICLE

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Improving production efficiency of farmed Atlantic salmon (*Salmo salar* L.) by isoenergetic diets with increased dietary protein-to-lipid ratio

Rúni Weihe[1,2] | Jens-Erik Dessen[2,3] | Regin Arge[2,4] | Magny Sissel Thomassen[2] | Bjarne Hatlen[5] | Kjell-Arne Rørvik[2,3]

[1]Havsbrún, Fuglafjørður, Faroe Islands

[2]Department of Animal and Aquaculture Sciences, Norwegian University of Life Sciences, Ås, Norway

[3]Nofima, Ås, Norway

[4]Fiskaaling, Hvalvík, Faroe Islands

[5]Nofima, Sunndalsøra, Norway

Correspondence
Rúni Weihe, Havsbrún P/F, Fuglafjørður, Faroe Islands.
Email: ruw@havsbrun.fo

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Abstract

The effects of isoenergetic diets with high (HP) and low (LP) protein-to-lipid ratios on feeding rate (SFR), feed conversion (FCR), growth (TGC) and relative- and absolute nutrient retention were investigated using both whole-body weight (BW) and carcass weight (CW) to assess the production efficiency. Three different feeding trials in seawater were conducted: two large-scale trials with yearling smolt (S1) and under-yearling smolt (S0) and one small-scale with S1 smolt. The initial body weights in the trials were 105, 319 and 978 g, respectively, and the fish were fed and monitored until they reached harvest weights. In all three trials, the dietary HP group attained significantly higher ($p < .05$) CW at harvest based on fish with equal BW. Also, fish fed the HP diets significantly improved FCR ($p < .05$) when based on CW. In the small-scale trial, fish fed HP diet, especially during late autumn and spring, significantly ($p < .001$) improved FCR_{BW} and FCR_{CW}. Improved FCR coincided with significantly higher ($p < .05$) relative energy retention in the dietary HP group. In all three trials, the HP groups had significantly higher ($p < .05$) TGC with regard to both BW and CW. Taken together, the present studies indicate that growth performance and feed utilization in modern salmon farming has the potential to be further improved by increasing the dietary protein-to-lipid ratio. In addition, dietary influence is more precisely assessed when using carcass as the weight denominator when analysing feed utilization and growth performance.

KEYWORDS
Atlantic salmon, carcass weight, dietary protein-to-lipid ratio, isoenergetic diets, nutrient retention, seasonal variation

1 | INTRODUCTION

In modern aquaculture production of Atlantic salmon, the dietary protein-to-lipid ratio generally decreases inversely with increasing body weight. Small salmon, such as, parr and smolt, are usually fed a diet with relative high protein content (>40%) and low lipid content (<30%). The commercial practice, especially in Norway, has been to give the salmon high-fat diets (≥35% lipid, ≤35% protein) from a body weight of approximately 1 kg (grower diets), while the protein content is reduced so that protein-derived energy is spared in favour of fat. A historical retrospective from the Norwegian aquaculture industry displays an approximately four times increase in lipid inclusion in the feed for salmon since the start of the industry in the 1970s (Tacon & Metian, 2009; Torrisen et al., 2011). Thus, during the relative short lifespan of the salmon farming industry, the dietary protein-to-lipid ratio in the grower diets has changed from near 5 to

1. With a shift towards higher content of lipid, the feeds have necessarily become denser in energy.

High-fat diets have previously been demonstrated to have beneficial effects on key production parameters such as growth rate and feed conversion (FCR) ratio (Hillestad, Johnsen, Austreng, & Åsgård, 1998; Karalazos, Bendiksen, & Bell, 2011; Karalazos, Bendiksen, Dick, & Bell, 2007). But studies have also indicated that high dietary fat intake may result in increased lipid content in both muscle and intestinal tissues of salmonids (Hillestad & Johnsen, 1994; Jobling, 1998, 2001; Jobling, Larsen, Andreassen, & Olsen, 2002; Refstie, Storebakken, Baeverfjord, & Roem, 2001). This may be undesirable since body lipids may act as a negative feedback signal on feed intake and thus impair growth (Johansen, Ekli, & Jobling, 2002; Johansen, Sveier, & Jobling, 2003; Silverstein, Shearer, Dickhoff, & Plisetskaya, 1999). Also, increased fat deposition in the visceral tissues may reduce the overall production yield.

Salmonids are poikilothermic, which means that their feed intake and growth are highly influenced by water temperatures (Brett, 1979; Jobling, 1997). Both sea temperatures and day length vary throughout the year, and previous experiments have demonstrated that Atlantic salmon responds greatly to the seasonal changes with regard to energy demand, feed intake, nutrient retention and growth (Alne, Oehme, Thomassen, Terjesen, & Rørvik, 2011; Hemre & Sandnes, 2008; Lysfjord, Jobling, & Solberg, 2004; Måsøval et al., 1994; Mørkøre & Rørvik, 2001; Oehme et al., 2010). In general, these studies seem to depict a high production efficiency during the autumn, which coincides with decreasing day lengths and peak sea temperatures in the salmon producing countries situated in the North Atlantic Ocean such as Norway, the British Isles and the Faroe Islands.

In general, it is a goal for all producers of animal proteins to increase utilization of feed resources. Thereto, a high turnover rate of production is crucial in most businesses. This is especially momentous in animal farming when the production area is limited. The Faroese aquaculture industry encounters significant limitations in biomass growth due to the relative limited coastline of the Faroe Islands (1,117 km), and virtually all potential farming areas are presently utilized. Currently, lack of well-established farming technology makes it difficult to farm salmon in exposed areas that surrounds the islands. Thus, the only realistic, short-term possibility for biomass increase for the Faroese aquaculture industry is through higher growth rate of salmon (shorter production cycle from sea transfer to harvest) and increased carcass-to-body weight yield.

Since final carcass is the primary tradable commodity, carcass weight and not only body weight, should be considered as the weight denominator when evaluating the dietary effects on FCR and growth performance. Thus, using the carcass weight as a biometric measurement of dietary effects, a more complete picture, both nutritional and economical, may be achieved when assessing overall feed efficiency in salmon production. Previous experiments have displayed high carcass-to-body weight yields (≥90%) (Einen & Roem, 1997; Hillestad & Johnsen, 1994; Hillestad et al., 1998; Wathne, 1995). Although there might be a lack of detailed definition of carcass weight in these studies, these results may indicate that the carcass-to-body weight ratio has been somewhat higher compared with some of the yields (~ 83%) recently observed in the industry (Waagbø et al., 2013). Therefore, it may be questioned whether the changes seen in the dietary protein-to-lipid composition have been in favour of obtaining high carcass growth throughout the marine production phase of salmon. In this context, diets with low protein-to-lipid ratios may not utilize the full potential of carcass growth in salmon, and thus the industry has not been assessing what protein-to-lipid composition is needed to achieve a more optimal production throughout the whole seawater phase, especially in the grow-out phase from approximately 1 kg until harvest. During this phase of production, the dietary protein-to-lipid ratio is at the lowest, however, most of the weight gain is generated as the fish is harvested between 4 and 6 kg (Nystøyl, 2017).

The aim of the present work was, consequently, to examine the effects of different dietary protein-to-lipid ratios on feed utilization and fish growth rate using both whole-body weight and carcass weight in assessing the feed effects on overall production efficiency. In addition, the effect of seasonal influence on biometric performance was examined together with the potential interaction of dietary effects.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Three dietary high protein-to-lipid ratio (HP) and three lower protein-to-lipid ratio (LP) feeding strategies were first tested in two different commercial large-scale farming sites in the Faroe Islands with yearling (S1) and under-yearling smolt (S0) following a small-scale trial which was conducted in Norway using S1 smolt. In all three experiments, the protein and lipid contents in the LP diets were designed to resemble those of a typical commercial diet for the respective sizes of fish, whereas the HP diets had higher protein and lower lipid contents. The total energy from lipid, protein and carbohydrates were targeted to be equal in the HP and LP diets for each pellet size.

Compared with large-scale feeding experiments in commercial conditions in general, small-scale trials ensure more accurate measurements of feed intake, biomass and equal slaughter time. Therefore, the present small-scale trial was conducted to test the reproducibility and validity of the dietary influences as well as to complement the observations from the large-scale experiments with a more scientific approach with regard to feed intake, feed utilization and dietary retention of nutrients.

2.2 | Experimental diets

All feeds were produced by Havsbrún (Fuglafjørður, Faroe Islands). Multiple batches of feed were produced throughout the large-scale experimental period and two feed batches per dietary treatment were produced for the small-scale trial (Table 1). The main dietary raw materials used in the large-scale experiments, ranked from highest to lowest inclusion level, were fishmeal, fish oil, wheat, soy protein concentrate, wheat gluten and sunflower meal. In the small-scale experiment, the ingredients used were, fishmeal, fish oil, rapeseed oil, wheat, krill meal and porcine blood meal. For all three trials, premixes containing pigments, minerals and vitamins were included in the diets to fulfil the minimum nutritional requirements in accordance with the National Research Council (1993, 2011). The estimated feed digestibility was calculated in compliance with Morris et al. (2003) assuming apparent digestibility coefficients for protein and lipid to be 0.86 and 0.94 (Einen & Roem, 1997), respectively, and 0.50 for nitrogen-free extractives (NFE) (Arnesen & Krogdahl, 1993). The feed production process included standard manufacturing routines regarding the control of physical pellet quality as well as the monitoring and control of proximate feed composition. Table 1 states the proximate composition of the experimental diets. These were based on the weighted mean from each feed batch supplied to the fish farming sites. The 3 and 4 mm HP diets in the S1 large-scale were intended to be the same (52% protein and 24% lipid). The relative large compositional deviation of the 3 mm HP feed was caused by manufacturing problems in addition to wrongful handling of feed during transport, which resulted in the dietary HP fish group being supplied with some 3 mm LP feed instead of HP feed. Thus, the dietary HP group was fed a combination of both HP and LP feed for approximately 4 weeks.

2.3 | Fish and facilities—large-scale trials

In the large-scale S1 trial, salmon smolt were supplied by Bakkafrost hatchery station in Glyvradalur and transferred to the Bakkafrost commercial seawater site at Lambavík (62°08′N, 06°41′W), Faroe Islands, during April 2009. Duplicate 128 m circumference cages with a water volume of 18,500 m^3 were used for rearing the fish per dietary treatment. Mean number of fish per net pen was 66,627 (*SEM* = 213). The fish were subjected to 1000 W artificial light (L:D 24:0) from 10 December 2009 until 21 March 2010. We identified an error regarding the body weight measurement of the stocked fish 5 months after the trial initiation which caused unequal starting weights between the dietary treatments, showing that the dietary LP group was 8% bigger (LP = 104 ± 10 g vs. HP = 96 ± 2 g, n = 2). To achieve equal starting weights per dietary treatment, a triplicate cage, also fed HP diet since sea transfer, was included. This was considered necessary to achieve reliable data to examine dietary influence based on comparable fish groups with equal starting weights. Thus, mean body weight at sea transfer for the fish group fed the LP diet was 104 g (*SEM* = 10, n = 2) vs. 105 g (*SEM* = 10, n = 3) after adjustment of the HP fed smolt group. Feeding of the fish in the experimental cages started in week 19 (May 2009). There was a great algal bloom during the period July–August 2009 at the S1 trial site causing a severe decrease in feeding rate within both dietary treatments. The average sea water temperature through the S1 experimental period was 8.5°C with a maximum and minimum of 11.1°C and 5.7°C respectively (Figure 1a). Salmon-fed HP feed had an average production period of 452 ± 11 days and 3,752 ± 109 day degrees, whereas the production duration of the dietary LP group was 477 ± 27 days and 3,971 ± 266 day degrees.

S0 smolt from Luna's hatchery station in Fútaklettur had been transferred to Luna's commercial sea farming site in Sørvágur (62°04′N, 7°20′W), Faroe Islands, in October 2008. In March 2009, when the feeding trial started, the fish had a mean body weight of 319 g (*SEM* = 5, n = 4) with a mean number of 60,392 fish per cage (*SEM* = 245). Duplicate cages per dietary treatment of 24 m × 24 m, with a water volume of 6,912 m^3, were used in the beginning of the trial. In June 2009, all the fish were transferred by towing the cages approximately 1 km southwest across the fjord (62°04′N, 07°22′W) and restocked in 128 m circumference cages with a water volume of 18,500 m^3, maintaining the same experimental groups. The transportation time was approximately 3.5 hr per cage. The S0 experimental fish were subjected to 1,000 W artificial light (L:D 24:0) from 14 December 2009 until 15 March 2010. The average sea water temperature through the S0 experimental period was 8.4°C where the peak temperature was 10.7°C and the lowest temperate was 5.8°C. The average production period for the dietary HP group was 429 ± 6 days and 3,597 ± 42 day degrees whilst the dietary LP group had a production period of 439 ± 11 days and 3,688 ± 97 day degrees respectively. Figure 1a gives an overview of the temperature and day length in both large-scale trials.

Four different pellet sizes were used within the dietary treatments in the S1 large-scale experiment, whereas two pellet sizes were used within the dietary treatments in the S0 large-scale trial (Table 2). The pellet sizes were adjusted to fit the fish weight according to the guidelines of the feed manufacturer.

2.4 | Fish and facilities—small-scale trial

The small-scale experiment with S1 post-smolt was conducted at Nofima's research station at Ekkilsøy (currently owned by Marine Harvest Fish Feed AS) on the west coast of mid-Norway (63°03′N, 07°35′E) in 2012. In all, 150 post-smolt salmon weighing 978 g (*SEM* = 1, n = 6) were randomly distributed in each of six cages measuring 5 m × 5 m × 5 m. Prior to this, the fish had been transferred to sea as yearling (S1) smolt (95 g) in April 2012 from Salmar's hatchery station in Straumsnes, and then been involved in an earlier feeding trial (Dessen, Weihe, Hatlen, Thomassen, & Rørvik, 2017) and fed the same high-protein diets through three different periods from April to September. During the last period from 23 July to 24 September in this pre-trial, the post-smolt grew 658 g, ending up with a final body weight of 926 g and a whole-body composition of 17.6% protein and 16.0% fat.

The experimental diets (HP and LP 9 mm, Table 1) used in the small-scale trial were fed to triplicate groups of fish from 27 September 2012 until trial termination on 10 June 2013. The trial was split into three feeding periods representing three different seasons; 1: 27 September–4 December (late autumn), 2: 7 December–8 April (winter) and 3: 11 April–10 June (spring) respectively (Figure 1b). Fish were fed to satiation daily using automatic feeders four times a

TABLE 1 Proximate feed compositions (wet weight) used in all three experiments. Brackets demonstrate the number of feed batches used in the experiment per pellet size per dietary treatment. Values are given as weighted means per diet. HP: dietary high protein-to-lipid ratio strategy. LP: dietary low protein-to-lipid ratio strategy

Smolt group	Large-scale S1		Large-scale S0		Small-scale S1	
Diet	HP	LP	HP	LP	HP	LP
Pellet size 3 mm	(*n* = 4)	(*n* = 2)				
Dry matter, %	93.3 ± 0.1	93.1 ± 0.2				
Crude protein, %	49.9 ± 0.7	46.6 ± 0.3				
Lipid, %	25.6 ± 1.4	27.2 ± 0.2				
Ash, %	9.4 ± 0.5	8.7 ± 0.2				
Starch, %[a]	6.7 ± 0.1	8.6 ± 0.2				
DP. %[b]	42.4 ± 0.6	40.0 ± 0.2				
DE, MJ/kg[b]	20.3 ± 0.4	20.5 ± 0.0				
DP:DE, g/MJ[b]	20.9 ± 0.7	19.5 ± 0.1				
Protein-to-lipid ratio	1.95	1.71				
Pellet size 4 mm	(*n* = 5)	(*n* = 2)				
Dry matter, %	94.1 ± 0.1	93.4 ± 0.2				
Crude protein, %	52.1 ± 1.4	45.8 ± 0.3				
Lipid, %	22.1 ± 1.8	28.7 ± 0.6				
Ash, %	11.0 ± 0.2	8.6 ± 0.3				
Starch, %[a]	6.9 ± 0.2	8.7 ± 0.3				
DP. %[b]	44.8 ± 1.2	39.4 ± 0.3				
DE, MJ/kg[b]	19.6 ± 0.4	20.9 ± 0.2				
DP:DE, g/MJ[b]	22.9 ± 1.0	18.9 ± 0.3				
Protein-to-lipid ratio	2.36	1.60				
Pellet size 6 mm	(*n* = 7)	(*n* = 2)	(*n* = 2)	(*n* = 7)		
Dry matter, %	95.6 ± 0.1	94.2 ± 0.1	94.1 ± 0.3	93.9 ± 0.2		
Crude protein, %	46.6 ± 0.5	41.9 ± 0.2	44.4 ± 0.3	42.7 ± 0.5		
Lipid, %	27.6 ± 0.4	32.4 ± 0.2	30.8 ± 0.7	31.6 ± 0.4		
Ash, %	9.5 ± 0.4	8.1 ± 0.2	8.2 ± 0.2	7.8 ± 0.1		
Starch, %[a]	8.6 ± 0.7	8.9 ± 0.0	8.3 ± 0.4	9.0 ± 0.0		
DP. %[b]	40.1 ± 0.5	36.1 ± 0.2	38.2 ± 0.3	36.7 ± 0.5		
DE, MJ/kg[b]	20.8 ± 0.1	21.6 ± 0.1	21.4 ± 0.2	21.5 ± 0.1		
DP:DE, g/MJ[b]	19.3 ± 0.1	16.7 ± 0.1	17.9 ± 0.3	17.1 ± 0.2		
Protein-to-lipid ratio	1.69	1.29	1.44	1.35		
Pellet size 9 mm	(*n* = 71)	(*n* = 10)	(*n* = 20)	(*n* = 10)	(*n* = 2)	(*n* = 2)
Dry matter, %	93.7 ± 0.2	94.1 ± 0.1	94.0 ± 0.2	94.2 ± 0.1	94.1 ± 1.0	94.3 ± 0.5
Crude protein, %	42.0 ± 0.2	35.4 ± 0.1	40.2 ± 0.3	34.5 ± 0.2	42.7 ± 0.1	35.4 ± 0.4
Lipid, %	32.6 ± 0.2	35.9 ± 0.1	34.4 ± 0.2	35.8 ± 0.2	32.1 ± 0.7	36.0 ± 0.6
Ash, %	8.1 ± 0.1	6.4 ± 0.1	8.0 ± 0.1	6.7 ± 0.1	7.9 ± 0.2	7.1 ± 0.2
Starch, %[a]	8.4 ± 0.2	9.6 ± 0.1	9.1 ± 0.1	9.8 ± 0.8	8.5 ± 0.2	11.0 ± 0.4
DP. %[b]	36.1 ± 0.1	30.4 ± 0.1	34.6 ± 0.3	29.6 ± 0.2	36.7 ± 0.1	30.4 ± 0.3
DE, MJ/kg[b]	21.6 ± 0.1	22.0 ± 0.0	21.9 ± 0.1	21.8 ± 0.1	21.6 ± 0.3	21.9 ± 0.3
DP:DE, g/MJ[b]	16.7 ± 0.1	13.9 ± 0.1	15.8 ± 0.1	13.6 ± 0.1	17.0 ± 0.2	13.9 ± 0.0
Protein-to-lipid ratio	1.29	0.99	1.17	0.96	1.33	0.98

[a]Starch content was not analysed in all feed batches. The stated value is the average of the analysed batches.
[b]Digestible protein and digestible energy were calculated, based on the measured proximate feed composition, assuming 23.7, 39.5 and 17.2 MJ per kg of protein, lipids and nitrogen-free extractives (NFE) respectively. The apparent digestibility coefficients used for protein, lipid and NFE in Atlantic salmon were 0.86 (Einen & Roem, 1997), 0.94 (Einen & Roem, 1997) and 0.50 (Arnesen & Krogdahl, 1993).

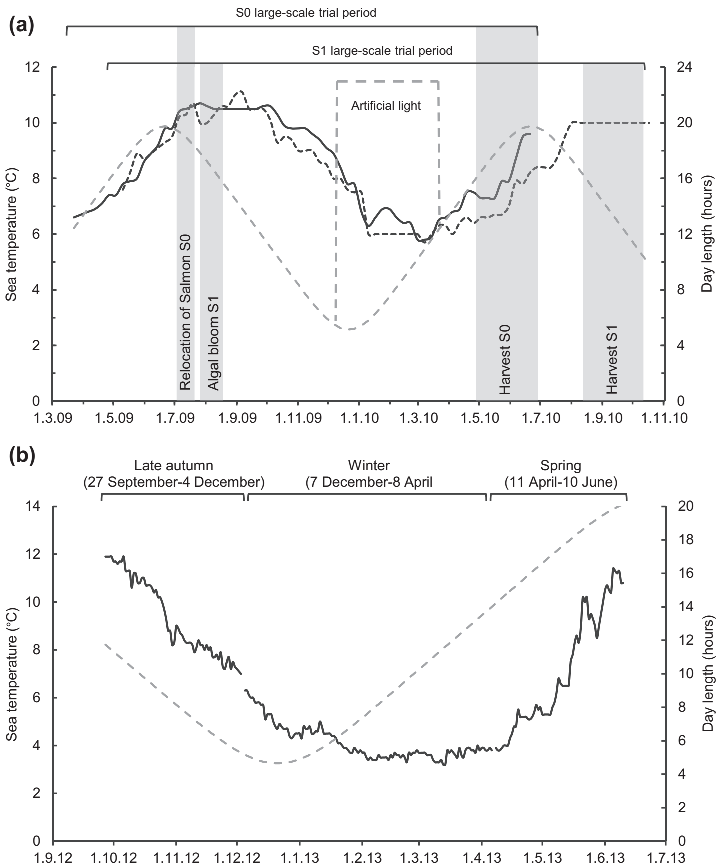

FIGURE 1 (a) Weekly seawater temperature (°C) for the large-scale S0 trial (solid black line) and the large-scale S1 trial (broken black line) displayed on the *y*-axis. (b) Daily seawater temperature (solid black line) during the S1 small-scale experiment is displayed on the *y*-axis where the sampling periods that identify the three feeding periods are noted above the figure. Average day length per week (hr) for the large- and small-scale experiments are illustrated with broken grey line displayed on the *z*-axis

day from 27 September to 25 October. Subsequently, until trial termination in June, the fish were fed three rations per day. The daily feed rations were approximately 10% in excess of the feed eaten the day before. Waste feed was collected daily as described by Einen, Mørkøre, Rørå, and Thomassen (1999) and analysed for recovery of dry matter as described by Helland, Grisdale-Helland, and Nerland (1996). The average sea water temperature in the three experimental periods was 9.4°C (612 day degrees), 4.1°C (490 day degrees) and 7.1°C (427 day degrees) respectively. Figure 1b illustrates the changes in temperature and day length during the small-scale trial.

2.5 | Sampling procedures large-scale

Fish from the experimental cages were harvested following standardized routines of the farming respective companies (Bakkafrost and Luna). This included a starvation period of 3–5 days prior to slaughter, and the average harvesting time per cage in the S1 and S0 trials was two and 4 weeks respectively. In the S1 large-scale trial, the fish were transported with well boat to the Bakkafrost harvesting facilities in Klaksvík (62°23′N, 06°59′W) during the period from week 28 (July) to week 41 (November) 2010. The experimental S0 fish were harvested at Luna's harvesting facility in Sørvágur (62°07′N, 07°32′W) from week 17 (April) to week 25 (June) 2010 after dragging the experimental cages approximately 2 km from the production site to the harvesting facilities at the head of the fjord. At both harvesting facilities, the salmon were killed and bleed using an automated swim-in system (SI-7 Combo, killing and bleeding machine) and subsequently transported to a bleeding tank with a water temperature between 0°C and −1°C to bleed out.

At the first day of slaughter of each experimental cage in the S1 trial, 30 fish were sampled and divided into three weight classes á 10 fish of 4.5 kg, 5.5 kg and 6.5 kg average weight respectively. All the sampled fish were handpicked from the bleeding tank at the harvesting facilities. In one experimental unit (cage no. 4) in the large-scale S1 trial fed HP feed, only 10 fish, respectively, of 4.5 kg and 5.5 kg were sampled. In the S0 experiment, 30 fish from all experimental cages were sampled 8 April (week 14), and divided into the mentioned weight classes. All samples in both large-scale experiments were recorded and measured for body weight, length and carcass weight. Carcass weight was defined as the weight after removal of blood, viscera, heart and kidneys. The measured body weights were corrected for 2.7% blood loss in accordance with Einen, Waagan and Thomassen (1998) to calculate live weight at slaughter.

During the harvest period, the total number of fish and gutted biomass was recorded and harvest reports were generated for each experimental unit and the body weight of fish and biomass within each cage was calculated. We chose to use the carcass-to-body weight ratio per cage, measured at first day of harvest, to convert the carcass weights in the harvest reports to whole-body weight and biomass within each experimental cage. The harvest reports depict a difference within the smolt groups regarding the number of production days in the experimental units and thus a difference in day degrees were used to achieve about the same body weight within dietary treatments at harvest.

2.6 | Sampling procedures small-scale

At the end of each feeding period (Figure 1b), all fish within each experimental unit were anaesthetized (MS 222 metacaine 0.1 g/L, Alpharma, Animal Health, Hampshire, UK) and bulk-weighed for determination of specific feeding rate (SFR), growth rate (presented as thermal growth coefficient, TGC) and FCR. When sampling fish in the first two periods, 10 fish representing the average body weight in each unit were stunned with a blow to the head and bled out. These fish were then individually weighed, length measured and gutted, and carcass weight registered. In line with the large-scale trials at trial termination, 30 fish from each cage were collected and divided into three weight classes. Because the experimental fish did not grow as big as the fish in the large-scale trials, the three groups of 10 fish were divided in subgroups of 2.4, 3.2 and 4.0 kg. Also, an additional 10 fish (not bled) representing the mean body weight per experimental unit were sampled for whole-body analysis of protein, fat and energy. The fish were starved for 4 days prior the sampling in December, whereas the fish were starved for 3 days prior to the

TABLE 2 Overview of the feeding period for each pellet size within both dietary treatments in the large-scale trials. The pellet sizes are fed in relation to the preferred fish weight intervals which is also given

	Pellet size used (mm)	Preferred fish weight (g)	First feed delivery	Feeding period
Large-scale S1				
HP	3	~ 100–150	07.04.2009	9 weeks (week 15–week 24)
	4	~ 150–300	16.06.2009	11 weeks (week 24–week 35)
	6	~ 300–800	28.08.2009	6 weeks (week 35–week 41)
	9	~ 800+	08.10.2009	44 weeks (week 41–week 33)
LP	3	~ 100–150	27.03.2009	10 weeks (week 13–week 23)
	4	~ 150–300	04.06.2009	11 weeks (week 23–week 34)
	6	~ 300–800	18.08.2009	7 weeks (week 34–week 41)
	9	~ 800+	19.10.2009	49 weeks (week 41–week 38)
Large-scale S0				
HP	6	~ 300–800	18.03.2009	16 weeks (week 12–week 28)
	9	~ 800+	09.07.2009	35 weeks (week 28–week 21)
LP	6	~ 300–800	04.03.2009	20 weeks (week 10–week 30)
	9	~ 800+	26.06.2009	39 weeks (week 26–week 23)

TABLE 3 Differences in specific feeding rate (SFR), feed conversion (FCR) and growth rate (TGC) based on live body weight (BW) and carcass weight (CW) in S1 and S0 Atlantic salmon in the large-scale experiments. Significant differences between dietary treatments (D) and smolt group (SG) and the interaction (D × SG) in the two-way ANOVA are given whilst the values in brackets depict statistical trends, and non-significant differences are highlighted as ns. Dietary statistics within smolt group is visualized by *p*

Smolt group	S1			S0			Two-way ANOVA			
Dietary group	HP (n = 3)	LP (n = 2)	*p*	HP (n = 2)	LP (n = 2)	*p*	D	SG	D ×SG	R^2
SFR	0.55 ± 0.01	0.56 ± 0.02	ns	0.51 ± 0.02	0.52 ± 0.02	ns	ns	0.03	ns	.50
FCR_{BW}	1.29 ± 0.03	1.36 ± 0.03	ns	1.21 ± 0.03	1.25 ± 0.02	ns	(.06)	0.01	ns	.73
FCR_{CW}	1.47 ± 0.04	1.57 ± 0.01	ns	1.40 ± 0.02	1.47 ± 0.03	ns	.03	0.04	ns	.67
TGC_{BW}	3.18 ± 0.04	2.98 ± 0.07	(.06)	3.16 ± 0.03	3.09 ± 0.09	ns	.04	ns	ns	.46
TGC_{CW}	3.05 ± 0.03	2.84 ± 0.07	(.06)	2.99 ± 0.03	2.91 ± 0.09	ns	.02	ns	ns	.59

samplings in April and June. At each sampling, all fish with obvious signs of wounds, runts or sexual maturity were removed (weights and number of these fish was recorded).

2.7 | Feed chemical analyses

In all three experiments, the feeds were analysed for moisture (drying loss at 103°C to stable weight; ISO 6496), ash (combustion at 550°C, ISO 5984), crude protein (N × 6.25, combustion according to the Kjeldahl principle, ISO 5983) and crude fat was analysed using pre-extraction and post-extraction in petroleum ether after HCL hydrolysis (98/64/EC). In the large-scale trials, total- and gelatinized starch was analysed as d-glucose following enzymatic cleavage with gluco-amylase after full gelatinization by cooking with NaOH. In the small-scale trial, the total starch content was analysed as glucose after enzymatic hydrolysis employing the Megazyme K-TSTA 07/11 kit (Megazyme International, Ireland) in accordance with AOAC method 996.11. The energy content was determined using a Parr 6400 Oxygen Bomb Calorimeter (Parr Instrument Company, USA) following the NS-EN 14918:2009 standard. Nitrogen-free extractives were calculated as dry matter—(protein + lipid + ash).

2.8 | Fish chemical analyses

Homogenates of whole fish samples were analysed for crude protein and energy as described for feeds. Whole-body crude fat was analysed using a semi-automatized Soxhlet extractor (Tecator Soxtec Avanti 2055) with petroleum ether as the extracting solvent. Whole-body energy content was assessed using bomb calorimetry (Parr, Moline, IL, USA).

2.9 | Calculations

SFR together with FCR and TGC based on whole-body weight (FCR_{BW}, TGC_{BW}) were measured in all three trials in accordance with the calculations in Dessen et al. (2017) in addition to the calculations of nutrient retention in the small-scale trial. The overall SFR, TGC, FCR and retention means in the small-scale trial were calculated as the weighted arithmetic mean of the three seasons to balance the values in relation to their relative contribution to the weight gain. In the large-scale trials, the calculations were based on the data given by the production programme FarmControl (AKVA Group, Norway) which was used on both farming sites, whereas the calculations in the small-scale trial were based on the bulk weighings of the experimental fish at the end of each feeding period. Feed conversion based on carcass weight (FCR_{CW}) in the large-scale trials was calculated as follows: feed eaten (kg) × (biomass increase (kg) + biomass of dead fish (kg) × 0.83)$^{-1}$ where 0.83 is a standard estimation of carcass-to-body weight ratio within the industry to calculate the carcass weight of the dead fish. In the small-scale trial, the measured carcass-to-body weight ratio was used for each feeding period. Growth based on the gutted biomass (TGC_{CW}) was calculated as the TGC_{BW} using carcass weight (CW) instead of whole-body weight.

2.10 | Statistical analysis

In the large-scale trials, data were analysed using two-way analysis of variance (ANOVA) with interaction using the general linear model (GLM) procedure, in which the two class variables were dietary treatment (D; HP and LP) and smolt group (SG; S1 and S0), and the dependent variables were SFR, FCR, TGC, BW and CW. Two-way ANOVA was also used to analyse the data in the small-scale trial based on a randomized block design, using season (S), diet (D) and the potential interaction between season and diet as class variables to assess their influence on the production performance. If only two means were compared, Student's *t*-test was applied to test dietary differences within season (small-scale experiment) and smolt group (large-scale experiment). Only significant models are presented and the proportion of total variation explained by the model is expressed as R^2, which was calculated as between-group sum of squares divided by the total sum of squares (type III). All analyses were conducted using SYSTAT® 13 software package (SYSTAT Software Inc., USA) and SAS software package (SAS institute Inc., 1990). Fish cage mean was used as the experimental unit. Results are presented as mean ± *SEM* if not otherwise stated. $p \leq .05$ was chosen as level of significance and $p \leq .10$ was considered as a trend.

3 | RESULTS

3.1 | Large-scale experiments

3.1.1 | Mortality

In the S1 trial, cages fed the HP diet had a lower (p = .03) mortality rate (4.5 ± 0.1%) compared with the LP-fed fish (6.3 ± 0.3%). In January and February 2010, the number of dead fish was considerably higher than in the rest of the trial period. Most of the dead fish in this period had visible wounds and damages derived from seal predation. No mortality differences between dietary treatments within the S0 smolt group were detected (HP: 2.2 ± 0.4% vs. LP: 1.6 ± 0.1%).

3.1.2 | Feed intake, feed conversion and growth performance

The S1 smolt group had a significantly higher feeding rate than the S0 group, but there were no differences between the dietary treatments within the smolt groups (Table 3).

FCR_{BW} was significantly higher in the S1 than in the S0 smolt group (Table 3). There was also strong trend (p = .06) towards higher FCR_{BW} in fish fed the LP diet than those fed the HP diet. This trend became significant between the dietary treatments when assessing the FCR based on carcass weight (Table 3). Thus, the 5.4% and 3.3% improvement in FCR_{BW} for the salmon provided with HP feed in the S1 and S0 groups, respectively, increased to 7.3% and 4.8% when carcass weight was used as the conversion weight denominator.

There were no significant interaction effects of smolt group and diet on FCR_{BW} or FCR_{CW}.

Salmon fed the HP diet grew significantly faster both in terms of body weight (TGC_{BW}) and carcass weight (TGC_{CW}) (Table 3). The dietary influence on carcass growth within both smolt groups may be visualized by the significant higher carcass weight within the dietary HP groups of the sampled fish at harvest which had virtually equal body weight as the dietary LP groups (Figure 2).

3.2 | Small-scale experiment

3.2.1 | Mortality

Three fish died in the dietary HP group, and no mortality was registered within the fish-fed LP diet throughout the trial.

3.2.2 | Feed intake, feed conversion and growth performance

Diet, season and their interaction significantly explained 99% of the variation in feed intake during the trial (Table 4). Both dietary fish groups had the highest feeding rates during late autumn where the LP group had significantly higher SFR than dietary HP group. Feed intake decreased in all the experimental units during the winter period, following a SFR increase during the spring season until harvest in June.

Throughout the trial, both dietary treatments had an increase in FCR_{BW} and FCR_{CW} and decrease in TGC_{BW} and TGC_{CW} with increasing body weight (Figure 4a, Table 4). Overall, both season and diet significantly influenced FCR ratios as well as growth rates. Based on the overall weighted mean, the dietary HP group had significantly better FCR and growth rate measured with both whole-body weight and carcass weight (Table 4). During the late autumn period, salmon fed the HP diet attained both lower FCR and higher TGC compared with the LP group, resulting in significant body weight differences between the dietary treatments in December (Figure 3a,b). During the winter period, the dietary HP group had numerically better FCR based on both BW and CW and maintained a significant higher CW (Figure 3b), whilst there were virtually no differences in TGC between the dietary treatments. From April and onwards, the dietary HP group had significantly lower FCR ratios and numerically better growth rates than the dietary LP group. Thus, fish fed the HP feed attained significantly higher BW and CW than the dietary LP group at trial termination (Figure 3a,b). Corresponding with the results in the large-scale trials, the relative differences between the dietary treatments in feed utilization became more apparent when FCR and TGC were calculated with basis on CW (Table 4). Within dietary treatments, a significant negative linear relationship between FCR_{BW} and TGC_{BW} was observed in the dietary HP group, and a virtual significant relationship was detected for the LP group as well (Figure 4a). There was no significant interaction between season and diet on FCR or TGC.

3.2.3 | Nutrient retention

Overall, the dietary LP group had significantly higher RnR_P whilst no difference was observed for AnR_P (Table 4). Despite the numerical higher RnR_P for the dietary LP group during the winter and spring feeding periods, season had not a significant influence on RnR_P or AnR_P. The season × diet interaction had no significant influence on protein retention.

Both RnR_L and AnR_L were highest during the late autumn and decreased throughout the trial period and were significantly influenced by season (Table 4). The overall weighted mean of RnR_L was virtually significantly higher ($p = .07$) for the dietary HP group, whereas there were no differences in the AnR_L. In the winter period, the dietary LP group had significantly higher AnR_L, but except for this observation, there were no significant dietary differences between the dietary treatments within season. No significant interaction effects of season and dietary treatment were observed on lipid retention. Within the dietary LP group, a near significant negative linear relationship was observed between the absolute retention of lipid and FCR_{BW}, whilst a similar and steeper pattern was observed within the dietary HP group although not significant (Figure 4b). A significant positive linear relationship was detected between AnR_L and TGC_{BW} (Figure 5a), and an overall negative linear relationship between AnR_L and FCR_{BW} (Figure 6a).

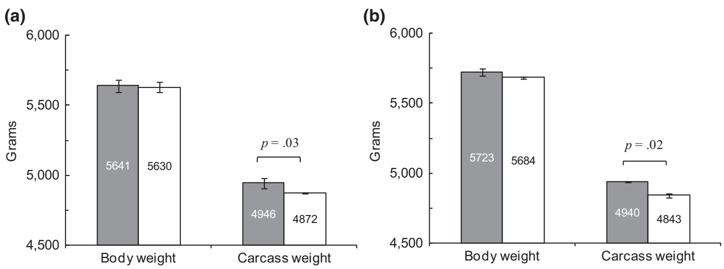

FIGURE 2 Average body weight and carcass weight of S1 (a) and S0 (b) Atlantic salmon in the large-scale trial sampled on the harvest line with respect to achieving identical weight classes of 4.5, 5.5 and 6.5 kg respectively. Grey and white bars illustrate the dietary HP and LP fish groups respectively. Brackets denote significant differences between dietary treatments. Values are presented as means ± *SEM*.

TABLE 4 Seasonal differences in specific feeding rate (SFR), feed conversion (FCR) and growth rate (TGC) based on live body weight (BW) and carcass weight (CW), relative nutrient retention (RnR: % of ingested) and absolute nutrient retention (AnR: g per 100 feed for protein and fat, and MJ/kg feed for energy) of protein (P), lipid (L) and energy (E), respectively, in S1 Atlantic salmon from September to June in small-scale experiment (mean ± *SEM*, $n = 3$)

Period	Sep–Dec		Dec–Apr		Apr–Jun		Two-way ANOVA				Overall weighted mean	
Dietary group	HP	LP	HP	LP	HP	LP	D	S	D × S	R^2	HP	LP
SFR	0.87 ± 0.01[b]	0.93 ± 0.01[a]	0.31 ± 0.01	0.33 ± 0.01	0.43 ± 0.01	0.44 ± 0.00	<0.001	<0.001	<0.01	.99	0.55 ± 0.01[b]	0.58 ± 0.00[a]
FCR_{BW}	0.94 ± 0.01[b]	1.05 ± 0.01[a]	1.04 ± 0.03	1.09 ± 0.01	1.13 ± 0.02[b]	1.22 ± 0.02[a]	<0.001	<0.001	ns	.89	1.03 ± 0.00[b]	1.12 ± 0.00[a]
FCR_{CW}	1.07 ± 0.01[b]	1.21 ± 0.02[a]	1.19 ± 0.04	1.26 ± 0.02	1.30 ± 0.05[b]	1.46 ± 0.02[a]	<0.001	<0.001	ns	.87	1.18 ± 0.02[b]	1.30 ± 0.00[a]
TGC_{BW}	3.71 ± 0.06[a]	3.52 ± 0.02[b]	3.18 ± 0.09	3.18 ± 0.05	2.53 ± 0.06	2.41 ± 0.03	0.05	<0.001	ns	.97	3.19 ± 0.00[a]	3.08 ± 0.02[b]
TGC_{CW}	3.55 ± 0.05[a]	3.36 ± 0.02[b]	3.03 ± 0.08	3.02 ± 0.02	2.41 ± 0.09	2.23 ± 0.03	0.02	<0.001	ns	.96	3.04 ± 0.02[a]	2.92 ± 0.02[b]
RnR_P	38.9 ± 2.9	38.8 ± 3.8	40.4 ± 4.1	50.9 ± 3.5	32.4 ± 4.8	43.5 ± 1.4	0.05	ns	ns	.22	37.7 ± 0.9[b]	44.7 ± 0.2[a]
RnR_L	77.7 ± 1.4	68.0 ± 4.7	61.0 ± 5.5	64.4 ± 6.2	53.8 ± 4.7	40.3 ± 8.6	ns	0.002	ns	.57	65.0 ± 0.9	58.9 ± 2.3
RnR_E	57.0 ± 0.9[a]	53.0 ± 1.4[b]	46.5 ± 3.0	51.1 ± 1.5	45.0 ± 3.3[a]	40.1 ± 2.9[b]	ns	0.001	ns	.61	49.9 ± 0.9	48.8 ± 1.7
AnR_P	16.6 ± 1.2	13.7 ± 1.4	17.3 ± 1.8	18.0 ± 1.2	13.8 ± 2.0	15.4 ± 0.5	ns	ns	ns	—	16.1 ± 0.4	15.7 ± 0.1
AnR_L	25.0 ± 0.3	24.5 ± 1.0	19.6 ± 1.8[b]	23.2 ± 2.2[a]	17.3 ± 1.5	14.5 ± 3.1	ns	0.001	ns	.59	20.9 ± 0.3	21.2 ± 0.8
AnR_E	12.3 ± 0.1	11.6 ± 0.2	10.0 ± 0.7	11.3 ± 0.3	9.7 ± 0.7[a]	8.8 ± 0.6[b]	ns	0.001	ns	.63	10.8 ± 0.2	10.7 ± 0.4

Significant differences between dietary treatments within season are denoted with small letters. Significant *p*-values in the two-way ANOVA and non-significant differences are highlighted as ns.

Comparable with the results of lipid retention, both RnR_E and AnR_E were highest during the late autumn and decreased throughout the trial (Table 4). Together with a block influence ($p < .01$), the HP group salmon had significantly higher RnR_E during the late autumn whilst the differences in AnR_E were not observed. During the spring season, both RnR_E and AnR_E were significantly higher for the dietary HP group. The dietary LP group had numerically higher energy retention, both relative and absolute, during the winter season. Trends ($p = .10$) were observed for the season × diet interaction in both RnR_E and AnR_E. Analogues with AnR_L results, there was an overall positive linear relationship between AnR_E and TGC_{BW} (Figure 5b), and an overall negative linear relationship between AnR_E and FCR_{BW} (Figure 6b).

4 | DISCUSSION

Several studies have previously explored the effects of dietary protein and lipid content on fish growth performance (Azevedo, Leeson, Cho, & Bureau, 2004; Einen & Roem, 1997; Hillestad & Johnsen, 1994; Hillestad et al., 1998; Karalazos et al., 2007, 2007) but virtually all studies consider fish performance on live fish weight basis only. Because fresh, head-on gutted salmon (HOG) is the primary commodity in the industry, achieving a certain defined harvest weight is a central production focus. Thus, evaluating the dietary protein-to-lipid influence on fish performance based on carcass weight is vital so that it can be better understood how dietary combinations influence the growth of the product as well as the growth of the fish. The present study documents that dietary influences may not be detected unless the biometric performance is assessed on carcass weight. This was clearly demonstrated with the sampling of the dietary fish groups which had equal body weights at harvest but had significantly different carcass weights, and thus illustrating how different protein-to-lipid ratios influence the weight gain of whole body and carcass differently.

Regardless of whether the growth rate is calculated based on whole-body weight or carcass weight, all presented experiments demonstrated that increased dietary protein-to-lipid ratios contributed to significantly improved growth, becoming even more evident when based on carcass weight. Corresponding with the recommendations from Einen and Roem (1997), the presented results display that DP:DE ratios >16 g/MJ improves fish growth and increases the carcass growth in relation to whole-body growth. This stands in contrast with the dietary composition used in the modern salmon farming industry (Tacon & Metian, 2009; Torrisen et al., 2011; Ytrestøyl, Aas, & Åsgård, 2015) where the general increase in dietary energy is derived from higher proportions of lipid. Therefore, it is likely that within the farming industry, the intake of fat might be excessive and that this fat is to a greater extent deposited into visceral tissue (Hillestad & Johnsen, 1994; Jobling, 1998, 2001; Jobling et al., 2002; Refstie et al., 2001) and thus not converted into tradeable carcass. Proteins and amino acids are the major organic compounds in fish tissue (National Research Council 2011;

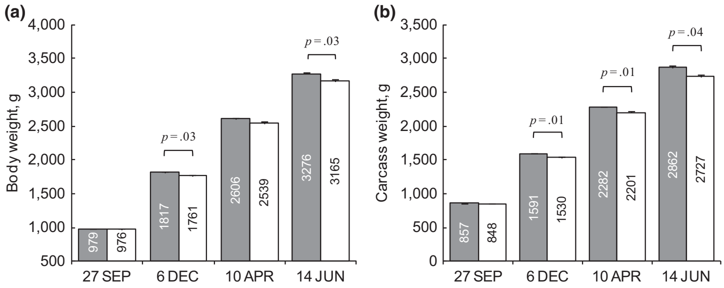

FIGURE 3 Average body weight (a) and carcass weight (b) of Atlantic salmon fed isoenergetic diets with high (HP: grey bars) and low (LP: white bars) protein-to-lipid ratio in the small-scale trial. Brackets denote significant differences between dietary treatments within sampling periods. Values are presented as means ± *SEM*, *n* = 3

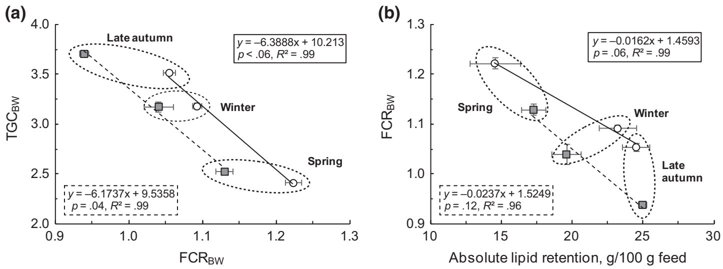

FIGURE 4 Relationships between feed conversion (FCR_{BW}) and growth (TGC_{BW}) responses (a), and absolute retention of dietary lipid and feed conversion (FCR_{BW}) (b) in Atlantic salmon fed isoenergetic high dietary protein-to-lipid ratio (HP: shaded squares) and low dietary protein-to-lipid ratio (LP: open circles) during late autumn, winter and spring in the small-scale trial. Values are presented as means ± *SEM*, *n* = 3

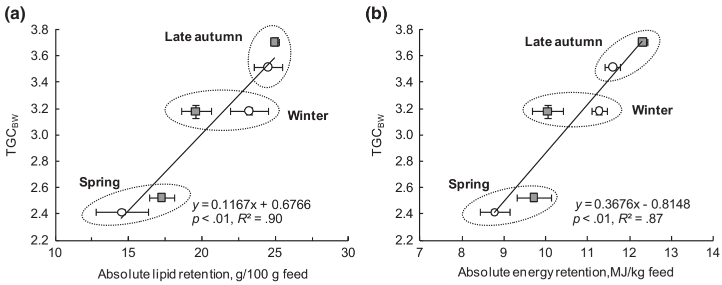

FIGURE 5 Growth response (TGC_{BW}) in relation to the absolute retention of dietary lipid (a) and dietary energy (b) in Atlantic salmon fed isoenergetic high dietary protein-to-lipid ratio (HP: shaded squares) and low dietary protein-to-lipid ratio (LP: open circles) throughout three seasons in the grow-out period, respectively, late autumn, winter and spring. Values are presented as means ± *SEM*, *n* = 3

Wilson, 2002) and like most fish species, salmon continue growing through most of the life (Kiessling, Ruohonen, & Bjørnevik, 2006). Therefore, sufficient amount of dietary proteins and amino acids are necessary to support optimal salmon growth and to convert feed into tradeable carcass. According to Einen, Holmefjord, Åsgård, and Talbot (1995), a satisfying growth rate for well performing farmed salmon has a TGC_{BW} of 3.3. Unfortunately, the sea temperature in the winter period in the small-scale trial was the lowest recorded in a 15-year long period. In poikilotherms, lower temperatures impair feed intake and restrict availability of nutrients which ultimately decreases metabolic processes (Bureau, Kaushik, & Cho, 2002; Kestemont & Baras, 2001). Thus, the record low temperature has likely hindered potential feed effects within both treatments.

Within both smolt groups in the large-scale studies, salmon fed the dietary HP feeds had both shorter production period and higher harvest weight than the LP fed salmon. Due to differences in time of slaughter and day degrees, dietary influence on the final body weight differences can be objectively assessed and estimated using

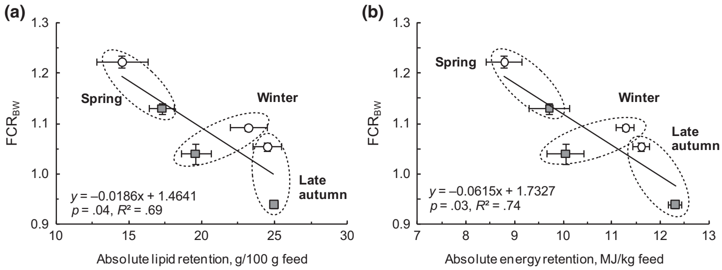

FIGURE 6 Relationships between feed conversion (FCR_{BW}) and absolute retention of dietary lipid (a) and dietary energy (b) in Atlantic salmon fed isoenergetic high dietary protein-to-lipid ratio (HP: shaded squares) and low dietary protein-to-lipid ratio (LP: open circles) during late autumn, winter and spring in the small-scale trial. Values are presented as means ± *SEM*, $n = 3$

the TGC_{BW} formula. This was performed using the same initial body weight within in each smolt group (S1: 105 g, S0: 319 g), the obtained TGC_{BW} (S1: HP = 3.18 vs LP = 2.98, S0: HP = 3.16 vs LP = 3.09) for each dietary treatment together with the same total day degrees used in the production of the dietary LP groups (S1: 3971, S0: 3688) respectively. The calculation demonstrated that the dietary HP group attained an increased body weight of 685 g and 261 g relatively to the LP group, in the S1 and S0 smolt group respectively. Hence, considering the presented results together with the recommendation from Einen and Roem (1997) indicate that the overall production of salmon carcass in the farming industry has a great potential to improve by increasing the protein-to-lipid ratio throughout the whole production period whilst maintaining an overall high-energy dense feed composition.

The FCR_{BW} tended towards being lower for the HP groups compared with the LP groups in the large-scale trials, but by the improvements in carcass weight among the HP groups the difference became significant when assessed as FCR_{CW}. Dessen et al. (2017) also made such an observation, which again highlights the importance of considering carcass weight as the weight denominator when assessing feed influence on biometric fish performance. Nonetheless, the dietary improvements for the HP groups, in the large-scale trials, all FCRs were generally high compared with the overall average conversion rates in the Faroese salmon industry (Nystøyl, 2017). A reason for this might be that there has been some overfeeding. In commercial production, great effort is put into controlling feeding quantities so that no feed is wasted. The opposite is applicable in small-scale experiments, where overfeeding is used to ensure that all fish is fed to satiation with a subsequent collection of the uneaten feed (Einen et al., 1999; Helland et al., 1996). The differences in dietary effect on FCR between the HP and LP treatments correspond in all three experiments and the relative improved influence of the HP diet are considered valid since the large-scale results were reproduced in the small-scale experiment.

Within the small-scale trial, both dietary treatments had the best biometric performances during the late autumn. Corresponding with the presented results, this is a period associated with fast growth (Mørkøre & Rørvik, 2001) and high retention of dietary energy, whereof most is derived from fat (Alne et al., 2011). However, there were no significant differences in either relative or absolute retention of nutrients between the dietary treatments during the autumn, suggesting that the higher FCR in the dietary LP group was related to higher feed intake. Previous studies have indicated an inverse relationship between inclusion rates of protein and lipid and the relative retention of these nutrients, respectively (Bendiksen, Berg, Jobling, Arnesen, & Måsøval, 2003; Einen & Roem, 1997; Hillestad & Johnsen, 1994; Hillestad et al., 1998; Karalazos et al., 2007), but this was not observed within any of the three feeding periods. Nonetheless, the dietary LP group had an overall significantly higher RnR_P and the dietary HP group had nearly overall significantly higher RnR_L ($p = .07$). Despite this, there were no differences between the dietary groups in the absolute retention of either protein or lipid and no correlations of relationship identified between the AnR_P and growth performance. This might indicate that the salmon needs a relative stabile intake of protein, and because the dietary LP group had lower protein content in the diet, the group had to compensate by moderately increasing the feed intake to ensure necessary proteins for maintenance, whereas the dietary HP group had sufficient proteins to increase carcass weight beyond maintenance requirements. However, apart from the late autumn, the were no dietary differences in feed intake in the other trial periods, stressing that feed responses are a results of feed composition, intake and utilization, especially in periods with high lipid retention. The latter may be visualized by improved FCR for the dietary HP group in the late autumn period and revealing an overall relation between FCR and the absolute retention of lipids, and overall strong correlations between FCR and TGC within both dietary treatments.

Although the HP group in the S1 large-scale trial were exposed to predator attacks in January and February, the mortality rates in the large-scale trials were generally low and consistent with the rates observed within the Faroese salmon industry (Nystøyl, 2017). Dietary-related differences in mortality were not observed in any of the three experiments.

In summary, high dietary protein-to-lipid ratios (≥1.2) throughout the whole production period of Atlantic salmon significantly improves both growth and feed utilization compared with an isoenergitic diet with lower protein-to-lipid ratio (≤ 1). A high protein-to-lipid feeding strategy induces greater carcass weight gain, and the improvements in FCR and growth rate become larger and more evident when calculated based on carcass weight. The fish performance is also greatly influenced by season whereof autumn seems the period where feed utilization and growth have the highest potential to be optimized. Thus, the presented study indicates that it is possible to attain faster growth and improved FCR in modern Atlantic salmon industry, by increasing the current dietary protein-to-lipid ratios, especially during the autumn.

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ORCID

Rúni Weihe http://orcid.org/0000-0002-2760-4352
Jens-Erik Dessen http://orcid.org/0000-0002-0667-4183
Regin Arge http://orcid.org/0000-0001-5657-8352

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Paper III

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Increased protein-to-lipid ratio in energy dense diets improves slaughter yields and muscle thickness of different weight classes of farmed Atlantic salmon (*Salmo salar* L.)

Rúni Weihe[a,b,*], Jens-Erik Dessen[b,c], Regin Arge[b,d], Magny S. Thomassen[b], Bjarne Hatlen[e], Kjell-Arne Rørvik[b,c]

[a] *Havsbrún, Bakkavegur 48, Fuglafjørður, FO-530, Faroe Islands*
[b] *Department of Animal and Aquaculture Sciences, Norwegian University of Life Sciences, NO-1432, Ås, Norway*
[c] *Nofima, NO-1432, Ås, Norway*
[d] *Fiskaaling, Við Áir 11 Hvalvík, FO-430, Faroe Islands*
[e] *Nofima, NO-6600, Sunndalsøra, Norway*

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ABSTRACT

Two isoenergetic feeding strategies, with emphasis on the grow-out stage (> 1 kg), with dietary high (HP > 1.1) and low protein-to-lipid ratio (LP < 1.0) were tested by using year-old smolt (S1) and under-year-old smolt (S0) Atlantic salmon in two large-scale trials, respectively, and in one small-scale experiment using S1 smolt. This was done to investigate the dietary influence on slaughter yield, muscle fat content, condition factor and thickness of the hypaxial anterior muscle (HAM) in all three trials. In addition, effects on the viscerosomatic index (VSI) was included in the small-scale trial. The initial body weights in the three trials were 100 g, 319 g and 978 g, respectively. At harvest, fish for analyzes were sampled into three weight classes of 4.5 kg, 5.5 kg, 6.5 kg in the large-scale trials, and 2.4 kg, 3.2 kg and 4.0 kg in the small-scale trial. In all three trials, the dietary HP strategy significantly improved slaughter yield ($p < 0.01$). In the large-scale trials, fish of the HP groups had lower muscle fat ($p < 0.05$), higher condition factor (significant in the S1 group: $p < 0.01$) and a trend towards a thicker HAM ($p \le 0.10$) than the LP groups. In all three trials, there were a significant positive relation between condition factor and HAM. The small-scale trial verified the large-scale trials revealing significantly lower VSI ($p < 0.001$) among the HP groups, partly explaining the high increase in slaughter yield (1.1%) for the HP groups compared to LP groups in the large-scale study. Except for slaughter yield and VSI, weight class significantly influenced all quality traits. Overall, this study indicates that the salmon farming industry, which generally prefers using lipid dense grower feeds, can improve product yields by using isoenergetic feeds with dietary high protein-to-lipid ratio.

1. Introduction

Like in other meat productions, farming Atlantic salmon is about converting feed into edible tissue. In modern salmon farming the primary commodity is fresh head-on gutted salmon which value chain intermediates further process to consumer demanded products. The major energy carriers in feeds for salmon are protein and lipids. Modern commercial salmon grower feeds (made for fish > 1 kg) have most of their energy as lipids, whereas the protein content has decreased over the years, leading to a gradual reduction in the protein-to-lipid ratio in the diet. This dietary strategy has proven to be successful to support good growth and feed utilization (Hillestad et al., 1998; Karalazos et al., 2007, 2011). However, sufficient dietary protein content with a good balance of amino acids is vital to support muscle growth, protein deposition and weight gain (Bureau et al., 2002). Salmon appears to increase fat deposits in both muscle and visceral tissue with increasing lipid content in the feed (Bendiksen et al., 2003; Einen and Roem, 1997; Hillestad et al., 1998; Jobling et al., 2002a). Thus, diets with high and low protein-to-lipid ratios are likely to have divergent influence on the quality characteristics of farmed salmon.

Processing yields greatly influence the economic performance of the value chain for salmon. Therefore, it is important for actors along the value chain to purchase salmon with characteristics that contribute to high yields. Earlier findings have demonstrated that yields are influenced by characteristics such as body shape and muscle fat content (Einen, 1998; Mørkøre et al., 2001; Rørå et al., 1998, 2001). Slaughter yield is highlighted as a central quality trait (Rasmussen, 2001), and

* Corresponding author at: Havsbrún PF, Bakkavegur 48, 530 Fuglafjørður, Faroe Islands.
E-mail address: ruw@havsbrun.fo (R. Weihe).

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according to Einen and Roem (1997) and Hillestad et al. (1998), a low-protein/high-fat feeding strategy reduces slaughter yield in salmon. Although these results also seem to be influenced by fish size and feeding rate, lipid dense diets still seem to be the preferred grower feeds within the industry. High slaughter yields are desired since it is the carcass that is the primary source of income for salmon farmers, but these previous findings suggest that the preference of using a low dietary protein-to-lipid strategy leaves an unexploited product yield in the industry.

At harvest, farmed salmon is normally graded into weight classes based on their carcass weight (1-2 kg, 2-3 kg, 3-4 kg and onwards to 9+ kg), often referred to as head-on gutted (HOG) and the various weight classes are priced differently. Historically, salmon price per kg has increased with the increase in weight class (Fish Pool, 2018). Quality characteristics of farmed fish is highly influenced by body weight (Shearer, 1994). However, grouping salmon into different weight classes has rarely been conducted in scientific studies when evaluating dietary effects on quality characteristics and product yield. Quality characteristics such as slaughter yield, fillet yield and fat content can be modulated by starvation or by altering feed rations prior to harvest (Einen et al., 1998, 1999; Wathne, 1995). Today, starvation prior to harvest is done for a short period, mainly to deplete gut content. Although a prolonged starvation period modifies the quality attributes, this is not an optimal production strategy as it also decreases the overall productivity and biomass (Einen, 1998). To optimize the quality, it is necessary to gain knowledge on how the conversion of feed into edible salmon tissue is influenced by dietary composition. It is therefore important that the desired quality characteristics are attained during the feed conversion process itself and not through starvation or feed restriction, so that production losses are avoided.

In the salmon industry, smolts are regularly transported to sea in the spring as year-old smolt (S1) or in the autumn as under-year-old smolt (S0). Given that the smolt weights are relatively similar when transferred to sea, they will reach harvest weights at different times of the year. Because these two smolt types are produced under different circumstances, their growth patterns diverge (Mørkøre and Rørvik, 2001; Roth et al., 2005). However, irrespective of smolt type and time of harvest, the HOG products enter the same markets and follow the normal pricing mechanisms. With the use of lipid dense feeds in modern salmon farming, this paper questions if the potential of farmed salmon destined for further value-added processes is fully utilized. Hence, the main objective of the present study was to evaluate the effects of iso-energetic diets differing in protein-to-lipid ratio on slaughter characteristics of different weight classes of farmed S1 and S0 Atlantic salmon. In an earlier study, we analyzed the dietary influence on specific feeding rate, feed conversion and growth rate (Weihe et al., 2018) which are considered as key performance indicators in salmon production. Since salmon is usually subjected to value adding processes after harvest, by using the same dataset as in Weihe et al. (2018) the present study focuses on slaughter yield, muscle fullness and body shape as key quality attributes. To ensure commercial relevance, the present study used data from two commercial salmon production cycles in addition to data collected through a more controlled study in small-scale.

Table 1
Chemical feed compositions (as is) in all three experiments. Brackets demonstrate the number of feed batches used in the experiment per pellet size per dietary treatment. Values are given as weighted means per diet. HP: dietary high protein-to-lipid ratio strategy. LP: dietary low protein-to-lipid ratio strategy.

Experiment (smolt group)	Large-scale S1		Large-scale S0		Small-scale S0	
Diet	HP	LP	HP	LP	HP	LP
Pellet size 3 mm	*(n = 2)*	*(n = 4)*				
Dry matter, g kg^{-1}	933	931				
Crude protein, g kg^{-1}	499	466				
Lipid, g kg^{-1}	256	272				
Ash, g kg^{-1}	94	87				
Starch, g kg^{-1a}	67	86				
DE, MJ kg^{-1b}	20.3	20.5				
Protein-to-lipid ratio	1.95	1.71				
Pellet size 4 mm	*(n = 3)*	*(n = 5)*				
Dry matter, g kg^{-1}	941	934				
Crude protein, g kg^{-1}	521	458				
Lipid, g kg^{-1}	221	287				
Ash, g kg^{-1}	110	86				
Starch, g kg^{-1a}	69	87				
DE, MJ kg^{-1b}	19.6	20.9				
Protein-to-lipid ratio	2.36	1.60				
Pellet size 6 mm	*(n = 2)*	*(n = 7)*	*(n = 2)*	*(n = 9)*		
Dry matter, g kg^{-1}	956	942	941	939		
Crude protein, g kg^{-1}	466	419	444	427		
Lipid, g kg^{-1}	276	324	308	316		
Ash, g kg^{-1}	95	81	82	78		
Starch, g kg^{-1a}	86	89	83	90		
DE, MJ kg^{-1b}	20.8	21.6	21.4	21.5		
Protein-to-lipid ratio	1.69	1.29	1.44	1.35		
Pellet size 9 mm	*(n = 10)*	*(n = 70)*	*(n = 10)*	*(n = 19)*	*(n = 2)*	*(n = 2)*
Dry matter, g kg^{-1}	937	941	940	942	941	943
Crude protein, g kg^{-1}	420	354	402	345	427	354
Lipid, g kg^{-1}	326	359	344	358	321	360
Ash, g kg^{-1}	81	64	80	67	79	71
Starch, g kg^{-1a}	84	96	91	98	85	110
DE, MJ kg^{-1b}	21.6	22.0	21.9	21.8	21.6	21.9
Protein-to-lipid ratio	1.29	0.99	1.17	0.96	1.33	0.98

aStarch content was not analysed in all feed batches. The stated value is the average of the analysed batches
bDigestible energy (DE) was calculated based on the measured proximate feed composition, assuming 23.7, 39.5 and 17.2 MJ kg of protein, lipids and nitrogen-free extractives (NFE), respectively. The apparent digestibility coefficients used for protein, lipid and NFE were 0.86 (Einen and Roem, 1997), 0.94 (Einen and Roem, 1997) and 0.50 (Arnesen and Krogdahl, 1993).

2. Material and methods

2.1. Experimental design and -diets

Three feeding experiments were conducted using a dietary high protein-to-lipid (HP) and a dietary low protein-to-lipid (LP) feeding strategy in all three trials. Two of the trials were conducted in large-scale commercial seawater facilities using year-old smolt (S1) and under-year-old smolt (S0), and the third feeding trial was carried out in a small-scale facility using S1 smolt. The proximate composition of protein and lipid in the LP diets in all three trials were designed to resemble common commercial diets. The HP diets were designed to have similar energy as the LP diets but with a greater proportion of the energy deriving from protein, increasing the dietary protein-to-lipid ratio.

All the experimental feeds were produced by Havsbrún (Fuglafjørður, Faroe Islands). Because the large-scale trials were conducted in commercial/industrial conditions, multiple batches of feed were produced throughout the experiments whereas in the small-scale trial, two batches per dietary group were used (highlighted with brackets in Table 1). In accordance with standard commercial feed manufacturing, the physical and nutritional quality was monitored throughout the production process. Also, in line with industrial practice, quality specifications and definitions of the feed ingredients were updated quarterly together with the respective raw material prices. Ranked from highest to lowest inclusion level, the main feed ingredients in the large-scale trials were: fishmeal, fish oil, wheat, soy protein concentrate, wheat gluten and sunflower meal. The same ranking in the small-scale trial was: fishmeal, fish oil, rapeseed oil, wheat, wheat gluten and soy protein concentrate. Within all three trials, the HP and LP feeds were supplied with identical vitamin- and mineral premixes. Based on the intended dietary protein and lipid balance, all feeds were composed and produced on a least-cost production strategy.

Feed digestibility was calculated in accordance with Morris et al. (2003), assuming that the apparent digestibility coefficients for protein, lipid and nitrogen free extractives were 0.86, 0.94 (Einen and Roem, 1997) and 0.50 (Arnesen and Krogdahl, 1993), respectively. The chemical composition of the experimental feeds is shown in Table 1. These are based on the weighted mean from each batch supplied to the farming sites. The 3 mm and 4 mm HP diets in the S1 large-scale experiment were intended to contain 52% protein and 24% lipid. The relative large deviation in protein and lipid composition in the 3 mm HP feed was caused by production problems as well as wrongful transport handling which lead to some LP feed being supplied to fish in the dietary HP group. Consequently, fish in the HP group were fed a combination of both HP and LP feed for approximately 4 weeks.

To investigate if feed intake would influence the quality traits, feed intake was measured as specific feeding rate (SFR) in all three experiments. In the large-scale trials, the SFR was measured for the whole experimental periods only, whereas the SFR was split into three feeding periods in the small-scale trial. There were no differences in SFR between the dietary treatments within the large-scale experiments, whereas differences in feeding rate between the dietary groups was observed in the small-scale trial during the initiating autumn period from September to December. An overview of the SFR for all three experiments is given in Table 2.

2.2. Fish material and rearing conditions in the large-scale trials

The S1 and S0 large-scale feeding trials were conducted at commercial farming sites in Lambavík (62°08′N, 06°41′W, Bakkafrost PF) and Sørvágur (62°04′N, 07°22′W, Luna PF), on the east coast and west coast of the Faroe Islands, respectively. Duplicate cages per experimental diet were used on both sites. The S1 trial started when the smolts were stocked in May 2009, whereas the S0 trial started in March 2009 after the smolts had been stocked in October 2008 and fed 3 mm (48% protein, 27% lipid) and 4 mm (46% protein, 30% lipid) feeds until March, similar to the diets described in Dessen et al. (2017). The weight and number of fish in the S1 group at trial initiation was 100 ± 5 g and 66,627 ± 213 (mean ± SEM), respectively, whereas in the S0 smolt group, the weight and number of fish was 319 ± 5 g and 60,371 ± 243 (mean ± SEM), respectively. Feeding and production on both sites followed the routines of the respective fish farming companies Bakkafrost and Luna. An overview of the design is presented in Fig. 1. See Weihe et al. (2018) for more details about the rearing conditions in the large-scale trials.

Table 2

Specific feeding rate (SFR) for year-old (S1) and under-year-old (S0) Atlantic salmon in the two large-scale trials as well as in the small-scale trial, fed diets with either high protein-to-lipid ratio (HP) or low protein-to-lipid ratio (LP). Significant differences between the dietary treatments are depicted with p-values, whereas non-significance is abbreviated as ns. Data are given as mean ± SEM. (n = 2 in the large-scale trials; n = 3 in the small-scale trial).

		SFR, %		ANOVA	
		HP	LP	P	R^2
Large-scale S1		0.55 ± 0.00	0.56 ± 0.02	ns	–
Large-scale S0		0.51 ± 0.02	0.52 ± 0.02	ns	–
Small-scale S1	Sep - Dec	0.87 ± 0.01	0.93 ± 0.01	0.006	0.88
	Dec - Apr	0.31 ± 0.01	0.33 ± 0.01	ns	–
	Apr - Jun	0.43 ± 0.01	0.44 ± 0.00	ns	–

2.3. Fish material and rearing conditions in the small-scale trial

The third feeding trial was conducted in 2012 with S1 smolt in small-scale facilities at Nofima's research station at Ekkilsøy (currently owned by Marine Harvest Fish Feed AS) on the west coast of Norway (63°03′N, 07°35′E). At trial initiation, 150 S1 salmon weighing 978 ± 1 g (mean ± SEM) were randomly distributed in six 5 × 5 x 5 m cages in three blocks, and the two dietary treatments were fed to triplicate groups of fish from 27th of September 2012 to 10th of June 2013 divided into three feeding periods: (1) 27th of September to 4th of December, (2) 7th of December to 8th of April, and (3) 11th of April to 10th of June (Weihe et al., 2018). Fish were fed to daily satiation by approximately 10% overfeeding based on the feed consumption from the day before, following a subsequent collection of excess feed which was analyzed for recovery and dry matter as described by Einen et al. (1999) and Helland et al. (1996), respectively. The fish material had previously been stocked in sea as 95 g smolt in April 2012 and were fed high-protein diets in an earlier feeding experiment until September 2012 which is presented in Dessen et al., (2017). During this earlier trial, the salmon had a body weight gain of 850 g, ending up with a body composition of 17.6% protein and 16.0% lipid. Fig. 1 gives an overview of all three feeding experiments, and Weihe et al. (2018) have more details about the rearing conditions in all three trials.

2.4. Sampling procedure in the large-scale trials

The final sampling in the S0 trial was conducted 8th of April 2010 (~12 months after trial initiation and ~17 months after stocking) which represented the date when the commercial harvest started. In the S1 trial, fish from three experimental units were sampled during 12th-20th of August (~16 months after initiation) whereas the fourth unit was sampled 1st of October (~17 months after initiation). The sampling of fish in each experimental unit was conducted at the first day of harvest of each unit. Here, 10 fish with virtually identical body weight (Tables 3 and 4) within each weight class of 4.5 kg, 5.5 kg and 6.5 kg were sampled (30 fish in total from each cage), aiming to attain equal average body weight for each of the two dietary groups within each weight class. By changing body weight from being a continuous variable to a class variable with 10 fish making up the mean weight of each weight class, it would be easier and more reproducible to visualize

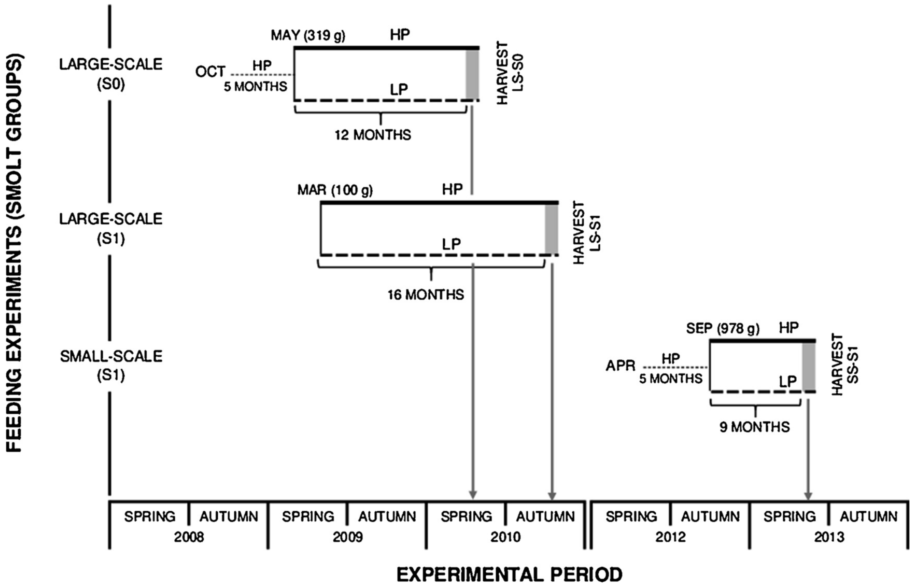

Fig. 1. Schematic overview of the three feeding trials in the experimental design specified with scale size (large-scale or small-scale), smolt group (S1 or S0) and the respective body weight of the salmon and month at trial initiation. The large-scale S0 trial started after the smolt had been in the sea for five months (thin dotted line) fed high-protein feed (HP), whilst this was also the case for the small-scale S1 salmon which had been fed HP feed prior to the trial initiation. For each feeding experiment, the high protein-to-lipid ratio feeding strategy (HP) is marked with a thick black line whereas the low protein-to-lipid ratio feeding strategy (LP) is marked with a black broken line. The shaded area represents the harvesting periods in each experment and highlighted with grey arrowed lines whether this was in the spring or autumn.

potential dietary influence on quality traits. Based on the operative software feeding systems that were used on the farming sites, which continuously estimated body weight development of the fish based on daily feeding quantities, in combination with the fact that the Faroese salmon generally has a harvest weight between 5 kg and 6 kg (Nystøyl, 2018), the chosen weight classes were considered to represent the main weight classes of the overall harvested fish. Larger weight classes yield higher prices per kilo (Fish Pool, 2018), and it is therefore reasonable to assume that salmon producers aim to attain high harvest weights ($>$ 4 kg) compared to lower weights ($<$ 4 kg). Thus, sampling salmon smaller than 4 kg did not seem to be very relevant from a commercial perspective.

The 3 × 10 fish in each experimental unit were sampled from the bleeding tank within the commercial harvesting facilities after the salmon had been killed and bled out using an automated swim-in killing and bleeding system (SI-7 Combo) complying to standard procedures at the commercial harvesting sites. The weights of sampled fish were corrected for 2.7% blood loss in accordance with Einen et al. (1998). First, body weight (BW) and body length (BL: fork length) of each fish was measured. Thereafter, the fish were cut open, cleaned and rinsed with water and carcass weight (CW) recorded. Carcass weight was defined as the weight after the fish was bled and all visceral contents removed, including heart, liver and kidneys. The thickness of the hypaxial anterior muscle (HAM) was measured before muscle samples were taken (Norwegian Quality Cut, NQC, NS 9401, Norwegian Standard, 1994a) for rapid analysis of muscle fat (MFAT).

2.5. Sampling procedure in the small-scale trial

At the end of each feeding period, all fish in the individual experimental cages were taken out and anesthetized in batches with MS-222 (Metacaine 0.1 g L^{-1}; Alpharma, Animal Health Ltd., UK) and subsequently bulk weighed and counted. Ten fish representing the average body weight in each cage were killed with a blow to the head and bled out. These fish were measured for body weight, visceral weight and analyzed for muscle fat.

At the final sampling 11th of June 2013, all fish in the individual experimental cages were anesthetized, bulk weighed and counted. The salmon were harvested over a three-day period, one block for each day. The fish in each block were starved for 3 days prior to harvest. Following the sampling procedure from the large-scale trials, thirty fish in each experimental unit were divided into subgroups of 10 fish, representing the weight classes of 2.4 kg, 3.2 kg and 4.0 kg and given a lethal dose of MS-222 before being individually measured for body weight and length. Thereafter, the sampled fish were cut open and visceral content weighed, followed up with measurements of carcass weight and muscle thickness (HAM), in accordance with the large-scale procedure. Finally, the NQC was cut for rapid analysis of muscle fat.

The seawater temperature during the trial were the coldest compared to the previous fifteen years at this location. Salmon are poikilothermic and therefore the colder temperatures had a negative influence on feed intake and subsequently growth. Thus, salmon in the small-scale experiment did not attain as high body weight as the salmon in the large-scale trials which ultimately resulted in sampling of smaller fish.

2.6. Fish analysis

The fat level (%) was predicted by digital image analyses, as described by Folkestad et al. (2008) by photographing the filleted left NQC cutlet using the PhotoFish box (PhotoFish, AKVAgroup, Bryne, Norway). The predictions made by the image analyses were calibrated against individual chemical analysis of fat (NS 9402, Norwegian Standard, 1994b) based on a great number of salmon with different body weight (0.5–7.0 kg) and levels of fat (3.6–22.9%). Highly significant correlation between the predicted and measured values for fat are documented ($p <$ 0.0001, $R^2 = 0.95$; MSE of 10 fish is 0.5%, Rørvik et al., 2014). This non-invasive method for determination of fat has been used successfully in previous studies of Atlantic salmon (Arge et al., 2012; Dessen et al., 2016; Rørvik et al., 2018). Muscle thickness (HAM) was measured with a slide caliper behind the pectoral fin above the belly flap, according to the section of the fish described by Einen et al. (1998).

2.7. Calculations

Specific feeding rate: SFR = (feed intake during the time period (kg) x average biomass weight during the time period (kg)) × 100^{-1}. Condition factor based on whole body weight: CF_{BW} = body weight (g)/body length (cm)^3. Condition factor based on carcass weight: CF_{CW} = carcass weight (g)/body length (cm)^3. Slaughter yield: SY = carcass weight (g)/body weight (g) × 100. Viscerosomatic index: VSI = visceral mass (g)/body weight (g) × 100.

2.8. Statistical analysis

The results from the large-scale trials were initially analyzed by the General Linear Model (GLM) procedure using dietary treatment (HP and LP, referred to as D) and weight class (4.5 kg, 5.5 kg and 6.5 kg, referred to as WC) as class variables (see Section 2.4) and their interaction (D x WC). The dependent variables were body weight (BW), body length (BL), muscle fat content (MFAT), slaughter yield (SY), condition factor based on body weight (CF_{BW}), condition factor based on carcass weight (CF_{CW}) and hypaxial anterior muscle thickness (HAM). As the statistical analysis showed no significant effects of the interaction term (D x WC) on the traits studied, the data were analyzed using D and WC as the experimental factors (similar to a two-way ANOVA). The small-scale trial was based on a randomized block design and the results were initially analyzed by the GLM using block (1, 2 and 3), D and WC (2.4 kg, 3.2 kg and 4.0 kg) as class variables (see Section 2.5). The dependent variables were the same as in the large-scale trial including viscerosomatic index (VSI) as a quality trait. Because the statistical analysis found no significant effects of block nor the interaction term between D and WC (except for VSI), the small-scale data were analyzed in the same way as the data in the large-scale statistical model. Cages were used as the experimental units, with two replicate cages (n = 2) per dietary treatment in the large-scale trials and three replicates (n = 3) in the small-scale trial.

All results are presented as means ± SEM unless otherwise stated and the proportion of total variation explained by the model is expressed as R^2. The level of significance was chosen as $p \leq 0.05$ whereas $p \leq 0.10$ was considered a trend and significant differences between means were determined by Tukey's HSD tests. The statistical analyses were carried out with the SYSTAT® 13 software package (SYSTAT Software Inc., USA) and Microsoft Office Excel 2016 (Microsoft Corporation, USA).

3. Results

3.1. Large-scale experiments

3.1.1. Body weight and body length

There were highly significant differences in body weight between weight classes, but no differences between the dietary treatments within smolt groups (Table 3,4). Within the S1 smolt group, fish in the dietary LP group was significantly longer compared to the HP group (Table 3).

3.1.2. Muscle fat content and slaughter yield

At harvest, salmon fed the LP diet had significantly higher muscle fat than those fed the HP diet in both smolt groups (Table 3,4). Muscle fat content increased significantly with increase in weight class in the

Table 3
Quality characteristics at harvest (mean ± SEM, n = 2) of year-old Atlantic Salmon (S1) after being produced in large-scale commercial environment and fed diets with high protein-to-lipid ratio (HP) or low protein-to-lipid ratio (LP). Significant differences between the dietary treatments are depicted with *p*-values, and trends of significance are highlighted with brackets. Non-significance is abbreviated as ns. BW: body weight, BL: body length, MFAT: muscle fat, SY: slaughter yield, CF_{BW}: condition factor based on body weight, CF_{CW}: condition factor based on carcass weight, HAM: thickness of the hypaxial anterior muscle, D: diet, WC: weight class.

LS-S1	HP			LP			ANOVA		
	4.5 kg	5.5 kg	6.5 kg	4.5 kg	5.5 kg	6.5 kg	D	WC	R^2
BW, kg	4580 ± 40	5625 ± 51	6602 ± 17	4649 ± 25	5595 ± 44	6622 ± 69	ns	< 0.001	0.99
BL, cm	71.0 ± 0.3	75.6 ± 0.1	78.9 ± 0.2	71.9 ± 0.2	76.0 ± 0.1	79.8 ± 0.4	< 0.01	< 0.001	0.99
MFAT, %	15.7 ± 0.6	16.2 ± 0.3	17.3 ± 0.4	17.2 ± 0.3	17.8 ± 0.7	18.0 ± 0.3	< 0.01	ns	0.45
SY, %	87.9 ± 0.1	87.8 ± 0.2	87.5 ± 0.2	86.8 ± 0.5	86.6 ± 0.7	86.6 ± 0.9	0.01	ns	0.49
CF_{BW}	1.28 ± 0.00	1.31 ± 0.02	1.35 ± 0.02	1.25 ± 0.00	1.28 ± 0.02	1.31 ± 0.00	< 0.01	< 0.01	0.84
CF_{CW}	1.13 ± 0.00	1.15 ± 0.01	1.18 ± 0.02	1.09 ± 0.00	1.11 ± 0.00	1.13 ± 0.02	0.001	< 0.01	0.86
HAM, mm	9.8 ± 0.2	10.8 ± 0.4	11.3 ± 0.3	9.6 ± 0.0	10.3 ± 0.1	11.0 ± 0.3	(0.10)	0.001	0.82

Table 4
Quality characteristics at harvest (mean ± SEM, n = 2) of under-year-old Atlantic Salmon (S0) after being produced in large-scale commercial environment and fed diets with high protein-to-lipid ratio (HP) or low protein-to-lipid ratio (LP). Significant differences between the dietary treatments are depicted with *p*-values, and trends of significance are highlighted with brackets. Non-significance is abbreviated as ns. BW: body weight, BL: body length, MFAT: muscle fat, SY: slaughter yield, CF_{BW}: condition factor based on body weight, CF_{CW}: condition factor based on carcass weight, HAM: thickness of the hypaxial anterior muscle, D: diet, WC: weight class.

LS-S0	HP			LP			ANOVA		
	4.5 kg	5.5 kg	6.5 kg	4.5 kg	5.5 kg	6.5 kg	D	WC	R^2
BW, kg	4678 ± 35	5692 ± 2	6697 ± 32	4644 ± 22	5651 ± 16	6755 ± 23	ns	< 0.001	0.99
BL, cm	71.6 ± 0.2	76.3 ± 0.0	78.1 ± 0.6	72.4 ± 0.4	76.4 ± 1.4	79.3 ± 0.6	ns	< 0.001	0.93
MFAT, %	16.1 ± 0.1	16.7 ± 0.5	18.2 ± 0.1	16.7 ± 0.1	17.9 ± 0.7	18.8 ± 0.7	0.05	< 0.01	0.80
SY, %	86.4 ± 0.5	86.8 ± 0.0	86.3 ± 0.8	85.7 ± 0.9	85.2 ± 0.2	85.0 ± 0.4	< 0.01	ns	0.51
CF_{BW}	1.28 ± 0.00	1.29 ± 0.00	1.41 ± 0.02	1.23 ± 0.03	1.28 ± 0.08	1.36 ± 0.00	ns	0.01	0.63
CF_{CW}	1.10 ± 0.01	1.12 ± 0.00	1.21 ± 0.01	1.06 ± 0.02	1.09 ± 0.07	1.16 ± 0.00	(0.08)	0.02	0.58
HAM, mm	9.7 ± 0.2	10.1 ± 0.3	10.8 ± 0.3	9.3 ± 0.3	9.8 ± 0.4	10.4 ± 0.4	(0.07)	0.02	0.59

S0 group (Table 4) and a similar trend ($p = 0.07$) was seen in the S1 group.

Within both smolt groups, fish fed the HP diet had significantly higher slaughter yield than those fed the LP diet (Table 3,4), in average 1.1% higher in the S1 group and 1.2% in the S0 group.

3.1.3. Body shape and muscle thickness

Condition factors (CF_{BW} and CF_{CW}) were markedly influenced by weight class in both large-scale experiments (Table 3,4), the biggest fish having the highest CF. In the S1 group, CF_{BW} and CF_{CW} were significantly higher in the HP group than in the fish fed the LP diet (Table 3). This was not observed within the S0 smolt group with regards to CF_{BW} (Table 4), but there was a trend ($p = 0.08$) towards increased CF_{CW} in the HP group (Table 3).

As expected, HAM thickness (mm) increased with increasing weight class (Table 3,4). ANOVA identified a significant influence of weight class together with a trend ($p \leq 0.10$) towards improved HAM thickness in the HP groups (Table 3,4). In all weight classes in both smolt groups, the HAM was numerically higher in the HP group than in the LP group, average 0.33 to 0.37 mm thicker in the HP group, resulting in *p*-values between 0.008 and 0.06 in a simple paired *t*-test analysis. There were positive and significant linear relationships between CF_{BW} and HAM (Fig. 2a, b) in both smolt groups. Based on the presented regression equations, an increase in CF_{BW} by 0.1 improves HAM by 1.8 mm in the S1 smolt group compared to 0.8 mm in the S0 smolt group. This linear relationship was also observed between CF_{CW} and HAM (S1: $p = 0.04$, S0: $p = 0.001$)

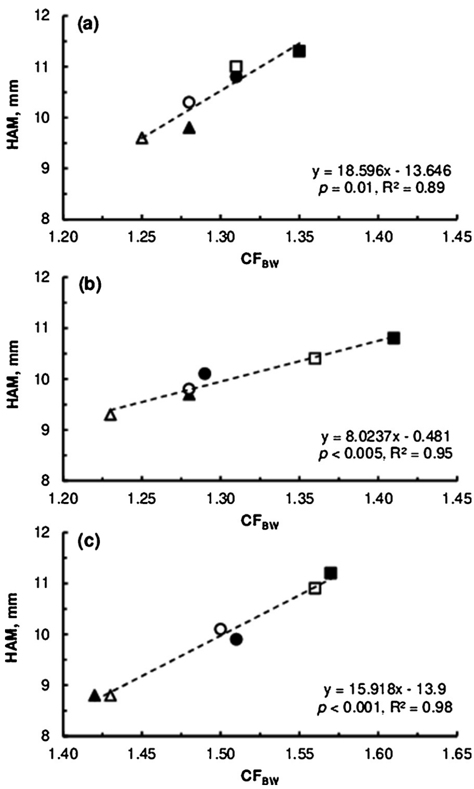

Fig. 2. Thickness of the hypaxial anterior muscle (HAM) in relation to condition factor based on body weight of year-old (a: S1) and under-year-old (b: S0) Atlantic salmon in large-scale production and year-old (c: S1) salmon in small-scale fed diets with either high protein-to-lipid ratio (HP: black symbols) or low protein-to-lipid ratio (LP: white symbols). Triangles, circles and squares represent weight classes, respectively, from lowest to highest in each experiment. Values are retrieved from the data in Table 3,4 and 5. Error margins are depicted in the respective tables.

3.2. Small-scale experiment

3.2.1. Body weight and body length

Body weight and body length were only influenced by weight class. Virtually all the variation in the model was explained by weight class (Table 5).

3.2.2. Fat content, viscerosomatic index and slaughter yield

During the first autumn period from September to December, there was a rapid decrease in VSI and a corresponding increase in muscle fat content for both dietary treatments (Fig. 3). The differences in VSI that emerged in the autumn lasted throughout the study revealing significantly higher VSI for the LP group than for the HP group in April and at harvest in June (Fig. 3, Table 5). At harvest, the muscle fat content varied with weight classes, but was not influenced by diet (Fig. 3, Table 5). However, when leaving out the 2.4 kg weight class, muscle fat content was significantly higher in the LP group than in the HP group ($p = 0.04$, ANOVA).

Corresponding with the large-scale harvest results, salmon fed the HP diet in the small-scale trial had significantly higher slaughter yield compared with the dietary LP group, irrespective of weight class (Table 5), in average 0.6% higher.

3.2.3. Body shape and muscle thickness

Weight class significantly influenced CF_{BW}, CF_{CW} and HAM (Table 5). As in the large-scale study, there was a positive and significant linear relationship between CF_{BW} and HAM (Fig. 2c) as well as between CF_{CW} and HAM ($p < 0.001$). Based on the presented regression equation, an increase in CF_{BW} by 0.1 improves HAM by 1.6 mm. In the small-scale study, numerically higher HAM for HP compared to LP was observed for salmon in the largest weight class only and no significant overall dietary effect was observed.

4. Discussion

Fish weight highly influenced quality characteristics of the harvested salmon. This corresponds with the conclusion from Shearer (1994) and underlines the importance of comparing data from equal sized fish when assessing the dietary influence on quality attributes. When assessing the quality potential in salmon from an industrial point of view, it is important to use harvest weights representative for the industrial practice. In addition, repetition in a controlled small-scale experiment may be necessary in order to validate observations from commercial data and to get more detailed information.

The duration of the experiments was relatively long (≥ 9 months) with an aim to produce fish up to harvest weight, and both feed raw material quality as well raw material prices may vary over such a long period. Although trying to maintain a stable dietary protein and lipid content and thus a steady protein-to-lipid ratio throughout the trial periods, some fluctuations in raw material inclusions in a least-cost formulation were unavoidable. However, this applied to both dietary groups in all three trials, and the repetition of results in the experiments supports that the dietary protein-to-lipid balance has a greater influence on salmon quality characteristics such as slaughter yield, rather than minor changes in raw material inclusion rates.

Overall, the present results demonstrate that high dietary protein-to-lipid ratio has a positive influence on key quality attributes. In compliance with earlier studies (Hillestad and Johnsen, 1994; Wathne, 1995; Einen and Roem, 1997; Hillestad et al., 1998; Einen et al., 1998), the presented results highlight that slaughter yield is clearly improved

Table 5
Quality characteristics at harvest (mean ± SEM, n = 2) of year-old Atlantic Salmon (S1) after being produced in small-scale research environment and fed diets with high protein-to-lipid ratio (HP) or low protein-to-lipid ratio (LP). Significant differences between the dietary treatments are depicted with *p*-values, and trends of significance are highlighted with brackets. Non-significance is abbreviated as ns. BW: body weight, BL: body length, MFAT: muscle fat, SY: slaughter yield, VSI: viscerosomatic index, CF_{BW}: condition factor based on body weight, CF_{CW}: condition factor based on carcass weight, HAM: thickness of the hypaxial anterior muscle, D: diet, WC: weight class.

SS-S1	HP			LP			ANOVA		
	2.4 kg	3.2 kg	4.0 kg	2.4 kg	3.2 kg	4.0 kg	D	WC	R^2
BW, kg	2442 ± 31	3208 ± 6	3981 ± 6	2461 ± 22	3207 ± 28	4006 ± 26	ns	< 0.001	0.99
BL cm	55.7 ± 0.3	59.7 ± 0.2	63.4 ± 0.1	55.7 ± 0.5	59.8 ± 0.6	63.6 ± 0.6	ns	< 0.001	0.98
MFAT, %	19.0 ± 0.0	19.9 ± 0.2	20.3 ± 0.0	18.9 ± 0.4	20.7 ± 0.4	21.4 ± 0.3	(0.10)	< 0.01	0.83
SY, %	87.1 ± 0.3	86.4 ± 0.1	86.9 ± 0.1	86.2 ± 0.2	86.2 ± 0.1	86.1 ± 0.1	0.001	ns	0.72
VSI, %	8.2 ± 0.2	8.8 ± 0.1	8.1 ± 0.2	9.3 ± 0.1	9.1 ± 0.1	8.9 ± 0.1	< 0.001	ns	0.75
CF_{BW}	1.42 ± 0.02	1.51 ± 0.01	1.57 ± 0.01	1.43 ± 0.02	1.50 ± 0.04	1.56 ± 0.04	ns	< 0.001	0.82
CF_{CW}	1.23 ± 0.02	1.31 ± 0.01	1.36 ± 0.01	1.23 ± 0.02	1.30 ± 0.04	1.34 ± 0.04	ns	< 0.001	0.81
HAM, mm	8.8 ± 0.1	9.9 ± 0.2	11.2 ± 0.3	8.8 ± 0.2	10.1 ± 0.2	10.9 ± 0.2	ns	< 0.001	0.94

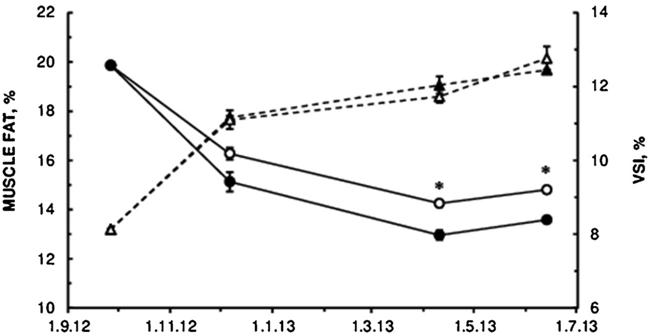

Fig. 3. Development in muscle fat content (triangles/broken line, y-axis) and viscerosomatic index (circles/solid line, z-axis) in year-old (S1) Atlantic salmon fed diets with either high protein-to-lipid ratio (HP: black symbols) or low protein-to-lipid ratio (LP: white symbols) in the small-scale trial. Values are presented as means ± SEM (n = 3). Values in June are based on the harvest data from Table 3. Asterisks denote significant ($p < 0.05$) differences between dietary treatments.

when the protein content in grower feed is increased above levels typically used in the industry over the last years. This improvement was evident in all three trials, despite the differences between them in smolt type, initial body weight, trial duration, rearing environment and management. The findings in the small-scale trial correspond with the results from Einen and Roem (1997), who found that the slaughter yield of salmon reared from 1 to 3 kg decreased with decreasing dietary protein-to-lipid level. Similar results have also been shown to be valid for post-smolt salmon up to nearly 1 kg (Dessen et al., 2017). In contrast to our findings, Einen and Roem (1997) found no influence of dietary protein-to-lipid ratio on slaughter yield for salmon reared from 2.5 to 5 kg. High slaughter yield is not only an expression of bigger muscle density. Elevated visceral fat deposition also contributes to slaughter yield differences between fish of equal body weights. This was depicted in the present small-scale trial. In this study, salmon deposited large amount of fat in the muscle and a relatively smaller proportion in the viscera first autumn in sea and about the same fat levels were observed in muscle for both the HP and LP groups, but significanlty lower VSI for the HP group. Hence, the deposition in the muscle appears irrespective of the fat content of the feed, whereas the decrease in visceral fat deposition appears partly relative to the fat content of the feed. The latter may also be affected by a significantly higher SFR in the LP group during the initiating autumn period. However, as the relative differences in dietary fat is about twice the difference in SFR (12.1% vs. 6.9%), the main explanation is probably the reduced dietary fat for the HP group. Increased VSI is usually related to higher visceral fat deposition (Hillestad et al., 1998; Bendiksen et al., 2003; Jobling et al., 1998, 2002b). Thus, the combination of both higher VSI and muscle fat content in the LP group indicates that there is an excess of lipid content in the LP diets. The results of Dessen et al. (2017) demonstrated how VSI in fish fed a HP diet in the autumn plateaued whilst muscle fat content still increased, whereas LP fed salmon during the same period deposited fat in both muscle and viscera, which ultimately increases both muscle fat and VSI and consequently reduces slaughter yield. The autumn represents the period with highest sea temperatures, high feed intake and high growth (Dessen et al., 2017; Weihe et al., 2018). Thus, the accumulation of dietary energy during this part of the year seems to be highly influenced by the season and the positive influence of a HP diet is greater and more evident in the latter part of the year compared to the spring season. Seasonal differences must therefore be accounted for in future studies when assessing feed influences on fish quality.

The present large-scale results of higher condition factor, significant correlation inn all groups between CF_{BW} and HAM, combined with a trend to greater HAM thickness and lower muscle fat content, indicate that a HP feeding strategy stimulates muscle development in salmon and that this, in combination with reduced VSI, is the main reason for the improvement in slaughter yields and overall product outcome. The results from the small-scale trial complemented the observations in the large-scale trial and depict that lipid dense diets increase the overall fat content in salmon, and this corresponds with earlier work (Einen and Roem, 1997; Hillestad et al., 1998; Jobling et al., 2002a; Bendiksen et al., 2003).

The majority of fish species grow continuously throughout their lives and the muscle growth is a combined effect of recruitment of more muscle fibers (*hyperplasia*) and increased size (*hyperthrophia*) of already existing fibers (Kiessling et al., 2006). Bearing in mind that proteins and amino acids are the building blocks in muscles, continuous muscle growth in farmed salmon will depend on the availability of dietary protein. With some exceptions, the development in CF_{BW} and muscle fat content typically correlates throughout the production cycle (Mørkøre and Rørvik, 2001; Alne et al., 2011). Rørå et al. (1998) indicated that fish with high fat content induced a higher degree of trimming of the fillet, consequently reducing fillet processing yields. Thus, high CF_{BW} based on increased fat content and not improved muscle development might be undesirable. The belly flaps below the HAM section is the fillet region with the highest fat content (Einen et al., 1998). These are typically cut of during fillet processing, and an increased degree of trimming will reduce the final weight and value of the fillet. Therefore, the relationship between HAM and CF_{BW} and reduced muscle fat in the HP group, indicate that an HP feeding strategy might induce higher fillet yields and subsequent greater economic value during processing.

The results in the large-scale and small-scale trials would probably have been more overlapping if the large-scale S0 trial had been initiated when the smolts were stocked in sea, and the small-scale S1 trial had been somewhat prolonged in time so that the experimental fish would reach bigger harvest weight. Also, it may be questioned if the 2.4 kg weight class in the small-scale S1 trial, which represented the smallest and most slowly growing part of the fish is representative to determine feed induced quality differences between the dietary treatments. Despite being smaller in body weight, salmon in the small-scale trial generally had higher muscle fat content and condition factors than the commercially produced salmon in large-scale. A potential explanation for this is the typical excess feeding conducted in small-scale trials (with subsequent feed collection), which ensures that all fish are fed to satiation. Further, an eight-times smaller perimeter of the small-scale cages compared to the commercial large-scale cages likely generates different behaviors (Huntingford et al., 2012). In contrast, commercial farmers avoid overfeeding to avoid additional feed costs.

In conclusion, this paper found that energy-dense diets with a high (>1.1) protein-to-lipid ratio (HP) significantly improves slaughter yield in Atlantic salmon and generates more primary product for further trade and processing, compared with isoenergetic diets with low (<1) protein-to-lipid ratio. In addition, muscle fat content can be significantly reduced by increasing protein on the expense of lipids. When adjusted for body weight of the fish, condition factor and muscle thickness are also positively influenced by HP diets. The overall results also highlight the importance of basing quality comparisons between different dietary treatments on fish of equal sizes.

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Paper IV

A model system to evaluate the economic performance of two different dietary feeding strategies in farmed Atlantic salmon (*Salmo salar* L.)

Rúni Weihe[1,2*], Kjell-Arne Rørvik[2,3], Magny S. Thomassen[2], Frank Asche[4,]

[1] Havsbrún, Bakkavegur 48, FO-530 Fuglafjørður, Faroe Islands

[2] Department of Animal and Aquaculture Sciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway

[3] Nofima, NO-1432 Ås, Norway

[4] Institute for Sustainable Food Systems and School of Forest Resources and Conservation, University of Florida, FL-32611-0180 Gainesville, USA

*Corresponding author: Rúni Weihe; Havsbrún P/F, Bakkavegur 48, 530 Fuglafjørður, Faroe Islands; Tel: +298 41 44 64; e-mail: ruw@havsbrun.fo

Abstract

This paper evaluates the feed cost differences in salmon farming based on two energy dense feed strategies: one resembles the industrial preference of using high fat diets whereas in the other strategy the dietary energy is to a greater degree derived from protein. Two different economical models are presented based on three different feeding experiments: one commercial large-scale and two small-scale trials. All trials were conducted with year old smolt (S1). Feed represents the biggest cost in salmon production. Production costs have increased from 2009 to 2016, and the presented data depict a general increase in price of feed proteins and oils. This is especially momentous for marine proteins and oils compared to the plant-based alternatives. Dietary proteins are more expensive than lipids and in isoenergetic diets, protein denser feeds are higher priced than the lipid dense alternative. Isoenergetic diets with high protein-to-lipid ratio lead to a higher feed deposition in carcass which results in a significantly lower feed conversion rate compared to the preferred isoenergetic high-fat commercial diets. Because of the improved feed to carcass conversion, the dietary protein dense feed strategy yields a lower feed cost. In addition, the high protein feed strategy induces faster growth which enables farmers to reduce the production cycle. A reduced production cycle represents an opportunity of reducing overall production costs. If improved growth is induced by dietary strategy, the reduction of overall costs should be assigned to the feed costs, i.e. a reduction of feed cost. Finally, dietary induced improvements in carcass weight yields more tradeable product which increases income. Thus, the present model system revealed that the traditional high-fat diets preferred in the salmon industry which are cheaper than the isoenergetic protein rich diets, are necessarily not precursors for lower feed costs.

Keywords: Atlantic salmon; feed cost; production cost; economic performance; dietary protein-to-lipid ratio

1. Introduction

Since the start of salmon farming in the 1970s, the industry has evolved quickly and developed into a modern intensive food production system (Asche et al., 2018a). Global production has increased from a few thousand metric tonnes in 1980 to approximately 2.4 million metric tonnes (FAO, 2018). From the start and through the 1980s, farmed salmon was mainly supplied to high-end markets as a luxury high-priced product. However, prices decreased towards the millennium following productivity growth in the industry (Asche, 2008; Kumar and Engle, 2016). This reflects the focus that has been in the industry on increasing production volumes to achieve scale advantages (Asche and Bjørndal, 2011). Such industrial competition typically results with a standard commodity where increased margins are achieved through cost reductions (Porter, 1980). Consequently, the majority of farmed salmon has been sold as fresh head-on gutted (HOG) salmon. Increased productivity and correspondingly lower prices repositioned salmon to become more available for other market segments as a competitive protein source relatively to other animal protein sources (Tveteras et al., 2012). Nevertheless, average HOG prices have seen an increase during the last decade as the demand growth seems to have been relatively higher than the growth in productivity (Brækkan et al., 2018), and several of the most important salmon producing nations experience restrictions on growth due to environmental concerns (Osmundsen et al., 2017).

The closed and controlled production cycle of farmed salmon has allowed systematic knowledge gathering and improvements with several factors that influence the overall productivity (Asche and Bjørndal, 2011). Feed is a crucial input factor and represents approximately 50 % of the total cost of production (Asche and Bjørndal, 2011). Like other production industries of animal protein, salmon farming is all about converting feed to food.

Compared to other aquaculture species and terrestrial animals, salmon is an efficient feed to food converter (Torrisen et al., 2011; Sarker et al., 2013). Salmon are carnivores and primarily utilize proteins and fats which are rich in energy. The cost focus in the industry has pushed the feed industry to compete on cost priced feeds, and although the cost share of feed has increased, the cost of feed has still been significantly reduced from the industry's early days.[1]

In line with improved nutritional knowledge and feed production technology, the energy in salmon feed has increased since the initiation of the industry (Tacon and Metian, 2009; Torrisen et al., 2011). The aquaculture sector has been a growing consumer of fishmeal and fish oil, and especially feeds for salmonids have relied heavily on the use of fishmeal and fish oil (Shepherd and Jackson, 2013). However, due to shortage and because of the foreseen necessity combined with an increased nutritional knowledge, these marine ingredients have been increasingly replaced by plant substitutes (Aas et al., 2015; Ytrestøyl et al., 2018). Concurrent with the development of energy denser diets, the fat content in the feeds has increased proportionally with a decrease in protein in the grower diets for salmon (> 1 kg), altering the dietary protein-to-lipid ratio significantly. Because plant proteins generally have lower protein concentrations compared to fishmeal (NRC, 2011), the shift towards high fat diets have not only reduced the cost of energy in the feed, but also made it easier to use cheaper plant proteins. This has enabled salmon farmers to buy cheaper sources of dietary energy without compromising feed utilization and growth performance (Hillestad and Johnsen, 1994; Hillestad et al., 1998; Azevedo et al. 2004; Karalazos et al., 2007; Karalazos et al., 2011). These earlier results contrast the findings of Weihe et al. (2018), who reported both improved feed conversion and faster growth with a high protein-to-lipid feeding strategy in full-scale trials. In addition, salmon increase the deposition of fat in both muscle and visceral tissue with increased dietary fat (Einen and Roem,

[1] Sandvold (2016) depics a similar development for smolt.

1997; Hillestad et al., 1998; Jobling et al., 2002, Bendiksen et al., 2003, Weihe et al., 2018) and increased visceral weight might be considered as productivity loss as this tissue is of lower value than the HOG product. These findings suggest that the potential productivity increase caused by improved nutritional knowledge primarily has been taken out by providing cheaper feed, and not by improving growth performance.

Because of its anadromous biology, the production of salmon is divided in to a freshwater phase and a seawater phase. An average total production time is approximately three years depending on the feed intake and subsequent growth performance, which are influenced by several biotic and abiotic factors (Houlihan et al., 2001). Continuous brood stock management, increased dietary energy and vaccine development are some key factors that have enabled the industry to produce salmon in high intensive closed conditions. However, keeping high animal density in captivity increases the risk of spreading diseases, and in the case of salmon production, there are great challenges related to sea lice infestation as well as viral diseases which increase the cost of production due to increased mortality, reduced growth performance, treatment and use of higher priced functional feeds (Costello, 2009; Aunsmo et al., 2010; Martinez-Rubio et al., 2012; Martinez-Rubio et al., 2013; Torrisen et al., 2013; Martinez-Rubio et al., 2014; Abolofia et al., 2017; Iversen et al., 2017). Thus, keeping salmon with high density in captivity possesses a high economical risk, and it is of great importance that the production cycle is as short as possible. In contrast with the general feeding strategy in the salmon industry where high-fat feeds are preferred to more expensive high-protein diets, recent results demonstrate that a dietary high protein-to-lipid feed strategy can improve growth performance (Weihe et al., 2018). Although such a feed strategy can reduce the duration of the production cycle and associated risks, dietary proteins are more expensive than dietary fat. Hence, it is a potentially important question what the trade-off between cost and growth performance is. As prices of ingredients and the feed vary significantly, it is also possible that this relationship is changing over time.

The objective in this paper is to present a feed cost evaluation of two different isoenergetic dietary feeding strategies with either high protein-to-lipid ratio (HP) or low protein-to-lipid ratio (LP) from three different feeding experiments. Two of the experiments were completed in small-scale research facilities and the third one was a large-scale full production cycle in sea from stocking of smolts to harvest. The cost evaluation is presented with two different models: (1) a model based on the results from the small-scale trials, which only includes the direct cost of feed price and feed conversion into tradeable carcass and (2) a model which builds partly on model 1 and incorporates the value of reduced production cycle together with a potential value of increased share of tradeable product. These values are regarded as opportunity cost. Before presenting the results of these models, the development of some feed ingredient prices as well as price development in the salmon market will be presented.

2. Methodology

2.1 Experimental feeding strategies

The evaluation of economic performance using a dietary high protein-to-lipid feeding strategy (HP) versus a dietary low protein-to-lipid (LP) feeding strategy, were based on data from three feeding experiment conducted from 2009 to 2013. The first trial was completed in large-scale commercial conditions in the Faroe Islands with year-old smolt (S1), and two small-scale trials in controlled research facilities in Norway with S1 smolts (Fig. 1).

The proximate composition of protein and lipid in the LP diets in all three trials were designed to resemble common commercial diets with much of the energy deriving from lipids. The HP diets were designed to have similar energy as the LP diets but with a greater proportion of the energy deriving from protein. Thus, the dietary strategies had almost similar dietary energy but different protein-to-lipid ratios (Table 1). The feed producing company Havsbrún (Fuglafjørður, Faroe Islands) supplied the experimental feeds in all three trials. All feed production followed standard commercial feed manufacturing, which included monitoring of nutritional and physical quality throughout the production process. Following industrial practice, quality specifications and definitions of the feed ingredients were updated quarterly together with the respective raw material prices. Based on the intended dietary protein and lipid balance, all feeds were composed and produced on a least-cost production strategy. The cost evaluations are based on the actual feed prices used during the trial periods. For further details about the feed experiment, see Dessen et al. (2017) and Weihe et al. (2018).

2.1 Biometric data

In the large-scale experiment, feed utilization and weight gain performance was based on the overall experimental period (Table 2), whereas in the small-scale trials, the performance was divided into three periods (Table 3 and 4). At trial initiation in the large-scale experiment, the mean number of the experimental fish was 66 883 ± 305 and the mean body weight was 104 ± 6 g. The feed trial started when the S1 smolts were stocked in the sea in April 2009 and continued until the fish reached commercial harvest weight (> 4 kg). In the first small-scale experiment, 8000 x 95 g S1 smolt were randomly divided into eight net pens in March 2012. Subsequently, the net pens were split into two quadrouple groups that were supplied with HP or LP feed through three feeding periods. In the second small-scale experiment, the HP fed salmon group from the small-scale trial were randomly restocked into six net pens in September 2012, 150 x 978 ± 1 g in each pen. Afterwards, these net pens were divided into two groups of three replicates to be fed the HP or LP feed strategy. As with the first small-scale experiment, the second small-scale trial was also split into three feeding periods to assess the dietary influence on fish performance.

2.2 Industrial data

The industrial cost data are based on the annual profitability statistics of the Norwegian salmon industry arranged by Norwegian Directorate of Fisheries (Directorate of Fisheries, 2018) and presented in Table 5. Data for production cycles/time are based on industrial performance of the Faroese salmon industry (Avrik, 2018) and presented in Table 6.

2.3 Calculations

2.3.1 Fish growth

The growth rate of the fish is presented as the thermal growth coefficient (TGC) as described by Cho (1992):

(1) $TGC = (W_1^{1/3} – W_0^{1/3}) \times (\sum T)^{-1} \times 1000$,

where W0 and W1 are the initial and final body weight, respectively. $\sum T$ is the sum of day degrees during the period and is calculated as average temperature (C°) in the period x number of feeding days in the period. A higher the TGC accordingly represents a faster growth rate and a shorter production period.

2.3.2 Feed conversion

The biological feed conversion ratio (FCR_{BW}) explains how much feed is consumed to produce 1 kg of whole body weight salmon:

(2) FCR_{BW} = feed intake (kg) x (biomass increase + biomass of lost fish (kg))$^{-1}$.

The biological feed conversion ratio based on carcass weight (FCR_{CW}) explains how much feed is consumed to produce 1 kg of head-on-gutted salmon (HOG):

(3) $FCR_{CW} = FCR_{BW}$ x harvest yield^{-1},

where harvest yield is calculated as carcass weight/whole body weight.

2.3.3 Feed cost excluding value of transferable product and production duration (direct cost)

This section provides the basic model that does not account for the opportunity cost of faster growth.

The difference in the feed price is given as:

(4) FC_P = (price kg^{-1} of LP feed) – (price kg^{-1} of HP feed).

The difference in feed cost based on whole body weight is:

(5) $FC_{P\ BW}$ = (price kg^{-1} of LP feed x FCR_{BW} in the LP group) – (price kg^{-1} of HP feed x FCR_{BW} in the HP group),

while the difference in feed cost based on carcass weight is:

(6) $FC_{P\ CW}$ = (price kg^{-1} of LP feed x FCR_{CW} in the LP group) – (price kg^{-1} of HP feed x FCR_{CW} in the HP group)

The direct feed cost calculations were initially conducted in Danish kroner (DKK) before being converted to US Dollars (USD) based on a DKK/USD exchange rate of 5.536, the average exchange rate in the 2012-2013 trial periods according to statistics from the National Bank of Denmark (http://nationalbanken.statistikbank.dk).

2.3.4 Feed cost including the value of faster salmon production cycle and increased sales value (opportunity cost)

This section provides the model that account for the opportunity cost of faster growth.

The difference in feed price:

(7) FC_P = (price kg^{-1} of LP feed) – (price kg^{-1} of HP feed).

The difference in feed cost based on whole body weight:

(8) $FC_{P\,BW}$ = (price kg^{-1} of LP feed x FCR_{BW} in the LP group) – (price kg^{-1} of HP feed x FCR_{BW} in the HP group)

The difference in FC_{BW} including reduced production cycle:

(9) $FC_{P\,BW\,T}$ = (price kg^{-1} of LP feed x FCR_{BW} in the LP group) – (price kg^{-1} of HP feed x FCR_{BW} in the HP group) – $COST_{TIME}$ kg^{-1},

where $COST_{TIME}$ is subtracted from the better performing feeding strategy and computed as:

(10) ((total operational cost – minus feed cost) x ($\sum T^{-1}$) in the LP feed strategy) - ((total operational cost – minus feed cost) x ($\sum T^{-1}$) in the HP feed strategy)

This is important as shorter production time increase the utilization of all fixed input factors. It is even more valuable when the regulatory system limit production capacity as in the Norwegian Maximum Total Biomass Regulations (MTB) (Asche et al., 2018b; Misund and Nygård, 2018).

The difference in $FC_{BW\,T}$ including the sales value of higher harvest yield:

(11) $FC_{BW\,T\,SV}$ = (price kg^{-1} of LP feed x FCR_{BW} in the LP group) – (price kg^{-1} of HP feed x FCR_{BW} in the HP group) – $COST_{TIME}$ kg^{-1} + SV kg^{-1},

where SV is the extra sales value of the harvested salmon of the better performing feeding strategy and computed as:

(12) (harvest weight of salmon x price kg^{-1} salmon in the LP group) – (harvest weight of salmon x price kg^{-1} salmon in the HP group)

Also here the alternative feed cost calculations were initially conducted in DKK before being converted to USD based on a DKK/USD exchange rate of 5.402, the average exchange rate in the 2009-2010 trial period (http://nationalbanken.statistikbank.dk). The inclusion of cost figures from the Norwegian industry as well as the salmon prices were based on an average NOK/USD exchange rate of 6.551 for the 2009-2016 period.

3. Price development

3.1 Feed ingredient prices

All raw materials display an increase in price from 2009 to 2016 (Fig. 2.). Except for a short period, in 2009, the marine ingredients fishmeal and fish oil have virtually been the most expensive protein and oil sources throughout the 2009 – 2016 period. Based on the energy content (MJ kg^{-1}), fishmeal and fish oil also display the highest relative price increase from 2009 to 2016. Fish oil has tripled the price from USD 0.018 kg MJ^{-1} to USD 0.06 kg MJ^{-1}, while fishmeal has had an increase of 63 %. This is important since the salmon production cost and price is highly influenced by the fishmeal and fish oil prices (Asche and Oglend, 2016; Misund et al., 2017). With regards to plant proteins, the energy derived from soy protein concentrate displays the highest increase in price (0.018 USD kg^{-1}), whereas wheat gluten and corn gluten, are the raw materials which display the lowest changes. The energy coming from rapeseed oil has had a 19 % price increase which is twelve times lower compared to price increase of fish oil in the same period.

3.2 Salmon prices

Salmon prices increased from 2009 to 2010 with a subsequent price decrease onwards to 2012. Thereafter, the price has increased since 2012 (Fig. 3). The three most commonly traded weight classes, 3-4 kg, 4-5 kg, and 5-6 kg, respectively, represent 75 % of the HOG salmon from 2009 to 2016 (Fig. 4). During this period, the Nasdaq index depicts that the price of HOG salmon generally increases with increasing weight classes. The relative increase is especially momentous in the smallest weight classes from 1-2 kg to 2-3 kg to 3-4 kg (Fig. 4.). Thus, by increasing the overall harvest weight within a given production cycle will not only lead to a greater tradeable biomass, but also an overall increase in value per kg salmon produced.

4. Results

4.1 Direct feed cost

4.1.1 Feed cost – Experiment 1 small-scale

Figure 5 depicts that the HP diets were higher priced compared to the LP diets throughout all feeding periods resulting in an overall higher weighted feed price (FC_P) for the HP feeding strategy (0.034 USD kg^{-1}). Because of better feed utilization and higher body weight gain, the calculations demonstrate a lower feed cost ($FC_{P\ BW}$) for the dietary HP group in the first (-0.007 USD kg^{-1}) and third (-0.001 USD kg^{-1}) period, whereas in the second period, the cost is higher, illustrating that there is a real trade-off between the two feed types. Overall, the $FC_{P\ BW}$ calculation demonstrated that the price difference of 0.034 USD kg^{-1} between the dietary strategies was reduced to 0.008 USD kg^{-1} when the difference in body weight gain was accounted for. When feed cost was based on carcass weight ($FC_{P\ CW}$) the HP strategy displayed a lower cost in the first (-0.035 USD kg^{-1}) and third (-0.058 USD kg^{-1}) period resulting in an overall lower feed cost (-0.039 USD kg^{-1}) for the whole experiment.

4.1.2 Feed cost – Experiment 2 small-scale

The HP feed was higher priced in all feeding periods (FC_P), resulting with an overall higher feed price of 0.111 USD kg^{-1} (Fig. 6). The HP strategy displayed a lower $FC_{P\ BW}$ in the autumn and spring periods and therefore decreasing the overall feed cost difference between the dietary strategies in these periods. However, the LP strategy demonstrated a lower $FC_{P\ BW}$ in the winter period, and therefore increasing the cost difference between the groups in the coldest period. Nevertheless, cold sea temperatures have a negative influence on feed intake in salmon and therefore the cost differences in the winter period had a relative low influence on the overall cost for the total period. Thus, the HP strategy displayed an overall lower $FC_{P\ BW}$ of 0.03 USD kg^{-1} compared to the LP feed strategy. Despite following the same pattern as the $FC_{P\ BW}$, the differences in $FC_{P\ CW}$ were even clearer because of higher carcass weight in the HP group. Overall, the HP feed strategy achieved a lower $FC_{P\ CW}$ of 0.07 USD kg^{-1}.

4.2 Feed cost including alternative cost

4.2.1 Feed cost – large-scale experiment

The overall weighted feed price for the HP dietary strategy was USD 0.162 kg^{-1} higher than the LP strategy (Fig. 7a). Because of better feed utilization in the HP group the feed cost difference ($FC_{P\ BW}$) was reduced to USD 0.102. Salmon in the dietary HP group had 219-day degrees shorter production cycle than the LP group, which reduced the cost difference ($FC_{P\ BW\ T}$) down to USD 0.016. The final average harvest weight class was 3-4 kg, which was priced at

USD 6.12 kg^{-1}. In addition to better feed utilization, the dietary HP group had 1.1 % higher harvest yield. This yield was equivalent to USD 0.065 kg^{-1} higher value of the produced biomass. Consequently, when the dietary induced production improvements were included in the overall feed cost evaluation ($FC_{P\,BW\,T\,SV}$), the HP strategy demonstrated an overall lower feed cost of USD 0.048 kg^{-1} (Fig. 7a).

Based on the data from 2009 to 2016 from the Norwegian salmon industry (Directorate of Fisheries, 2018), the feed prices increased with approximately 46 % in the period and the overall production cost excluding feed increased from USD 1.545 to 2.948 kg^{-1} (Table 5). In 2016, the average salmon prices for the 3-4 kg weight class was USD 9.10 kg^{-1} (Fig. 3). When repeating the same calculation with the biometric results from the large-scale feeding experiment with the actual salmon cost and salmon prices from 2016, the overall economic result was improved ($FC_{P\,BW\,T\,SV}$ = USD 0.076 kg^{-1}) despite even higher feed price difference (FC_P = USD 0.236 kg^{-1}) between the dietary HP and LP strategies (Fig. 7b).

5. Concluding remarks

From a cost perspective, feed is the most important input factor in salmon aquaculture. As aquafeed producers rapidly increased their share of the available fishmeal and fish oil in the 1990s, there were significant concerns with respect to the sustainability of the industry due to its dependence on marine ingredients in the feed (Naylor et al., 2000) and the competitiveness due to increased feed cost (Asche and Tveteras, 2004; Kristofersson and Anderson, 2004).

As one of the largest users of fishmeal and fish oil, salmon had been at the head of a development where improved nutritional knowledge reduced the share of marine ingredients in the feed (Ytrestøyl et al., 2015; Aas et al. 2018). The shift towards energy denser diets, especially in the grow out phase (> 1 kg) with less protein and more oil, has made it easier for the feed industry to use lower concentrated protein ingredients in the feed formulation for salmon. Until recently, the literature indicates that reducing the protein content and inverse increase of dietary oil has been achieved without sacrificing growth performance (Hillestad and Johnsen 1994: Hillestad et al., 1998; Azevedo et al., 2004, Karalazos et al., 2007; Karalazos et al., 2011). However, Weihe et al. (2018) nuance this conclusion by reporting improved feed conversion and faster growth with a high protein-to-lipid feeding strategy in full-scale trials, suggesting that the potential productivity increase caused by improved nutritional knowledge primarily has been taken out by providing cheaper feed, and not by improving growth performance. Hence, there is a trade-off between cheaper feed containing less protein and more expensive feed that improves growth performance. As feed prices varies significantly over time (Dahl and Oglend (2014) show that fishmeal is one of the most volatile commodities), this trade-off may also depend on the price levels of the different feed ingredients.

This trade-off is investigated in three experiments in this paper for two types of isoenergetic feed strategies: high and low protein-to-lipid ratio. The results indicate that there indeed is a trade-off as total cost per kg is lower in some periods with the commonly used low protein feed, while it is lowest in other periods with the high protein feed. When one accounts for the opportunity cost of secondary factors such as longer production time with the low energy feed leading to poorer capacity utilization, the high protein feed performs even better, but it still does not dominate the lower protein feed. This suggest that a mixed strategy with respect to feeding might be preferable for any farm, given that sufficiently informative forecasts of salmon as well as fish feed prices can be obtained. This is relatively straightforward for the salmon price given the existence of a futures market (Asche et al., 2016b; Ankamah-Yeboah et al., 2017), with contracts fixing prices with buyers as an alternative (Misund and Nygård, 2018). For feed it is harder given that the price forecast must be made inhouse, but also here contracts (with the feed producers) are an alternative. Nevertheless, feed intake and growth performance in a given period might be a response to the condition of the salmon which has been influenced by previous feeding periods (Dessen et al., 2017; Rørvik et al., 2018). Although the choice of feed in a single period might be the most rationale economic choice, it may not be the best solution seen over a whole production cycle.

It is also worthwhile to note that the regulatory system in several of the salmon producing countries limit the biomass at each farm (Asche and Bjørndal, 2011). Such regulations will further increase the opportunity cost of the longer production process associated with low protein feeds, as it leads to poorer capacity utilization within the available biomass restriction. This adds to the opportunity cost of a longer production time. This effect becomes even stronger when the number of farms or licenses are also limited as in Norway, or when it in practice is hard or impossible to get new licenses like in Scotland, as production cannot be increased by adding more farms.

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Table 1

Proximate composition in the diets in all three feeding experiments, given as the overall weighted mean. Brackets demonstrate the number of feed batches used in each experiment per pellet size per dietary treatment, and the nutritional values are given as weighted mean per diet. The feed compositions above the vertical line in the small-scale column were used in first small-scale feeding experiment (3 mm, 4 mm, 6 mm), whilst the dietary compositions below the line were used in second small-scale experiment (9 mm). HP: dietary high protein-to-lipid strategy, LP: dietary low protein-to-lipid strategy.

Trial	**Large-scale**		**Small-scale**		
Diet	**HP**	**LP**	**HP**	**LP**	
Pellet size 3 mm	*(n = 4)*	*(n = 2)*	*(n = 1)*	*(n = 1)*	Experiment 1
Crude protein, %	49.9	46.6	48.3	44.4	
Lipid, %	25.6	27.2	26.0	28.6	
Protein:lipid ratio	1.95	1.71	1.86	1.55	
DE, MJ/kg[a]	20.3	20.5	20.3	20.6	
Pellet size 4 mm	*(n = 5)*	*(n = 2)*	*(n = 1)*	*(n = 1)*	
Crude protein, %	52.1	45.8	45.2	41.3	
Lipid, %	22.1	28.7	28.5	32.8	
Protein:lipid ratio	2.36	1.60	1.59	1.26	
DE, MJ/kg[a]	19.6	20.9	20.6	21.5	
Pellet size 6 mm	*(n = 7)*	*(n = 2)*	*(n = 1)*	*(n = 1)*	
Crude protein, %	46.6	41.9	44.1	39.0	
Lipid, %	27.6	32.4	31.6	34.7	
Protein:lipid ratio	1.69	1.29	1.40	1.12	
DE, MJ/kg[a]	20.8	21.6	21.8	22.1	
Pellet size 9 mm	*(n = 71)*	*(n = 10)*	*(n = 2)*	*(n = 2)*	Experiment 2
Crude protein, %	42.0	35.4	42.7	35.4	
Lipid, %	32.6	35.9	32.1	36.0	
Protein:lipid ratio	1.29	0.99	1.33	0.98	
DE, MJ/kg[a]	21.6	22.0	21.6	21.9	

[a]Digestible energy was calculated based on the measured feed composition, assuming 23.7, 39.5 and 17.2 MJ kg-1 of protein, lipids and nitrogen-free extractives (NFE), respectively. The apparent digestibility coefficients used for protein, lipid and NFE were 0.86 (Einen and Roem, 1997), 0.94 (Einen and Roem, 1997) and 0.50 (Arnesen and Krogdahl, 1993), respectively.

Table 2

Key performance indicators of the overall dietary influence on salmon production in the large-scale trial. FCR_{BW}: overall feed conversion based on whole body weight, FCR_{CW}: overall feed conversion based on carcass weight (head-on-gutted weight, HOG), TGC: thermal growth coefficient. Brackets denote the number of experimental cages of fish used per dietary treatment.

Dietary strategi	HP	LP
Large-scale	*(n = 3)*	*(n = 2)*
Body weight at harvest, g	4610 ± 14	4489 ± 32
Head on gutted weight (HOG), g	4044 ± 12	3883 ± 25
FCR_{BW}	1.29 ± 0.03	1.36 ± 0.03
FCR_{CW}	1.47 ± 0.04	1.57 ± 0.01
TGC	3.18 ± 0.04	2.98 ± 0.07
Day degrees	3752 ± 109	3971 ± 266

Table 3

Key performance indicators of the periodic dietary influence on salmon production in the first small-scale trial (SS1). FCR_{BW}: overall feed conversion based on whole body weight, FCR_{CW}: overall feed conversion based on carcass weight (head-on-gutted weight, HOG), TGC: thermal growth coefficient. Brackets denote the number of experimental cages of fish used per dietary treatment.

Dietary strategi	HP	LP
April - June, 3 mm diet	*(n = 4)*	*(n = 4)*
Weight gain, g	67 ± 2	66 ± 1
End weight, g	162 ± 2	161 ± 1
FCR_{BW}	0.76 ± 0.02	0.79 ± 0.01
FCR_{CW}	0.88 ± 0.01	0.93 ± 0.02
TGC	1.62 ± 0.03	1.59 ± 0.02
June - July, 4 mm diet	*(n = 4)*	*(n = 4)*
Weight gain, g	126 ± 2	123 ± 2
End weight, g	288 ± 2	284 ± 1
FCR_{BW}	0.75 ± 0.01	0.74 ± 0.00
FCR_{CW}	0.88 ± 0.01	0.89 ± 0.01
TGC	2.49 ± 0.05	2.46 ± 0.04
June - July, 4 mm diet	*(n = 4)*	*(n = 4)*
Weight gain, g	568 ± 2	552 ± 9
End weight, g	945 ± 4	836 ± 11
FCR_{BW}	0.80 ± 0.00	0.81 ± 0.01
FCR_{CW}	0.93 ± 0.00	0.98 ± 0.01
TGC	3.82 ± 0.00	3.46 ± 0.03

Table 4

Key performance indicators of the periodic dietary influence on salmon production in the second small-scale trial (SS2). FCR_{BW}: overall feed conversion based on whole body weight, FCR_{CW}: overall feed conversion based on carcass weight (head-on-gutted weight, HOG), TGC: thermal growth coefficient. Brackets denote the number of experimental cages of fish used per dietary treatment.

Dietary strategi	HP	LP
September - December	*(n = 3)*	*(n = 3)*
Weight gain, g	838 ± 11	785 ± 11
End weight, g	1718 ± 11	1761 ± 13
FCR_{BW}	0.94 ± 0.01	1.05 ± 0.01
FCR_{CW}	1.07 ± 0.01	1.21 ± 0.02
TGC	3.71 ± 0.06	3.52 ± 0.02
December - April	*(n = 3)*	*(n = 3)*
Weight gain, g	785 ± 24	789 ± 27
End weight, g	2606 ± 12	2539 ± 26
FCR_{BW}	1.04 ± 0.03	1.07 ± 0.02
FCR_{CW}	1.19 ± 0.03	1.24 ± 0.02
TGC	3.16 ± 0.10	3.25 ± 0.11
April - June	*(n = 3)*	*(n = 3)*
Weight gain, g	664 ± 21	621 ± 3
End weight, g	3276 ± 19	3165 ± 29
FCR_{BW}	1.13 ± 0.02	1.22 ± 0.02
FCR_{CW}	1.30 ± 0.05	1.46 ± 0.02
TGC	2.53 ± 0.06	2.41 ± 0.03

Table 5

Cost development in the Norwegian salmon industry in the period from 2009 to 2016 given in American dollars (USD). Numbers are converted from Norwegian kroner (NOK) based on a NOK/USD average exchange rate of 6.551 for the period. (Sources: Norwegian Directorate of Fisheries, 2018; National Bank of Norway, 2018).

									Change	
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**USD**	**%**
Smolt	0.300	0.374	0.347	0.330	0.334	0.384	0.416	0.485	0.185	61.6
Feed	1.525	1.677	1.679	1.657	1.756	1.806	2.012	2.221	0.697	45.7
Insurance	0.021	0.023	0.021	0.018	0.017	0.016	0.019	0.019	-0.002	-8.6
Wage	0.199	0.258	0.245	0.236	0.274	0.293	0.316	0.347	0.148	74.4
Depreciation	0.153	0.177	0.167	0.175	0.188	0.193	0.242	0.275	0.121	79.1
Other operational cost	0.448	0.504	0.513	0.497	0.852	0.846	0.963	1.329	0.881	196.4
Net financial cost	0.059	0.044	0.029	0.033	0.042	0.030	0.024	-0.006	-0.064	-109.6
Harvest	0.364	0.433	0.385	0.408	0.403	0.375	0.450	0.498	0.134	36.7
Total	**3.070**	**3.491**	**3.386**	**3.355**	**3.867**	**3.944**	**4.442**	**5.169**	**2.099**	**68.4**
Costs excluding feed	1.545	1.814	1.707	1.698	2.111	2.138	2.430	2.948	1.403	90.8
% feed cost	49.7	48.0	49.6	49.4	45.4	45.8	45.3	43.0		

Table 6

Production performance in the Faroese salmon industry. TGC: Thermal growth coefficient, FCR_{BW}: Feed conversion ratio based on body weight. (Source: Avrik, 2018).

	2007	2008	2009	2010	2011	2012	2013	2014	2015
Production cycles	5	6	9	12	7	11	9	14	2
Smolts, pcs	4,313,803	5,398,788	8,738,417	12,382,975	8,442,617	12,086,618	12,353,715	8,711,549	2,325,180
Weight in, g	88 ± 28	97 ± 38	120 ± 44	89 ± 14	113 ± 23	123 ± 46	132 ± 52	135 ± 41	168 ± 50
Live weight out, g	6109 ± 651	6007 ± 610	5656 ± 468	6002 ± 497	6398 ± 709	6231 ± 352	6728 ± 550	6324 ± 536	6602 ± 332
Day degrees	4670 ± 329	4559 ± 343	4104 ± 164	4471 ± 301	4391 ± 346	4306 ± 279	4576 ± 242	4365 ± 338	4556 ± 60
TGC	2.92 ± 0.17	2.97 ± 0.15	3.11 ± 0.15	3.07 ± 0.20	3.12 ± 0.15	3.11 ± 0.18	3.02 ± 0.20	3.05 ± 0.18	2.92 ± 0.07
FCR_{BW}	1.07 ± 0.03	1.14 ± 0.03	1.14 ± 0.06	1.14 ± 0.05	1.14 ± 0.04	1.12 ± 0.07	1.16 ± 0.09	1.15 ± 0.03	1.14 ± 0.06

Figure captions

Fig. 1. Overview and duration of the three feeding experiments which form the basis of the biometrical data for the economic analysis of feed influenced fish performance. The two dietary strategies are depicted with thick black line (HP: high protein-to-lipid feeding strategy) and broken black line (LP: low protein-to-lipid feeding strategy), respectively. The number of experimental replicates per treatment per trial are denoted in brackets. The gray shaded areas represent the trial terminations, either as harvest (LS1 and SS2) or as restocking of HP fish group to another experiment (SS1).

Fig. 2. Price development in feed ingredients based on their energy content (MJ $kg^{-1)}$ from 2009 to 2016 (Sources: Chr. Holtermann ANS; Havsbrún; National Research Council, 2011).

Fig. 3. Annual prices of fresh head-on gutted (HOG) salmon from 2009 to 2016 divided into weight classes. Until week 13 in 2013, the 7+ weight class was the highest weight class which subsequently was divided into 7-8 kg, 8-9 kg, and 9+. Prices are originally given in NOK kg^{-1} (Norwegian currency) and converted to USD by the average NOK/USD exchange rate in the 2009-2016 period of 6.551 (Source: Fish Pool, 2018; National Bank of Norway, 2018).

Fig. 4. Distribution of fresh head-on gutted (HOG) salmon from 2009 to 2016. Until week 13 in 2013, the 7+ weight class was the highest weight class which subsequently was divided into

7-8 kg, 8-9 kg, and 9+ kg. The percentages represent the average increase in sales value of a given weight class when increased with 1 kg (Source: Fish Pool, 2018).

Fig. 5. Differences in direct feed cost development in post-smolt S1 salmon production from approximately 100 g to 950 g (small-scale experiment 1), using a dietary high protein-to-lipid feed strategy (HP) and a low protein-to-lipid feed strategy (LP). Negative and positive numbers represent a higher cost and lower cost, respectively, for the HP feed strategy. Difference in feed price (FC_P: white bars), difference in feed cost assessed after including the whole-body weight-based feed conversion ratio ($FC_{P\ BW}$: black bars), difference in feed cost assessed after including the carcass weight (head-on-gutted, HOG) based feed conversion ratio ($FC_{P\ CW}$: vertical striped bars), OWM: overall weighted mean.

Fig. 6. Differences in direct feed cost development in S1 salmon grow-out phase from approximately 1000 g to 3500 g, (small-scale experiment 2), using a dietary high protein-to-lipid feed strategy (HP) and a low protein-to-lipid feed strategy (LP). Negative and positive numbers represent a higher cost and lower cost, respectively, for the HP feed strategy. Difference in feed price (FC_P: white bars), difference in feed cost assessed after including the whole-body weight-based feed conversion ratio ($FC_{P\ BW}$: black bars), difference in feed cost assessed after including the carcass weight (head-on-gutted, HOG) based feed conversion ratio ($FC_{P\ CW}$: vertical striped bars), OWM: overall weighted mean.

Fig. 7. Development in feed cost differences in salmon production based on a dietary high protein-to-lipid feed strategy (HP) or dietary low protein-to-lipid feed strategy (LP), using the

actual cost figures from the large-scale experiment in 2010 (A) as well as basing the same calculations with operational cost figures for 2016 (B). Negative and positive numbers represent a higher cost and lower cost, respectively, for the HP feed strategy. Difference in feed price (FC_P: white bars), difference in feed cost assessed after including the feed conversion process ($FC_{P\ BW}$: grey bars), difference in feed cost assessed after including the feed conversion process and production time ($FC_{P\ BW\ T}$: vertical stribed bars), difference in feed cost assessed after including the feed conversion process, production time and extra sales value of the salmon ($FC_{P\ BW\ T\ SV}$: horizontal stribed bars).

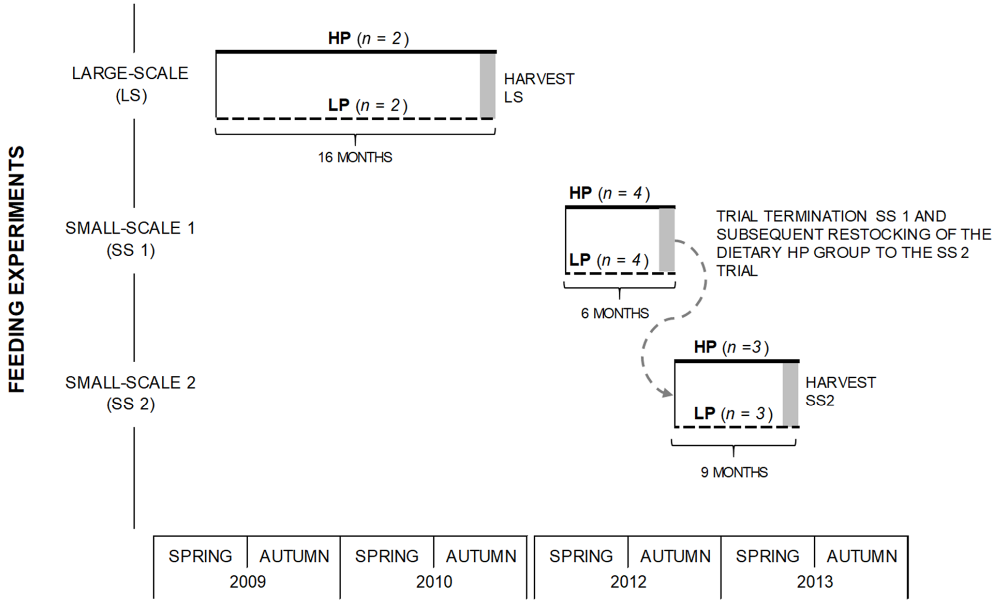

FEEDING EXPERIMENTS
LARGE-SCALE (LS)
SMALL-SCALE 1 (SS 1)
SMALL-SCALE 2 (SS 2)
HP (n = 2)
LP (n = 2)
HARVEST LS
16 MONTHS
HP (n = 4)
LP (n = 4)
6 MONTHS
TRIAL TERMINATION SS 1 AND SUBSEQUENT RESTOCKING OF THE DIETARY HP GROUP TO THE SS 2 TRIAL
HP (n =3)
LP (n = 3)
HARVEST SS2
9 MONTHS
SPRING
AUTUMN
SPRING
AUTUMN
2009
2010
SPRING
AUTUMN
SPRING
AUTUMN
2012
2013

Figure 1

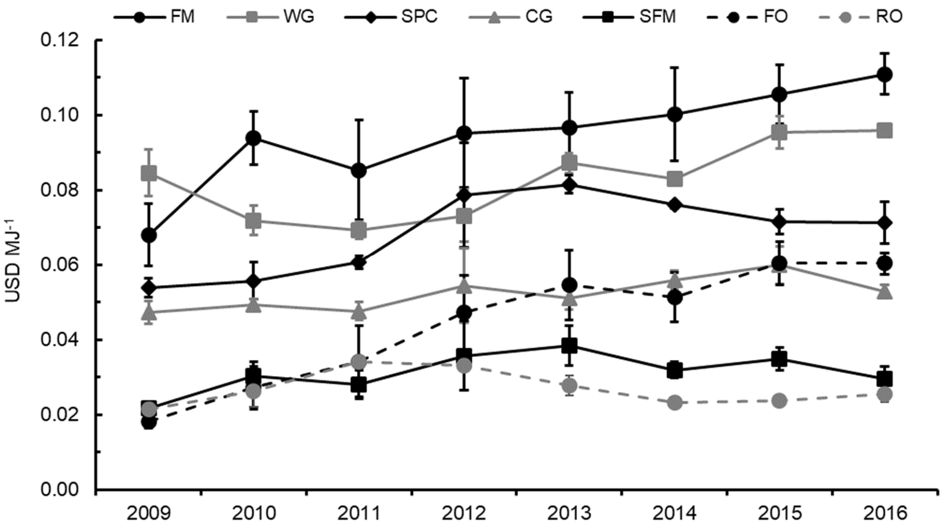

FM
WG
SPC
CG
SFM
FO
RO
0.12
0.10
0.08
0.06
0.04
0.02
0.00
USD MJ-1
2009
2010
2011
2012
2013
2014
2015
2016

Figure 2

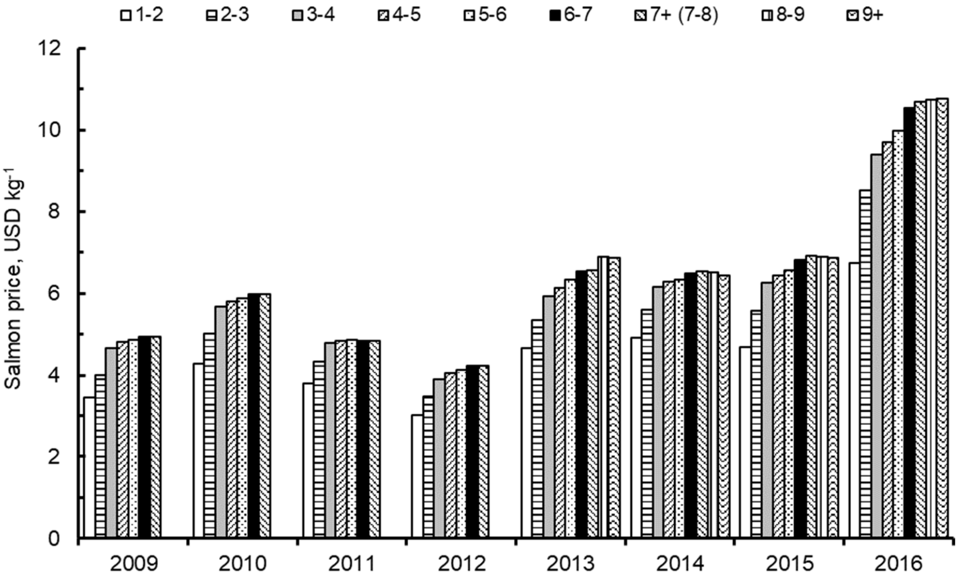
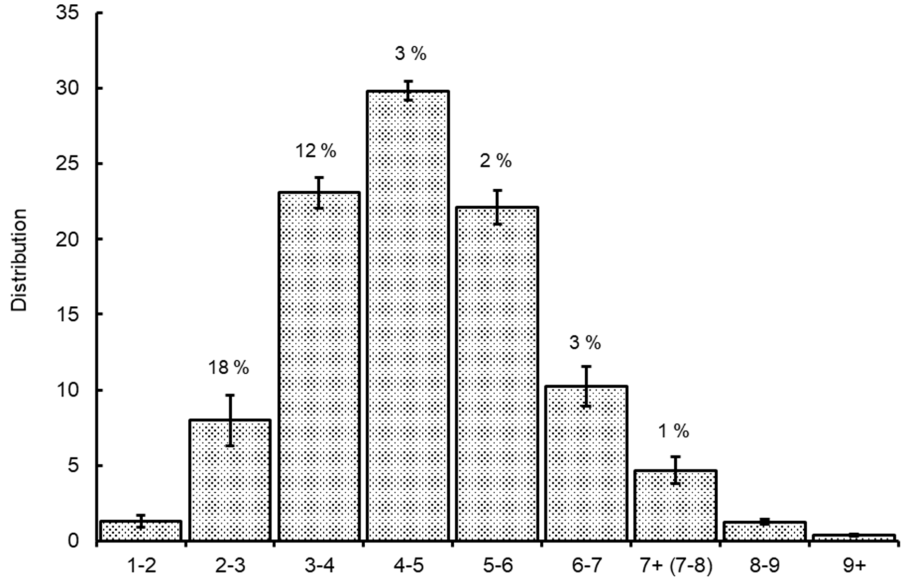
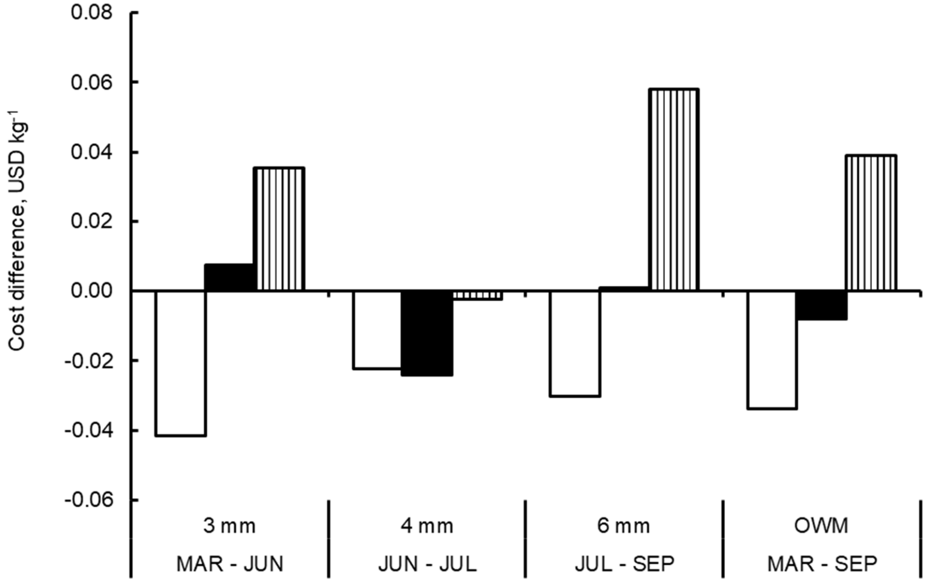

1-2
2-3
3-4
4-5
5-6
6-7
7+ (7-8)
8-9
9+
Salmon price, USD kg-1
0
2
4
6
8
10
12
2009
2010
2011
2012
2013
2014
2015
2016

Figure 3



Distribution
35
30
25
20
15
10
5
0
3 %
12 %
2 %
18 %
3 %
1 %
1-2
2-3
3-4
4-5
5-6
6-7
7+ (7-8)
8-9
9+

Figure 4



0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
Cost difference, USD kg^{-1}
3 mm
MAR - JUN
4 mm
JUN - JUL
6 mm
JUL - SEP
OWM
MAR - SEP

Figure 5

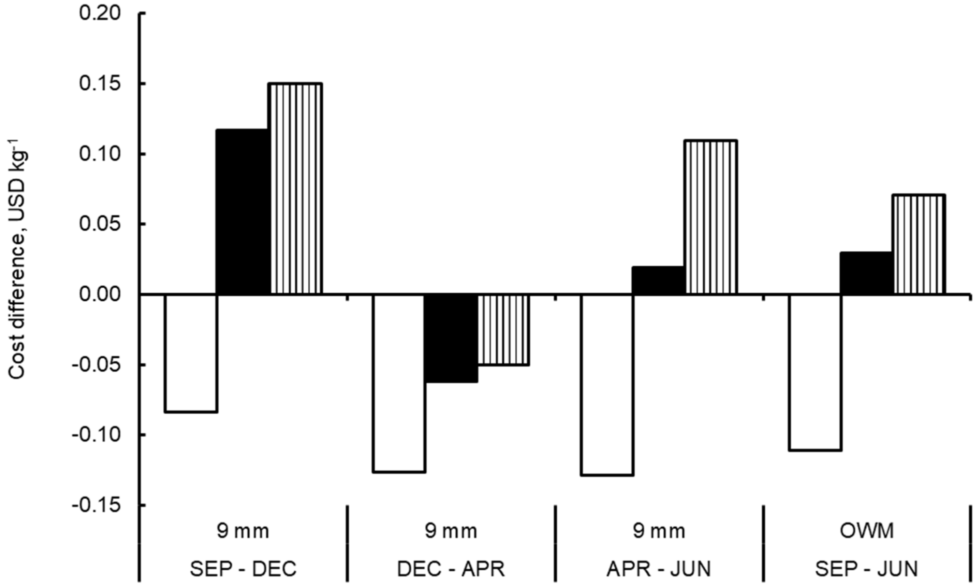

0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
Cost difference, USD kg-1
9 mm
SEP - DEC
9 mm
DEC - APR
9 mm
APR - JUN
OWM
SEP - JUN

Figure 6

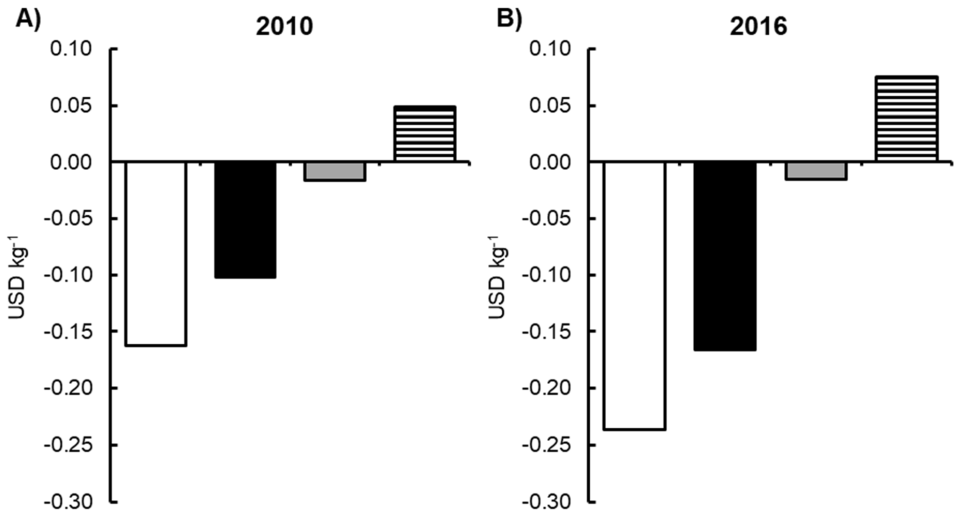


Figure 7

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

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