Norwegian University of Life Sciences
Faculty of Biosciences

Philosophiae Doctor (PhD)
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Novel indicator traits for individual feed efficiency in Atlantic salmon *(Salmo salar)*

Nye indikatoregenskaper for individuell fôreffektivitet hos Atlantisk laks *(Salmo salar)*

Hanne Dvergedal

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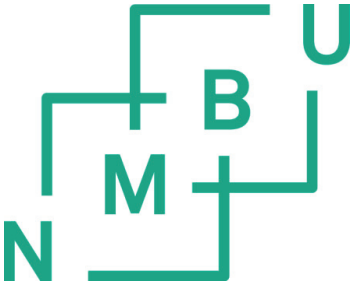
Department of Animal and Aquacultural Sciences

Ås 2019



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Hanne Dvergedal

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Summary

Selective breeding for enhanced feed efficiency in aquaculture production has so far been performed indirectly, largely through selection for an improved growth rate. However, a substantial fraction of the genetic variation in feed efficiency is due to other factors than growth, and there is room for improvement. Direct selection for improved feed efficiency would be beneficial. This requires both individual growth and feed intake routinely recorded for a large number of individuals. Recording of individual feed intake in fish is, however, extremely challenging. Hence, the overall aim of this study was to identify novel phenotypes that can be used as indicators for individual feed efficiency in fish breeding.

Stable isotopes enhance the ability to trace the processes involved in metabolic breakdown and growth of new tissue that occurs after a dietary switch between diets that differ in their isotope profiles. In one experiment with full-sib families kept in separate tanks and feed efficiency recorded on a tank level, growth rate and sampling day explained 62% of the between-tank variation in feed efficiency, and by extending the regression model with change in isotope profiles, the model was able to explain as much as 79% of this variation. This thesis proposes several new indicator traits with potential for large-scale evaluation of feed efficiency. By the use of stable-isotope profiling, indicator ratio traits for feed efficiency were defined as the ratios between growth and changes in the amount of isotope (nitrogen or carbon). These ratios were estimated with rather low heritabilities (0.06-0.11), but with genetic correlations to feed efficiency on a tank level approaching unity. Feed efficient fish are characterized by low maintenance costs and apparently high carbon metabolism in the liver. The underlying causative genes are still unknown, but several single-nucleotide polymorphisms were found to significantly associate with growth as well as nitrogen and carbon metabolism in the muscle and liver. Furthermore, individual digestibility was found to be heritable with an unfavorable genetic correlation to growth.

Based on the findings of the thesis, it can be concluded that indicator traits based on stable-isotope profiling can be used to assess individual phenotypes for feed efficiency that has a potential in selective breeding programs. However, to be commercially relevant, implementing isotope-based indicator traits in selective breeding requires that large quantities of feeds of contrasting isotope

profiles can be produced cost-effectively. Individual indicator phenotypes for feed efficiency might be costly to record, but the potential financial benefit for the aquaculture industry is significant.

Sammendrag

Seleksjon for forbedret fôreffektivitet i akvakultur har så langt vært indirekte, gjennom hovedsakelig å selektere for økt tilvekst, men en stor andel av den genetiske variasjonen i fôreffektivitet skyldes andre faktorer enn tilvekst, og det er derfor muligheter for forbedring. Direkte seleksjon for forbedret fôreffektivitet ville ha vært fordelaktig, men dette krever individuell vekst- og fôropptaksregistrering på et stort antall fisk. Registering av individuelt fôropptak hos fisk er imidlertid ekstremt utfordrende. Derfor var hovedformålet med studiet å identifisere nye fenotyper for individuell fôreffektivitet hos fisk.

Ved bruk av fôr med kontrasterende isotop-profiler kan prosessene involvert i metabolsk nedbrytning og vekst av nytt vev, spores, etter et fôrskifte. I et forsøk hvor fullsøskenfamilier ble holdt i separate tanker og fôreffektivitet ble registrert på tank-nivå forklarte vekstrate og prøvetakningsdag 62% av variasjonen i fôreffektivitet mellom-tanker, og ved å utvide regresjonsmodellen med endring i isotop profil kunne modellen forklare så mye som 79% av denne variasjonen. Denne avhandlingen foreslår flere nye indikatoregenskaper med potensial for storskala registrering av individuell fôreffektivitet. Ved bruk av stabil-isotop profilering kan man definere indikator ratio egenskaper for fôreffektivitet som: forholdet mellom vekst og endring i isotop mengde (nitrogen og karbon). Ratioene ble estimert med relativt lave arvegrader (0.06-0.11), men med en genetisk korrelasjon til fôreffektivitet på tank-nivå som var tilnærmet en. Fôreffektiv fisk karakteriseres av et redusert vedlikeholdsbehov og tilsynelatende økt karbonmetabolisme i lever. De underliggende genene er fortsatt ukjent, men flere polymorfier ble funnet å være signifikant assosiert med vekst så vel som for nitrogen- og karbonmetabolisme i muskel og lever. Videre ble individuell fordøyelighet funnet å være arvelig med en ugunstig genetisk korrelasjon til vekst.

Basert på resultatene i avhandlingen kan det konkluderes med at indikatoregenskaper basert på stabil-isotop profilering kan benyttes til å registrere individuelle fenotyper for fôreffektivitet. For at indikatoregenskapene skal være kommersielt relevante, kreves det imidlertid at store mengder fôr med kontrasterende isotop-profiler kan produseres kostnadseffektivt. Individuelle

indikatorfenotyper for fôreffektivitet kan være kostbare å registrere, mens det økonomiske potensialet i akvakultur synes betydelig.

List of abbreviations

^{12}C	The vast majority of naturally occurring carbon
^{13}C	The less common stable isotope of carbon
^{14}N	The vast majority of naturally occurring nitrogen
^{15}N	The less common stable isotope of nitrogen
AAC	Atom percentage ^{13}C in adipose tissue
ALC	Atom percentage ^{13}C in liver
ALN	Atom percentage ^{15}N in liver
AMC	Atom percentage ^{13}C in muscle
AMN	Atom percentage ^{15}N in muscle
APE	Atom percentage excess
CO_2	Carbon dioxide
EA-IRMS	Element Analyzer Isotope Ratio Mass Spectrometry
EBV	Estimated breeding value
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FW	Final weight
GEBV	Genomic estimated breeding value
GS	Genomic selection
GWA	Genome-wide association
H_2O	Water
IA %	Initial atom percentage
IFCR	Ratio of change in isotope content to body growth
IFER	Ratio of body growth to change in isotope content
IW	Initial weight
Mbp	Megabase pair
N_2	Nitrogen gas
NOx	Generic nitrogen oxide pollutants
O_2	Oxygen
QTL	Quantitative trait loci

RFI	Residual feed intake
RG	Relative weight gain
SNP	Single-nucleotide polymorphism
Ssa	*Salmo salar* autosome
T/C	Ratio between trypsin and chymotrypsin
WG	Weight gain
Y_2O_3	Yttrium oxide

List of papers

The present thesis is based on the papers listed below. The papers will be referred to by their roman numbers throughout the thesis.

I. **Dvergedal, H**., Ødegård, J., Øverland, M., Mydland, L.T., Klemetsdal, G., 2019. Indications of a negative genetic association between growth and digestibility in juvenile Atlantic salmon (*Salmo salar*). Aquaculture (Review submitted March 2019).

II. **Dvergedal, H**., Ødegård, J., Mydland, L.T., Øverland, M., Hansen, J.Ø., Ånestad, R.M., Klemetsdal, G., 2019. Stable isotope profiling for large-scale evaluation of feed efficiency in Atlantic salmon (*Salmo salar*). Aquaculture Research, 50, 1153-1161.

III. **Dvergedal, H**., Ødegård, J., Øverland, M., Mydland, L.T., Klemetsdal, G., 2019. Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling. Genetics Selection Evolution (Accepted).

IV. **Dvergedal, H**., Våge, D.I., Klemetsdal, G., Moen, T., Ødegård, J., 2019. Genome-wide association study for indicator traits of feed efficiency in Atlantic salmon (*Salmo salar*). (Manuscript).

1. General introduction

1.1 Why study feed efficiency?

In 2050, the global population is expected to increase to 9 billion people, implying that food production must increase by 70 percent (FAO, 2018). There will be increased pressure on limited natural resources (i.e., arable land and water), and humanity has to cope with climate changes (Foley et al., 2011; Rockström et al., 2009). Therefore, livestock and aquaculture production should be as efficient as possible in utilizing feed resources, i.e., the amount of feed needed to produce one unit of meat should be minimized. In salmonid aquaculture, feed amount to ~50% of the production cost per kg produced fish (Figure 1) in the grow-out phase in the sea (Directorate of Fisheries, 2018). Therefore, reducing feed costs relative to growth is central in developing future sustainable aquaculture.

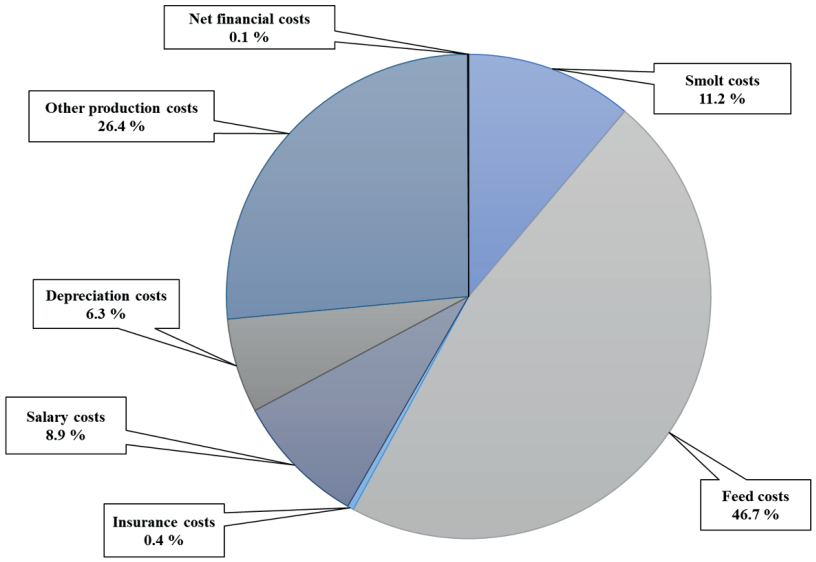

Figure 1. Average production costs per kg produced fish in Norway in 2017 (Directorate of Fisheries, 2018).

1.2 Feed efficiency - an economically important trait in the selection of Atlantic salmon

Feed efficiency can be defined as feed conversion ratio (FCR), which is the amount of feed consumed per unit of growth, or as the feed efficiency ratio (FER), which is the growth per unit of feed consumed (Halver & Hardy, 2002). Since feed efficiency contains major cost and income variables, it is one of the most economically important traits in breeding (Gjedrem, 2005). Improved feed efficiency, by improved growth or other means, will reduce production costs and reduce the environmental footprint per unit produced (Besson et al., 2016; de Verdal et al., 2011b). Through selective breeding, animals may reach a given slaughter weight at a younger age, resulting in saved feed for maintenance and thus improved FCR. Although selecting for growth is practiced, FCR in salmonids in Norway has only been slightly improved over the last nine years, with deterioration over the last three years (Figure 2) (Directorate of Fisheries, 2018). The reasons behind this are complex and likely largely environmental, e.g. changes of diet composition towards a more plant-based diet (European Commission, 2012) and health challenges (i.e., frequent sea lice treatments, infectious diseases, etc.). Nevertheless, there is a need to evaluate whether one can select more efficiently for feed efficiency in aquaculture breeding programs.

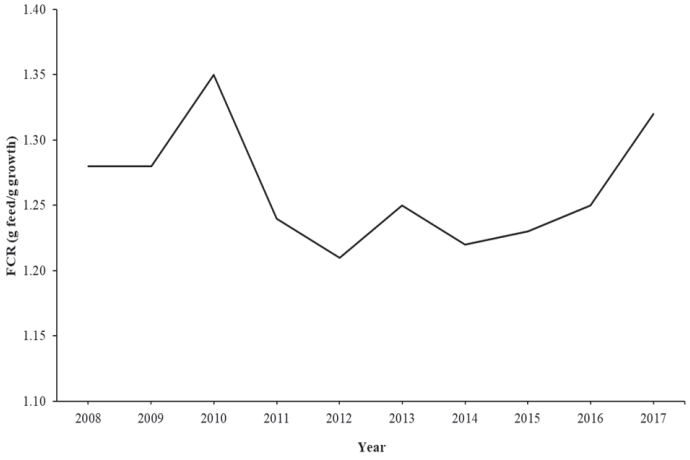

Figure 2. Changes in feed conversion ratio (FCR) over the last nine years in salmonids production in Norway (Directorate of Fisheries, 2018).

Feed efficiency as the ratio between feed intake and growth is described in Figure 3. The figure illustrates that digestion of feed leads to fecal loss. The remaining nutrients are either used for maintenance (e.g., basal metabolism, activity, etc.) or growth. However, the efficiency will also be affected by the composition of growth, i.e., protein, water, and lipids.

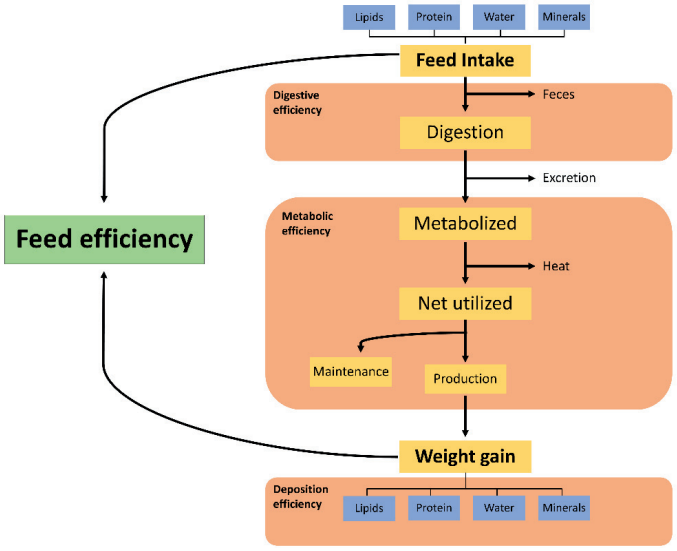

Figure 3. Schematic overview of the relationship between feed intake and growth with the ratio defining feed efficiency (Adapted from Gjedrem, 2005).

Direct genetic selection for improved feed efficiency in a commercial breeding program implies that both individual growth and feed intake must be routinely recorded for a large number of individuals. In aquaculture, recording of individual growth rate is relatively straight-forward, while recording of individual feed intake can be extremely challenging. Fish are typically kept in large units and fed communally by dispersing feed into the water, making a recording of individual feed intake difficult. However, it is possible to measure feed intake at a group (family) level (Helland et al., 1996), which alleviates estimation of the genetic variation between families (Kolstad et al., 2004). To obtain individual feed intake of fish, two methods, X-radiography and video recording, have been used experimentally. The first method is based on radio-opaque ballotini glass beads

for which the number of ingested beads are subsequently detected by x-raying, and feed intake predicted (Jobling et al., 2001; Kause et al., 2016; McCarthy et al., 1993, 1994). For a single meal the prediction has been shown to be highly accurate (McCarthy et al., 1993), but the method requires repeated handling of the fish, increasing the stress and exposing the fish for injuries and diseases. In addition, as Atlantic salmon show a high variation in feed intake from day-to-day (McCarthy et al., 1992; Thodesen et al., 1999), between 3-6 measurements are needed to obtain a good average estimate of the individual feed intake (Kause et al., 2006), potentially even more if the aim is to assess feed intake over a longer period. This intense handling of the fish limits its use in commercial settings. The second method used for recording individual feed intake of fish held in groups is video recording, with manual feeding of one and one pellet and retrospective identification of individual fish and number of pellets eaten from video analysis (de Verdal et al., 2017, 2018a, 2018b). However, even if the individual feed intake can be recorded over a longer period of time, without disturbing the fish, the time required to analyze the video is extensive. In addition, the method requires external tagging and tracking of the individual fish, which limits the group size to 10-20 individuals (de Verdal et al., 2018a), which is not close to commercial conditions, where thousands of fish are kept in the same tank or net-cage in the sea.

Residual feed intake (RFI) has been widely used in livestock as an alternative approach to FCR or FER (Aggrey et al., 2010; Arthur et al., 2001; Johnson et al., 1999; Wolc et al., 2013), and heritability has been estimated in range of 0.1-0.4 in various species (Aggrey et al., 2010; Arthur et al., 2001; Johnson et al., 1999; Saintilan et al., 2013; Wolc et al., 2013). The RFI trait is defined as the deviation between the observed and expected feed intake. The latter is normally estimated from the observed growth and the expected need for maintenance (Archer et al., 1999; Arthur et al., 2001; Koch et al., 1963). The most feed efficient animals are expected to have a negative RFI (Koch et al., 1963; Rauw, 2012). It has for example been found that pigs selected for low residual feed intake have reduced backfat in the carcass and a reduced heat production due to less activity and lower basal metabolic rate (Barea et al., 2010; Boddicker et al., 2011). For rainbow trout, using feed intake predicted by X-raying, RFI explained 23% of the genetic variation in feed efficiency (Grima et al., 2008). However, the main issue by implementing RFI in the selection of fish is that individual feed intake still has to be recorded individually. Because of this difficulty, selective

breeding for improved FCR has relied on indirect selection for increased growth rate (Thodesen et al., 1999, 2001; Gjedrem & Baranski, 2010).

1.3 Growth - the pivotal trait in the breeding scheme of Atlantic salmon

With large-scale individual feed recording being challenging advanced selective breeding programs for Atlantic salmon have been carried out since the early 1970s (Gjedrem et al., 1991), with growth as the pivotal trait through indirect selection for improved feed efficiency (Thodesen et al., 2001). Selection for an improved growth rate has been shown to improve feed retention and feed conversion rates (Neely et al., 2008; Ogata et al., 2002; Silverstein et al., 2005; Thodesen et al., 1999). After five generations of selection, Thodesen et al. (1999) compared selected salmon to wild salmon, reporting a 25% improvement in feed efficiency, defined as growth per unit of feed. A faster-growing animal will likely be more feed efficient by using a relatively larger fraction of the feed for growth and relatively less for maintenance of existing body tissue (Gjedrem & Baranski, 2010). On a family group level, a favorable phenotypic correlation between growth and feed efficiency has been estimated to range 0.6-0.9 in fish (de Verdal et al., 2017; Kolstad et al., 2004; Thodesen et al., 1999, 2001). However, the number of studies estimating the genetic correlation between feed efficiency and growth are limited: Two studies have shown that the genetic correlation between feed efficiency and growth varies between 0.63-0.99 in rainbow trout (Henryon et al., 2002; Kinghorn, 1983), indicating that growth rate and feed efficiency is partly under the same genetic control. Selection for an improved growth rate is expected to indirectly improve feed efficiency, mainly through reduced time to slaughter, reducing the maintenance requirement per unit produced, but also through increased retention of energy and protein (Gjedrem & Baranski, 2010). However, the size of some of these estimates proposes that a substantial fraction of the genetic variation in feed efficiency is due to other factors than growth, with ample room for improvement. In addition, Thodesen et al. (2001) observed a decreasing response in feed efficiency with increasing growth rates, suggesting that direct selection for improved feed efficiency would be beneficial.

Although genetic variation in feed efficiency exists within species (Archer et al., 1999), the underlying mechanisms (apart from growth) are largely unknown. Individual variability in growth

efficiency is partly explained by differences in digestion, absorption, utilization, and metabolism (Austreng & Refstie. 1979; Barreto-Curiel et al., 2018; Buchheister & Latour, 2010; Carter et al., 1993b; Kause et al., 2016; MacAvoy et al., 2005; McCarthy et al., 1994; Neely et al., 2008; Sun et al., 2012; Xia et al., 2013). The growth rate is a result of the net difference between the absorption of new nutrients from feed and excretion of degraded nutrient components, while efficiency depends on the ratio between growth and feed intake. Although growth and feed efficiency are correlated, the growth rate does not explain all variation in feed efficiency. Hence, it is timely to address indicator traits potentially related to feed efficiency in order to improve selective breeding for feed efficiency in aquaculture. Potential novel phenotypes for feed efficiency might require high capital spending, but the potential financial benefit for the aquaculture industry is significant. As an example, improving feed efficiency by 1% will increase the present annual value in Norway alone by EUR ~23 million (1.8 million tons of feed, 1.21 EUR/kg) (Directorate of Fisheries, 2018).

1.4 Traits causing variation in feed efficiency in Atlantic salmon

Figure 3 indicates that variation in feed efficiency is mainly affected by variation in digestive efficiency and metabolic efficiency (synthesis and degradation loss).

1.4.1 Apparent digestibility as an indicator trait for individual feed efficiency in Atlantic salmon

The apparent digestibility coefficient can be defined as the amount of feed eaten that is absorbed and not excreted with the feces, without correcting for endogenous fecal excretions (Halver & Hardy, 2002). Carnivorous fishes, such as Atlantic salmon, are dependent on high crude protein content in the diets for metabolic energy and growth (Halver & Hardy, 2002). To obtain high growth rates in the freshwater phase, an optimal dietary crude protein level of 55% in diets for Atlantic salmon was reported by Grisdale-Helland and Helland (1997). The utilization of the protein is likely dependent on the functioning and morphology of the gastrointestinal tract (Lemieux et al., 1999). An enhanced digestibility of the protein ingredients will potentially improve feed efficiency, and thereby reduce the production costs. However, in recent years, a reduction in both protein and lipid digestibility have been observed (Krogdahl et al., 2003; Refstie

et al., 1998). These results are often seen as a consequence of replacing fishmeal with plant feed ingredients in salmonids diets, and a substantial genetic variation in utilizing plant-based diets for growth has been observed in rainbow trout (Pierce et al., 2008). The apparent digestibility of both protein and lipids depends on the availability of the digestive enzymes such as pepsin, trypsin, chymotrypsin and lipase, and these enzymes ability to degrade the complex macronutrients into free amino acids and fatty acids so that they can be absorbed from the digestive tract and into the blood (Lemieux et al., 1999). In fact, a higher trypsin and chymotrypsin ratio (T/C ratio) has been shown to be phenotypically correlated to a higher feed conversion efficiency in Atlantic salmon (Rungruangsak-Torrissen, 2007; Sunde et al., 2001, 2004).

In rainbow trout, a significant variation in protein digestibility has been found on a family group level (Austreng & Refstie, 1979; Rasmussen & Jokumsen, 2009). Further, a moderate positive phenotypic correlation between high growth rate (and FCR) and protein digestibility has been reported in rainbow trout (Kinghorn, 1983; Rasmussen & Jokumsen, 2009). However, these latter results are somehow in contradiction with the result obtained by Thodesen et al. (1999), where Atlantic salmon selected for growth showed a significantly lower protein digestibility than wild fish. However, yet another study, Thodesen et al. (2001) found no significant effect of family on apparent digestibility of protein. Thodesen et al. (1999) concluded that the reduction in digestibility would probably be too small to have a major impact on the FCR. Moreover, Rasmussen & Jokumsen (2009) have suggested that the variation seen in protein digestibility can be explained by differences in feed intake.

In terrestrial animals, such as poultry (de Verdal et al., 2011a, 2011b; Mignon-Grasteau et al., 2004, 2010; Rougière et al., 2009), and in dairy cattle (Berry et al., 2007), genetic variance of digestibility has been estimated, with low to moderate heritability (0.10-0.47). This indicates that there might be some potential to indirectly improve feed efficiency by selecting for improved digestibility in fish.

The heritability of digestibility and its genetic correlation to other important traits, such as growth, are essential for evaluating the potential of digestibility as a trait in selection for improved feed efficiency in salmon. The ability to record individual phenotypes of digestibility, therefore,

becomes imperative. In this context, the stripping method for recording individual apparent digestibility coefficients with an inert marker, yttrium oxide (Y_2O_3), has already been established by Austreng et al. (2000).

1.4.2 Lipid deposition as an indicator trait for individual feed efficiency in Atlantic salmon

In fish, a relationship between feed efficiency and body fat has been found: Selection for growth in Coho salmon resulted in improved feed efficiency with the priority of dietary lipids for energy (sparing of protein for growth), meaning that fish gained less body fat (Neely et al., 2008). However, its relation to feed efficiency would depend on the physiological age and feed composition. As lipid deposition is highly variable, older fish tend to deposit more fat, meaning that during the grow-out period in the sea lipid metabolism might explain more of the variation in feed efficiency in Atlantic salmon. At this life stage, the relative weight gain (RG) (per time unit) is expected to be smaller (Davidson et al., 2014; Gjedrem & Gunnes, 1978; Santosh, 1999), and a relatively larger fraction of the feed is allocated to energy and lipid deposition (Einen & Roem, 1997). As proposed by de Verdal et al. (2018a) there are several instruments (i.e., Distell Fish Fatmeter®. Internal ultrasound, 2D external imagery and computed tomography (CT) scans) to measure fat content and lipid deposition in fish.

Quinton et al. (2007b) showed that selecting for both growth and reduced lipid content would accelerate the improvement in the daily gain/daily feed intake ratio, over just selecting for growth alone. Moreover, Kause et al. (2016) have shown in rainbow trout of 2-3 kg that selecting against muscle lipid % and for growth is expected to increase the genetic response in FCR by 49% compared to selecting for growth alone. In the same study, feed intake was recorded by use of X-radiography with ballotini beads, and selection for weight gain (WG) and against feed intake would be expected to increase genetic gain in FCR by 50% compared to growth alone. These selection index results predict that fish with genetically low body and muscle lipid percentages are more efficient by allocating the ingested protein to growth, and indicate that muscle lipid percentage might be essential in later life-stages and should be considered to enhance genetic progress for feed efficiency in fish.

1.4.3 Protein metabolism as an indicator trait for individual feed efficiency in Atlantic salmon

Fish display a high efficiency in the conversion of dietary protein into body protein (Tacon & Cowey, 1985). The body has a pool of protein, which is dynamically regulated i.e., new proteins enter the pool by synthesis while others are removed by degradation. The net change in the size of the protein pool is termed protein growth (Fraser & Rogers, 2007). The fraction of protein growth in different tissues depends on the rate of protein synthesis to degradation, which may be termed protein metabolism (describing the three interrelated biochemical processes; synthesis, degradation, and growth) (Fraser & Rogers, 2007). Skeletal muscle is the main protein accretion site in fish (Verri et al., 2011), and 80% of the synthesized proteins in muscle are accumulated as growth (Houlihan & Laurent, 1987). Due to this, muscle growth is a direct reflection of the whole-body growth (Peragón et al., 1999).

Several studies have shown a relationship between protein metabolism and feed efficiency, where high growth efficiency has been associated with lower protein degradation (Carter et al., 1993a; McCarthy et al., 1994; Morgan et al., 2000). A lower rate of protein degradation represents the basis for reduced energy requirements for maintenance in efficient animals (Carter et al., 1993a), and individual differences in protein metabolism are proposed to be genotype-dependent (Hawkins et al., 1989). This indicates that individual variation in feed efficiency can partly be explained by differences in protein metabolism of individual fish. Hawkins and Day (1996) proposed that developing genetic lines with high efficiencies of protein growth would result in improved feed efficiency in aquaculture species. If protein metabolism can be shown to be genetically related to feed efficiency, it would likely be an important trait in the selection of future breeding candidates of aquaculture populations. This requires, however, that protein metabolism can be individually assessed, either in the broodfish candidates themselves or in a training sample of test-fish (sib-testing). The change in isotope ratio can be used to determine the rate of nitrogen and carbon metabolism in various tissues, as a result of nutritional state, partitioning of nutrients, physical activity, and growth (Bloomfield et al., 2011).

1.4.3.1 Stable-isotope profiling to assess protein metabolism

Isotopes are variants of the same chemical element that differ in the number of neutrons. Several isotopes may exist for a given chemical element, and these isotopes can either be stable or radioactive. Nitrogen and carbon isotopes are the most relevant when assessing feed efficiency; by definition, all organic compounds contain carbon, while nitrogen is common to all amino acids. For nitrogen, two stable variants exist; ^{14}N and ^{15}N (natural abundance 99.63% and 0.37%, respectively) (Lide, 2005). Likewise, carbon has two stable variants; ^{12}C and ^{13}C (natural abundance 98.93% and 1.07%, respectively) (Lide, 2005). Molecules containing ^{14}N and ^{15}N differ in mass, and the ratio of these isotopes can be detected with an element analysis isotope ratio mass spectrometry (EA-IRMS). First, the sample is subjected to element analysis by being dropped into a heated reactor, which contains an oxidant such as copper or chromium oxide. Samples are combusted at 1000 ℃ to produce N_2, NOx, H_2O, O_2, and CO_2. The abundances of different isotopes in the sample are then determined by mass spectrometry (Iso-Analytical, 2018).

Using feed enriched with certain isotopes (i.e., with altered ratios of $^{14}N/^{15}N$ and/or $^{13}C/^{12}C$) and monitoring the subsequent rate of change in isotope profile of different tissues, the relative contribution of the nutrients to protein growth can be assessed (Houlihan et al., 1995; Le Vay & Gamboa-Delgado, 2011; MacAvoy et al., 2005). After a dietary switch, say towards a diet enriched with the heavier isotope, the isotopic signature of tissues samples can be used to assess the fraction of "new" protein in different tissues, using the atom percentage excess (APE) ^{15}N or ^{13}C (Fry, 2006) as an indicator:

$$APE\ ^{15}N = \left(\left(\frac{(\delta^{15}N_{Sample} + 1000)}{\left(\delta^{15}N_{Sample} + 1000 + \left(\frac{1000}{\delta^{15}N_{Standard}} \right) \right)} \right) 100 \right) - IA\ \%,$$

where (using nitrogen as an example) $\delta^{15}N_{Sample}$ and $\delta^{15}N_{Standard}$ are the proportion of ^{15}N divided by the proportion of ^{14}N in the sample and in the reference standard, respectively, and *IA %* is the initial atom percentage in $N_{standard}$ or tissue (prior to the dietary switch). The APE ^{15}N is thus the total atom percentage ^{15}N in the sample adjusted for the IA %. Feeding diets with enriched levels of specific isotopes will result in an isotopic change in the organism (Le Vay & Gamboa-

Delgado, 2011). Changes in the isotopic composition of tissues after a change in diet occurs through two processes (Fellerhoff, 2002; Jardine et al., 2003). The first process involves the metabolic breakdown of tissues that were synthesized during feeding on the previous diet and their subsequent replacement with tissues synthesized on a new diet. Secondly, the synthesis of new tissue after a diet switch will reflect the isotopic composition of the current diet and will contribute to the overall isotopic composition of the fish (Figure 4). Efficient fish should be characterized by a low ratio between total synthesis (to replace degraded nutrients and synthesis of new tissue) and growth, i.e., as much as possible of the synthesis should be allocated to growth and as little as possible to replace degraded nutrients. The APE reflects the fraction of newly synthesized nutrients, while APE multiplied with final weight (FW) reflects the amount of newly synthesized nutrients in the tissue in the body. This means that the ratio between the amount of newly synthesized nutrients and growth should be able to measure the efficiency of protein metabolism. Individual isotope-based indicator ratio traits for feed conversion ratio (IFCR) and its' inverse feed efficiency ratio (IFER), were defined as follows (taking ^{15}N as an example):

$$IFCR_{Ni} = \frac{FW_i * APE_{Ni}}{FW_i - IW_i},$$

$$IFER_{Ni} = \frac{FW_i - IW_i}{FW_i * APE_{Ni}},$$

where FW_i and IW_i are final and initial weights for individual i, and APE_{Ni} is the excess atom percentage ^{15}N in muscle for individual i adjusted for the IA % in the tissue. The IFCR is a ratio of the "metabolic costs" (synthesis allocated to growth and replacement of nutrients) to total body growth within the same time period. As the IFCR ratio is expected to be proportional to the amount of newly deposited body nutrients per g increase in body weight, fish that exchange a larger fraction of the body mass per unit of growth will be less feed efficient.

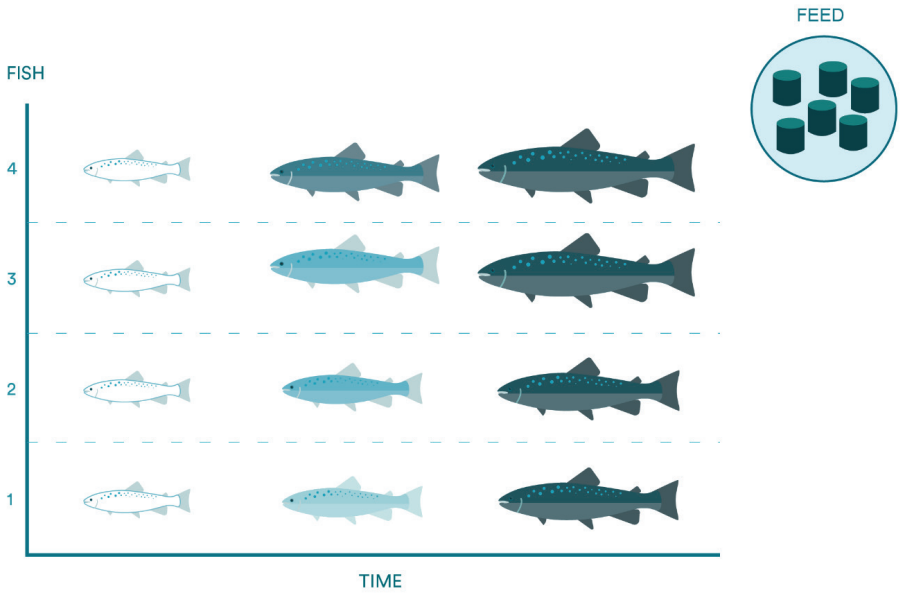

Figure 4. A schematic drawing illustrating the overall reflection of growth and nutrient incorporation over time. Increase in color illustrates the fraction of new nutrients after a dietary switch. Fish with similar growth, but different color illustrates the variation in feed efficiency, with the least efficient fish having the darkest color (Dvergedal et al., 2019).

1.5 Potential of individual phenotyping for feed efficiency in selective breeding

Since no direct measurement of feed intake/efficiency is available for large-scale aquaculture production systems, direct genetic improvement of this trait is difficult to obtain in aquaculture breeding programs. Thus, it is important to investigate potential indicator phenotypes explaining more of the genetic variation in feed efficiency than growth alone. Given that these indicator traits can be individually recorded on a massive scale, they can be used as phenotypic information in aquaculture breeding programs. However, the potential use of such a phenotype in the breeding program will depend on several factors such as:

- The cost of implementing such a trait in the breeding program

- The accuracy of the estimated breeding value (EBV) for the indicator trait (depends on the heritability of the indicator and the number of fish/relatives that can be phenotyped)
- The genetic correlation between the indicator trait and feed efficiency
- Genetic variation in feed efficiency

In fish breeding, indicator traits could be recorded on the selection candidates itself and/or by performing a slaughter test using full-sibs of the breeding candidates to collect phenotypic information. In the latter case, breeding values can be predicted on the untested selection candidates by the use of genomic selection (GS) methods. The GS method uses a large number of genomic markers such as single-nucleotide polymorphisms (SNPs) covering the whole genome to predict the genetic value of each individual (Meuwissen et al., 2001). When using indicator traits assessed by stable-isotope profiling of different tissues (e.g., liver and muscle), the tested fish usually has to be sacrificed to obtain the necessary samples. Practical selection will typically be among untested, albeit genotyped selection candidates. If quantitative trait loci (QTL) related to feed efficiency can be identified, individual selection based on QTL genotypes can still be performed (Vallejo et al., 2017; Goddard & Hayes, 2009). Even in the absence of identified QTL, the GS methods (Meuwissen et al., 2001) utilizing individual phenotypes and genotypes on training animals for selection among genotyped selection candidates is expected to be substantially more effective than traditional pedigree-based selection methods, as a result of increased accuracy and potentially also increased intensity of selection (Vallejo et al., 2017; Vela-Avitúa et al., 2015; Ødegård et al., 2014). The reliability of the genomic estimated breeding values (GEBV) depend on the genetic parameters (genetic and environmental variances) of the trait, the number of individuals in the reference population, as well as phenotypic and genomic data, and the statistical method used to estimate the SNP effects (Hayes et al., 2009). By use of GS, breeding values for all genotyped breeding candidates could be predicted for individually recorded feed efficiency indicator traits, which has the potential to increase the genetic improvement of feed efficiency. Another advantage of using genomic relationships is that genetic parameters can be estimated using fewer families (or in some cases even a single family), even when applied to selectively genotyped data (Ødegård & Meuwissen, 2012).

In addition, based on phenotyping of an indicator trait for feed efficiency, genome-wide association (GWA) studies can be performed to identify possible QTL of major importance to feed efficiency. A GWA study for associations between genetic markers (e.g. SNPs) distributed throughout the whole genome and the phenotype(s) is of interest. For Atlantic salmon, AquaGen AS has developed an SNP-chip with 56 177 SNPs, which can be used for GWA. The number of animals and genetic markers needed to ensure satisfactory power of the GWA depends on the size of the effect and to what degree the SNPs explain the genetic variance (Goddard & Hayes, 2009). To the best of my knowledge, no GWA studies have reported chromosomal regions or QTLs related to feed efficiency in Atlantic salmon.

2. Aim and outline of the thesis

The overall aim of the thesis was to identify novel phenotypes that can be used as indicators for individual feed efficiency in fish. The hypothesis was that the genetic variation in feed efficiency can to a large extent be explained by variation in nitrogen and carbon metabolism and growth. To test the overall hypothesis, three experiments were performed with the following specific aims:

1. Estimate the genetic variance and heritability of nitrogen and carbon digestibility parameters and their genetic and phenotypic correlations with growth in juvenile Atlantic salmon using individually recorded phenotypes and genotypes (Experiment 1, Paper I).

2. Assess whether individual stable-isotope profiling can be used to identify efficient animals without recording individual feed intake. Study the relationship between relative weight gain and atom percentage excess in the muscle, liver, and mid-intestine, to establish how phenotyping should be performed during the feed efficiency test (Experiment 2, Paper II).

3. Examine whether isotope profile data of nitrogen and carbon can explain more of the genetic variation in FCR beyond what can be achieved by growth alone, i.e. to explore the potential of using indicator traits in selective breeding for improved feed efficiency in Atlantic salmon. Estimate heritability and genetic correlations between FCR, growth and indicator traits, as functions of nitrogen and carbon metabolism in various tissues (Experiment 3, Paper III).

4. Perform GWA for indicator traits of feed efficiency in Atlantic salmon (Experiment 3, Paper IV).

3. A brief summary of Papers I-IV

3.1. Paper I

Digestibility is a part of the feed efficiency complex. Improved understanding of the genetics of digestibility and its relation to the most important traits in a breeding program, such as growth rate, is important for enhanced knowledge on the biological aspects of selective breeding for growth. The fish were kept in a common tank from start-feeding until the end of the experiment. All fish were individually tagged and genotyped (56K SNP-chip) at ~5-10 g body weight. A total of 129 fish from 14 families (average initial body weight of 194 g) were included in an individual digestibility trial carried out over 30 days. Individual digestibility was measured as apparent digestibility of nitrogen and carbon in fecal samples, using a fishmeal-based diet with yttrium oxide as an inert marker. To obtain enough feces per fish for digestibility analysis, the fish was stripped four times, i.e., once a week. Results showed significant differences between families with respect to digestibility. Heritabilities were 0.39 ± 0.17 and 0.51 ± 0.18 for digestibility of nitrogen and carbon, respectively. Digestibility showed adverse genetic correlations to the growth rate (-0.77 ± 0.24 to -0.85 ± 0.16). A possible explanation may be that a high growth rate is related to higher feed intake, increasing the passage rate in the gastrointestinal tract and thereby reducing the digestibility of the nutrients. This, however, does not imply that there is an adverse genetic relationship between growth and feed efficiency, as the latter is determined by a number of other factors in addition to digestibility.

3.2 Paper II

In growing animals, individual variation in feed efficiency may arise from individual differences in growth rate and protein metabolism. Over a period of time, these factors will affect the ratio between 'new' vs. 'old' protein, which can be quantified using isotope profiling. The aim of this study was to investigate the relationship between relative weight gain and atom percentage excess ^{15}N in the muscle, liver and mid-intestine. A 50-day experiment was conducted with a total of 375 fish initially fed a standard diet, subsequently replaced by one out of five experimental diets, enriched with ^{15}N. In general, fast-growing fish are expected to have a better

feed efficiency, and the results show that this is captured by isotope profiling in liver and muscle tissues. Furthermore, individual variation in isotope content, that is relative fraction of 'new' protein, among fish with comparable growth rates was observed, most expressed around ~50% isotope saturation, indicating differences in protein degradation and replacement not attributed to growth. The results suggest that isotope profiles can be used as individual indicator traits for feed efficiency and that inclusion levels of stable isotopes of 1%-2% gave the most reliable results.

3.3 Paper III

We used stable isotope profiling (^{15}N and ^{13}C) to obtain indicator phenotypes for feed efficiency in aquaculture. Our objectives were to (1) examine whether atom percent of stable isotopes of nitrogen and carbon can explain more of the variation in feed conversion ratio than growth alone, and (2) estimate the heritabilities of and genetic correlations between feed efficiency, growth and indicator traits as functions of nitrogen and carbon metabolism in various tissues. A 12-day experiment was conducted with 2281 Atlantic salmon parr, with an average initial weight of 21.8 g, from 23 full-sib families that were allocated to 46 family tanks and fed an experimental diet enriched with ^{15}N and ^{13}C. Using leave-one-out cross-validation, as much as 79% of the between-tank variation in feed conversion ratio was explained by growth, indicator traits, and sampling day, compared to 62% that was explained by growth and sampling day alone. The ratio of tissue metabolism, estimated by a change in isotope fractions relative to body growth, was used as an individual indicator for feed efficiency. For these indicator ratio traits, the estimated genetic correlation to feed conversion ratio approached unity but their heritabilities were low (0.06 to 0.11). These results indicate that feed-efficient fish are characterized by allocating a high fraction of their metabolism to growth. Among the isotope indicator traits, carbon metabolism in the liver had the closest estimated genetic correlation with feed conversion ratio on a tank level (-0.9) but a low estimated genetic correlation with individually recorded feed efficiency indicator ratio traits. The underlying determinants of these correlations are largely unknown. Our findings show that the use of indicator ratio traits to assess individual feed efficiency in Atlantic salmon has great prospects in selection programs. Given that large quantities of feeds with contrasting isotope profiles of carbon and/or nitrogen can be produced cost-effectively, the use of stable isotopes to monitor nitrogen and carbon metabolism in various tissues has potential for large-

scale recording of individual feed efficiency traits, without requiring individual feed intake to be recorded.

3.4 Paper IV

The objective was to search for putative quantitative trait loci affecting the following indicator phenotypes; relative weight gain, weight gain, atom % ^{13}C in muscle, atom % ^{15}N in muscle, atom % ^{13}C in liver, atom % ^{15}N in liver, atom % ^{13}C in adipose tissue and indicator ratio traits of feed conversion and efficiency ratios for atom % ^{15}N and ^{13}C in muscle. The material was a family experiment performed in the freshwater-phase, encompassing 2281 individuals from 23 full-sib families. Eggs from each family were hatched and families kept separately until start feeding. At start feeding 120 fry were randomly sampled from each family and reared together in a single tank until the start of the feed conversion test. During the 12-day feed conversion test, families were randomly allocated to family tanks (50 fish per tank and 2 tanks per family), and feed conversion ratio was registered on a family group level. Families were fed a fishmeal-based diet labeled with the stable isotopes ^{15}N and ^{13}C, with inclusion levels of 2% and 1%, respectively. For genotyping, a custom 56K single-nucleotide polymorphism array was used. Using a linear mixed-model algorithm, several significant associated single-nucleotide polymorphisms related to growth, and nitrogen and carbon metabolism in muscle and liver were identified on chromosomes 3, 5, 9, 11, 12, 15 and 20. The most important results from this study are the finding of a quantitative trait locus for growth on chromosome 9. Yet another finding was a quantitative trait locus for carbon metabolism in liver on chromosome 12, a trait closely related to feed conversion ratio on a tank level. However, the peak was broad, likely due to the few and numerous families in this study. For the indicator feed efficiency ratio traits, derived from the ratios between the fraction of stable isotopes (^{15}N and ^{13}C) in muscle and growth, no convincing quantitative trait locus was obtained.

4. General discussion

The estimated increase in the human population by 2030 (FAO, 2018) calls for making all parts of the food chain more efficient. In aquaculture with Atlantic salmon, efforts to reduce feed costs by genetically improving the salmon's ability to digest, absorb and utilize ingested nutrients for body growth is of high importance to both sustainability and profitability.

Selective breeding is dependent on phenotypes with a significant heritability that can be individually measured in large scale. To improve aquaculture profitability and resource use, there is an ongoing discussion on how to properly assess feed efficiency. Alternative indirect measurements of feed efficiency have been proposed with traits and phenotypes such as digestibility (Berry et al., 2007; de Verdal et al., 2011a, 2011b; Mignon-Grasteau et al., 2004, 2010; Rougière et al., 2009), residual feed intake (e.g. Rauw, 2012), lipid deposition (Kause et al., 2016) and protein metabolism (Hawkins & Day, 1996; McCarthy et al., 1994; Morgan et al., 2000), with potential to explain more of the feed efficiency complex than growth alone.

All the phenotypes mentioned above are part of the feed efficiency complex, which is heavily dependent on the animal's energy balance. The energy balance is affected by the catabolism of feed components to increase the level of energy and substrates for anabolic processes such as growth and other important processes in the organism (Halver & Hardy, 2002). The amount of energy left for growth depends on feed intake and the digestive as well as the metabolic efficiencies. The overall aim of the thesis was to identify phenotypes that can be used as indicators for individual feed efficiency in fish. Therefore, the thesis has investigated the genetic relationship between growth, apparent digestibility and indicator traits as potential phenotypes for individual assessment of feed efficiency in Atlantic salmon. The feed efficiency of growing animals can be divided into efficiency at two main levels: 1) Digestive efficiency and 2) Metabolic efficiency.

Digestive efficiency can be defined as the fraction of ingested nutrients that are absorbed, i.e., entering the metabolism of the animal, while metabolic efficiency is defined here as the fraction of absorbed nutrients that remain in body tissues at the end of a growth period. Selective breeding for growth may affect both levels. Increased growth will likely result in a correlated increase in

feed intake, which may have a (potentially unfavorable) effect on digestive efficiency. Secondly, it will likely increase the metabolic efficiency by allowing a relatively larger fraction of the absorbed nutrients to be used in anabolic processes (tissue growth).

4.1 Digestive efficiency

A living organism is dependent on inputs from its surroundings to maintain essential processes in the cells. Before nutrients can be metabolized, they need to be digested and absorbed from the gastrointestinal tract. Feed ingredients, which resist digestion, lead to high gross energy losses and excretion of undigested nutrients and components. As indicated in earlier studies there might be a genetic component in the animals ability to digest macronutrients in an efficient manner (Aas et al., 2017; Austreng & Refstie, 1979; Berry et al., 2007; Cook et al., 2000; de Verdal et al., 2011a, 2011b; Kinghorn, 1983; Mignon-Grasteau et al., 2004, 2010; Rasmussen & Jokumsen, 2009; Rougière et al., 2009; Thodesen et al., 1999, 2001). Given that genetic variation in digestibility of feed nutrients exists, the trait can be improved through selection, which is a tool for improving the utilization of feed resources. Thodesen et al. (2001) have investigated protein digestibility in Atlantic salmon and found no significant family effect for apparent digestibility of protein. Studies in Rainbow trout have shown significant effects in apparent digestibility of protein between families (Austreng & Refstie, 1979; Rasmussen & Jokumsen, 2009). However, none of the mentioned studies have estimated genetic parameters for apparent digestibility. Therefore, Paper I is to my knowledge the first study elucidating the genetic variation and heritability of apparent digestibility in Atlantic salmon.

Undigested feed components and endogenous losses from the body are compounding the fecal material. The fraction of undigested feed components and endogenous losses are influenced by the level of feed intake and feed characteristics (Austreng & Refstie, 1979; Halver & Hardy, 2002; Rasmussen & Jokumsen, 2009). Furthermore, growth is closely related to feed intake (0.98 on a family group level) (Kolstad et al., 2004), and positively phenotypically correlated to feed efficiency (0.60-0.79 on a family group level, respectively) (Kolstad et al., 2004; Thodesen et al., 2001). Hence, selection for a reduced feed intake jointly with increased growth might have the

potential to improve digestibility, although impractical due to the difficulty of recording individual feed intake.

Alternatively, one might phenotype directly for digestibility by use of an inert marker. Paper I, aimed to estimate the genetic variance and heritability of nitrogen and carbon digestibility and their phenotypic and genetic correlation to growth. Results show that the apparent digestibility of nitrogen and carbon were highly heritable (0.39 ± 0.17 and 0.51 ± 0.18, respectively), but both had a strong adverse genetic correlation to growth (-0.77 and -0.85; Paper I, Table 4). Based on these estimates, selection for growth is expected to impose a reduction in the ability to digest the ingested feed. Cook et al. (2000) compared growth-enhanced transgenic Atlantic salmon with non-genetically modified salmon and found no significant effects on digestibility of protein and energy. However, the tendency was slightly unfavorable for both protein and energy (2 and 3% percentage points reduction in digestibility, respectively), which is in accordance with the results in Paper I. The imposed reduction in digestion can likely be explained by the high genetic correlation that exists between growth rate and feed intake. Low feed intake and thus a correspondingly low growth is associated with a longer gastric evacuation time (Venou et al., 2009), which leads to longer time for digestion and absorption of nutrients, and improved digestibility (Aas et al., 2011; Adamidou et al., 2009). Aas et al. (2017) also revealed a large individual variation in gastrointestinal passage rate in Atlantic salmon, potentially also affecting the time for digestive enzymes to degrade macronutrients throughout the digestive tract. On the other hand, an increased growth rate is expected to improve utilization of digested nutrients after absorption (metabolic efficiency), as relatively more of the absorbed nutrients are allocated to the growth of new tissue and relatively less to the maintenance of existing body mass (Cook et al., 2000).

By selecting for increased growth rate, the net effect on feed efficiency is still expected to be favorable as the improvement of metabolic efficiency likely exceeds the unfavorable effect on digestive efficiency (see section: *4.2 Metabolic efficiency*). However, the unfavorable relationship between digestion and growth rate leaves uncertainty with respect to future improvement of feed efficiency through indirect selection on growth rate. As the estimated genetic correlations are strongly unfavorable, selection for both improved growth rate and improved digestibility is an alternative. It should be noted that the genetic relationship between growth and feed intake could

be affected by life stage, production environment, feeding practice and feed composition, which are topics that deserve more study.

4.2 Metabolic efficiency

After digestion and absorption, nutrient components are metabolized. Catabolic and anabolic processes are dynamically regulated by the cells in different tissues. The minimum level of catabolism and anabolism in the cell is termed basal metabolism (Halver & Hardy, 2002). Basal metabolism represents the metabolic activity in the cell needed to obtain the structure and function of the different organs and tissues. Maintenance costs are the sum of energy from feed to cover energy losses associated with the basal metabolism and heat increment of feeding (Baldwin et al., 1980). This restricts the fraction of the absorbed amino acids that are available for tissue growth. Several factors, such as age, nutrition, stress, disease, hormones and activity, influence the level of metabolism in the cell (Rathmacher, 2000). So far, the genetic variation in nitrogen and carbon metabolism and its importance for feed efficiency has not been widely studied in Atlantic salmon. The efficiency of growth has been estimated to range between 40-60% in fish (Halver & Hardy, 2002), which is influenced by the level of protein synthesis and degradation in the organism. Several studies have proposed that changes in protein synthesis and degradation, due to differences in maintenance costs, can explain the variable energy costs of growth, leading to individual variation in feed efficiency not attributed to growth. By genetically selecting animals for an efficient nitrogen and carbon metabolism (i.e., less protein degradation per unit of protein growth), feed utilization may be enhanced in Atlantic salmon as well as other aquaculture species. In this study, protein metabolism is defined as the fraction of newly deposited amino acids in the tissue, resulting from replacement of amino acids (degradation loss) and tissue growth (growth dilution), which is estimated using stable-isotope profiling after a diet switch with one or two stable isotopes (^{15}N and/or ^{13}C).

Paper II examined the use of stable isotopes in the feed to assess feed efficiency in Atlantic salmon through the relationship between RG and APE ^{15}N in muscle, liver, and mid-intestine at the end of the experiment. The change in nitrogen isotope profile can be explained by protein metabolism, i.e., accumulation of “new” protein originating from the isotope-enriched feed through growth

dilution and replacement of existing body tissues (losses). It is not surprising that the content of the feed-enriched isotope increases with body growth. Results showed that fish of similar RG, but different growth rates (i.e., reaching the same RG at different time points), had clearly different isotope contents, with the fastest-growing fish having the lowest content of enriched isotope (Paper II; Figures 2b-e). This cannot be attributed to growth dilution (as RG is similar), implying that fast growth results in lower replacement of existing body tissue, and thus better FCR, likely due to lower maintenance costs from a shorter growth period (Herzka et al., 2001). The experiment indicates that individual differences in FCR are likely to be captured by individual differences in isotope profiles. Furthermore, within each time point (Paper II; Figures 2b-e) there is individual variation in protein degradation for fish of similar RG. This may be explained by variation in maintenance requirements and relates to individual differences in the efficiency of protein metabolism. These results are in accordance with earlier research in aquaculture species, which has shown that protein metabolism efficiencies vary between groups of individuals. Efficient fish obtain a higher growth rate due to reduced protein degradation (Carter et al., 1993a; Hawkins et al., 1989; McCarthy et al., 1994; Morgan et al., 2000), which form the basis for reduced energy requirement for efficient animals. None of the mentioned studies tried to elucidate the genetic component of protein metabolism between efficient and inefficient fish.

Paper III explored to what extent variation in nitrogen and carbon metabolism assessed with stable-isotope profiling could be explained by inheritance, with the objective to establish indicator phenotypes that are more closely related to feed efficiency than growth alone. Results showed between-family variation in nitrogen and carbon metabolism (Paper III; Figure 2a-e) which could potentially affect feed efficiency. By predicting the observed tank-FCR using a multiple regression model, growth, isotope-based indicator traits and sampling day combined explained 73% of the variance in masked tank-FCR records (compared to 46 to 55% by growth variables and sampling day alone) (Paper III; Table 3; $\hat{R}^2$). Hence, by including nitrogen and carbon metabolism traits in different tissues, the prediction of FCR data improved substantially above what can be obtained by growth data alone (Paper III; Table 3).

The obtained genetic correlations (Paper III; Table 5) revealed, as mentioned, that fast growth is favorably associated with improved feed efficiency (-0.74 to -0.82). Further, nitrogen and carbon

metabolism in muscle (AMN and AMC, respectively) and nitrogen metabolism in the liver (ALN) had highly positive estimated genetic correlation to growth, indicating that genetic variation in these traits is largely controlled by growth dilution (i.e., fraction "old" nutrients being diluted by synthesis of "new" nutrients). It turned out that the ratio of tissue metabolism to body growth or its inverse (based on a change in isotope fractions and relative body growth) could be used as a more direct individual indicator for feed efficiency (IFCR/IFER variables). The IFCR (and IFER) variables for AMN and AMC are expected to be approximately proportional to the (inverse) ratio of the mass of newly deposited nutrients in muscle to total growth in body mass and relates as such directly to the metabolic efficiency. The results indicate that the IFCR is close to perfectly genetically associated with FCR on a tank level. Feed-efficient fish are characterized by a high fraction of nutrients being allocated to growth. Among the nitrogen and carbon metabolism traits (i.e., isotope content), carbon metabolism in the liver (ALC) was estimated with a moderate heritability (0.15) and had the closest genetic correlation to FCR on a tank level (-0.9). However, ALC had a low estimated genetic correlation to the individually recorded IFCR/IFER traits, indicating that ALC might have a different biological basis. The IFCR/IFER indicators are intuitively appealing, and can easily be interpreted biologically, compared to ALC, for which the underlying determinants are largely unknown. The metabolic efficiency (Figure 5) and allocation of nutrients for growth are closely related to the feed efficiency complex: Using body tissue as fuel for e.g., maintenance will be less efficient than utilizing the nutrients absorbed and metabolized from the feed directly. Although processes related to maintenance will anyhow occur, i.e., heat increment, basal metabolism and cell maintenance (Halver & Hardy, 2002; Rauw, 2012; Sun et al., 2012), there seems to be a variation between individuals to what extent body tissues are used for maintenance (Barreto-Curiel et al., 2018; McCarthy et al., 1993; Sun et al., 2012; Xia et al., 2013). A reduced exchange of body tissue components would lead to a more efficient protein utilization and thus reduced feed costs (Barreto-Curiel et al., 2018). The IFCR/IFER variables allow for a direct measurement of carbon and nitrogen fluxes, by using stable-isotope profiling to trace the contribution and allocation of nutrients from feed to growth in animal tissue (Barreto-Curiel et al., 2018; Gamboa-Delgado et al., 2011; Xia et al., 2013). As the IFCR and IFER variables can be interpreted as the (inverse) fraction of metabolizable nutrients being allocated to body growth, they are likely to have universal relationships with FCR in growing animals and could be used independently of life-stages and species.

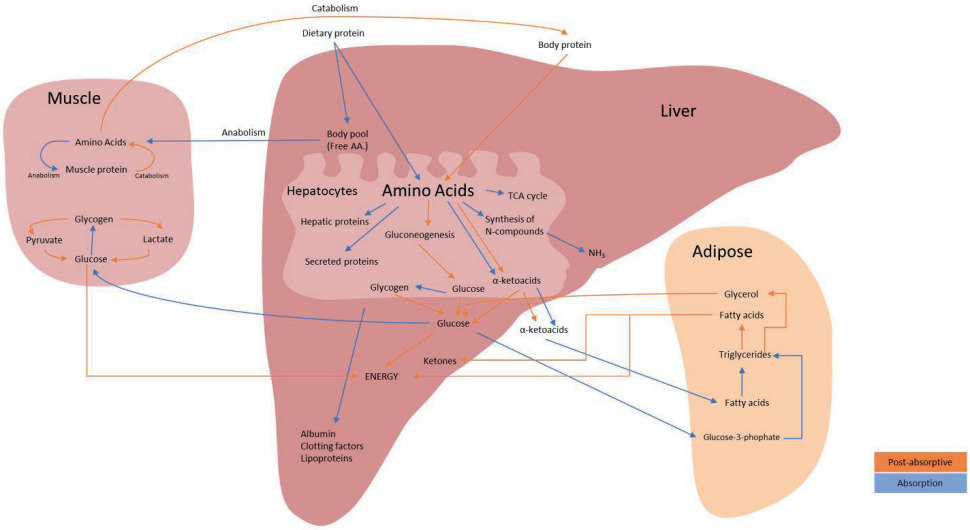

Figure 5. Metabolic allocation and utilization of dietary protein between tissues.

In livestock production, a lower activity is associated with an enhanced feed efficiency (Knap, 2009; Luiting, 1990), mainly due to reduced maintenance costs in less active animals (Braastad & Katle, 1989). The level of activity is likely included within the stable-isotope profiling approach. Increased activity will increase the nitrogen and carbon metabolism in the animal, i.e., increase the maintenance cost, which is likely to be captured by the IFCR/IFER variables. Several studies have shown that there is a genetic relationship between the behavior of the animal and feed efficiency (Lancaster et al., 2009; Rauw et al., 2000). Differences in energy used for swimming have been shown to explain over 20% of the observed differences in FCR between Atlantic salmon and Chinook salmon, likely due to differences in body shape (Petrell & Jones, 2000). Particular, submissive fish will use more energy fighting for the resources, leading to increased maintenance cost and reduced feed efficiency (Sloman et al., 2000). In the present study, families were commonly reared until the start of the experiment. Initial weights showed large differences between families. However, when fish were allocated into family tanks, some of the families with low initial weights compensated with accelerated growth rates and obtained a similar FCR as the families with higher initial weights, potentially due to a more favorable social environment through

more evenly sized fish in the tank. These results show that social behavior and activity can be important factors in the feed efficiency complex. However, specifically studying such factors are beyond the scope of this thesis, but the topic deserves more study.

Results obtained in both Paper II and III show that there is a large potential for improving components of the feed efficiency complex by selecting for reduced maintenance costs, through selection for variables derived from nitrogen and carbon metabolism in muscle, liver and adipose tissue. However, one should keep in mind that even if efficient animals have reduced maintenance costs, protein metabolism is essential for metabolic adaptation and development of cells and organisms (Hawkins, 1991). Thus, there should be room for examining the biological aspects, due to the role that protein metabolism plays in the adaptation and development of an organism. In addition, when using indicator traits assessed by stable-isotope profiling of different tissues (e.g., liver and muscle), the tested fish usually has to be sacrificed to obtain the necessary samples. However, the isotope profile in muscle may also be taken with a muscle biopsy on live animals, which would allow the indicator ratio traits to be recorded even on selection candidates. Alternatively, if test fish has to be sacrificed through sib-testing, this means that information on the full-sibs can be used to predict breeding values on the untested selection candidates. Hence, individual phenotyping is still of major importance, even for traits that cannot be recorded on the selection candidates. Thus, as mentioned in the introduction, in full-sib testing an indicator trait is efficient if the EBV for the indicator ratio trait is estimated with high accuracy (based on a considerable number of full-sibs), the indicator has a high genetic correlation to feed efficiency (as estimated for the IFCR indicator trait) and feed efficiency has a significant genetic variance (considered considerable, with 3 percentage point standard deviation for FCR). A slaughter test using full-sibs of the breeding candidates is already a part of the breeding program, and implementation of the indicator ratio traits can, therefore, be carried out in the existing test under field conditions. Given that, the isotope-enriched feed can be produced at an acceptable cost with a precise isotopic signature, this study presents indicator ratio traits (IFCR/IFER) for individual FCR that might be recorded on a massive scale, without requiring individual feed intake recording.

The potential improvement in feed efficiency would depend on the genetic variation (3 percentage point standard deviation for FCR) in feed efficiency. However, the coefficient of variation shows

that there is less variation between families in FCR (4.8%) than RG (24.6%) (Paper III; Table 2), and the heritability was low for the indicator ratio traits IFCR/IFER (0.06-0.11) (Paper III; Table 4). One reason for this is that the coefficient of variation in FCR was estimated on a tank-level and is therefore relevant for group means, implying that the coefficient of variation is lower than it would have been on an individual level, such as for the individual recorded traits. Some studies have, however, proposed that the low heritability and low genetic variation in FCR are due to fish being poikilotherms and that this might reduce the variability in the energy requirements for maintenance costs in fish compared to terrestrial animals (Gjedrem, 1983; Lupatsch et al., 2003; Quinton et al., 2007a).

There is an ongoing discussion on how to properly assess feed efficiency, as FCR is not taking into consideration differences in feed content, quality of the final product or *g* edible product (Jillian et al., 2018). Using FCR as a measure of feed efficiency, the specific intake of protein and calories is not compared to what is retained in the body tissue. Salmonids are efficient converters of dietary protein into body protein (Tacon & Cowey, 1985). However, because salmonids are carnivores, they depend highly on glucose synthesis from non-carbohydrate sources. The surplus of amino acids has a major role in energy metabolism as oxidative substrates in many tissues (Sjaastad et al., 2016). It has also been reported that a reduced capacity for body lipid deposition is favorably associated with high protein growth efficiency (Kause et al., 2016). Fish that store proteins as lipids would not be as efficient as a fish that utilize the dietary protein for growth. Use of carbon isotopes may also allow tracking the metabolism of dietary proteins into lipids. Results showed that converting carbon from a protein source into adipose tissue (AAC) was associated with reduced feed efficiency, however, the genetic correlation between FCR and AAC was moderate (Paper III; Table 5; $r_g = -0.43$) at this life stage (freshwater phase). However, in rainbow trout at a later life-stage (saltwater phase), it has been shown that selection against lipid deposition is associated with improved feed efficiency (Kause et al., 2016). Therefore, it seems that the metabolic processes related to feed efficiency may dependent on the physiological age in salmonids, meaning that lipid deposition potentially explain more of the variation in feed efficiency at a later-life stage. Older fish tend to deposit more fat, and a relatively larger fraction of the feed is allocated to energy and lipid deposition (Einen & Roem, 1997). Hence, the efficiency

of deposition of the dietary nutrients is also an important factor in the feed efficiency complex, which deserves more study.

4.3 Genome-wide association study for efficiency-related traits

The difficulty of obtaining phenotypic recordings on breeding candidates has made it difficult to assess feed efficiency in selective breeding of Atlantic salmon. If a QTL related to feed efficiency can be identified, individual selection based on QTL genotypes can be performed, which could have led to a rapid genetic improvement of feed efficiency.

Paper IV aimed to search for potential associations between growth, indicator traits, and SNPs. Results showed several genome-significant associated SNPs and traits of interest (Paper IV; Figure 1), generally spread over broad regions of chromosomes (Ssa). SNPs significantly associated with growth-related traits such as RG, WG, AMC, AMN, and ALN were mainly located at Ssa09. Gutierrez et al. (2012), who mapped QTLs related to body weight in Atlantic salmon at different time points, reported a genome-wide significant SNPs at Ssa09 for Atlantic salmon at the same age as in this study (~10 months). However, they could not find any relationship between their findings at different time points. Baranski et al. (2010) argued that the large number of different QTLs reported for body weight in Atlantic salmon imply that body weight actually can be considered a polygenic trait. The commercial interest of a QTL for body weight in the freshwater phase is, therefore, most likely limited.

Three genome-wide significant associations were found between SNPs and the indicator ratio trait IFER_AMN, at Ssa06, Ssa23, and Ssa27 (Paper IV; Figure 2d), while no genome-wide significant associations between SNPs and IFCR_AMC, IFCR_AMN, and IFER_AMC were identified (Paper IV; Figure 2a-c). At a chromosome level, a consistent pattern was obtained with all the IFCR/IFER traits having common significant SNPs at Ssa03, Ssa06, Ssa21, Ssa23, and Ssa27 (Paper IV; Table 2). The heritability of these traits was low, and the families were few (n = 23), meaning that if a “true” QTL exist in the population it might not be represented in the families used in this study. More families will increase the number of different haplotypes represented in the data, increasing the possibility to more accurately pinpoint the position of a QTL.

SNPs on a chromosomal region of Ssa12 associated significantly genome-wide with ALC, but SNPs were spread over a region of 40 Mbp (Paper IV; Figure 1e). This strengthens the suggestion that ALC might have another biological basis than the remaining indicator traits considered here. A QTL, which is not related to growth rate, could possibly improve feed efficiency, without inducing a correlated response in increased feed intake. However, ALC showed a clear association to FCR on the family level, albeit low associations to the other FCR indicators on an individual level. Hence, the association between this QTL and FCR is far from clear but cannot be ruled out. Given that a significant QTL affecting FCR can be identified in the saltwater phase, marker-assisted selection can be performed, even on unphenotyped selection candidates, to improve feed efficiency of future generations. The chromosomal regions identified in this study were rather broad and a new experiment is needed for more accurate positioning and identification of possible causative genes.

5. Concluding remarks

The results of this thesis indicate that there is a substantial potential for improved selection for feed efficiency beyond what can be obtained from growth alone, by basing selection on variables derived from nitrogen and carbon metabolism in muscle and liver tissues, measured as the fraction of deposited new nutrients. Since isotope profiling can be performed individually, the IFCR/IFER indicator traits are highly promising traits for assessment of individual feed efficiency in selective breeding of aquatic species. The IFCR/IFER indicator ratio traits quantify the fraction of metabolism allocated to growth, and as such are expected to be global indicators of feed efficiency in aquaculture species and potentially even in livestock. The results of the present study can be summed up as:

- Significant genetic variations in both digestibility of nitrogen and carbon exist.
- The estimated genetic correlations between digestibility and growth rate were strongly unfavorable, indicating that genetic selection for faster growth may reduce the fish's ability to digest, likely an indirect effect through increased feed intake and reduced passage time of ingested feed.
- Stable-isotope profiling after a dietary switch can be used to assess individual feed efficiency. Information on growth and isotope profiles can be combined into biomarkers for individual feed efficiency in fish.
- The indicator ratio trait IFCR (and its' inverse IFER) is measured as the ratio between the excess amount of a certain isotope after a dietary switch (i.e., proportional to newly synthesized nutrients) to growth.
- The nominator of this ratio contains total synthesis (involving both growth and replacement of degraded nutrients), while the denominator captures the difference between synthesis and degradation, meaning that this ratio should capture central aspects of the efficiency.
- Efficient fish have a reduced rate of nutrient replacement in body tissues relative to body growth, which improves FCR.
- Genetic variation exists for the derived feed efficiency variables (denoted indicator ratio traits).

- Switching to an experimental diet enriched on certain stable isotopes can be done without disturbing the fish, and can be performed on a large-scale, provided that large quantities of experimental feed can be produced cost-effectively with a contrasting isotope profile. The method allows feed efficiency to be individually assessed in fish without requiring individual feed intake recording. Hence, the technique has a large potential for use in commercial aquaculture breeding programs, even under field conditions.
- Several genome-wide significant SNPs related to body growth and nitrogen and carbon metabolism in muscle and liver of salmon parr were detected. However, the chromosomal regions were broad, and additional experiments would be needed to verify these QTLs.

6. Implications and future perspectives

This research has shown that the following need to be examined:

- The relationship between growth and digestibility should be estimated in a larger dataset.
- More cost-effective production of experimental feed with contrasting isotope profile should be tested.
- The genetic variance for feed efficiency indicator ratio traits needs to be estimated during the grow-out phase in the sea.
- Potential negative side effects of selection for improved feed efficiency need to be elucidated.
- The underlying biological factors relevant to feed efficiency should be examined.

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Paper I

Indications of a negative genetic association between growth and digestibility in juvenile Atlantic salmon (*Salmo salar*)

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Running title: Genetic variation of digestibility in Atlantic salmon

Abstract

Digestibility is a part of the feed efficiency complex. Improved understanding of the genetics of digestibility and its relation to the most important traits in a breeding program, such as growth rate, is important for enhanced knowledge on the biological aspects of selective breeding for growth. The fish were kept in a common tank from start-feeding until the end of the experiment. All fish were individually tagged and genotyped (56K SNP-chip) at ~5-10 g body weight. A total of 129 fish from 14 families (average initial body weight of 194 g) were included in an individual digestibility trial carried out over 30 days. Individual digestibility was measured as apparent digestibility of nitrogen and carbon in fecal samples, using a fishmeal-based diet with yttrium oxide as an inert marker. To obtain enough feces per fish for digestibility analysis, the fish was stripped four times, i.e., once a week. Results showed significant differences between families with respect to digestibility. Heritabilities were 0.39 ± 0.17 and 0.51 ± 0.18 for digestibility of nitrogen and carbon, respectively. Digestibility showed adverse genetic correlations to the growth rate (-0.77 ± 0.24 to -0.85 ± 0.16). A possible explanation may be that a high growth rate is related to higher feed intake, increasing the passage rate in the gastrointestinal tract and thereby reducing the digestibility of the nutrients. This, however, does not imply that there is an adverse genetic relationship between growth and feed efficiency, as the latter is determined by a number of other factors in addition to digestibility.

Keywords: aquaculture, genetic variance, heritability, genetic correlation, phenotyping, protein digestibility.

1. Introduction

Aquaculture is a rapidly growing industry, and the feed costs make up as much as ~50% of the total production cost in the grow-out phase in the sea (Directorate of Fisheries, 2018). The aquaculture sector steadily needs more protein and lipid ingredients for feed production (FAO, 2015). This is due to carnivorous fish, such as Atlantic salmon, being dependent on high levels of lipids and crude protein in the diets for metabolic energy and growth (Halver & Hardy, 2002). Grisdale-Helland and Helland (1997) reported that the optimum dietary lipid and crude protein levels for obtaining high growth rates of Atlantic salmon in the freshwater phase were 30 and 55%, respectively. In 2017, the total feed cost in Norwegian aquaculture was ~ € 2.3 billion, and ~1.8 million tons of feed were produced (Directorate of Fisheries, 2018). Hence, efforts to save feed costs by genetically improving the Atlantic salmon's ability to digest protein and lipid are highly relevant for improved feed utilization. Currently, genetic selection for improved feed efficiency is primarily targeting growth rate (Gjedrem & Baranski, 2010; Gjedrem et al., 2012; Janssen et al., 2017), and a positive correlation (0.60-0.90) on a family group level between growth rate and feed efficiency has been reported in several studies (Kolstad et al., 2004; Thodesen et al., 1999), likely because an increased growth rate implies that a relatively larger fraction of ingested nutrients are used for growth and less for maintenance (Gjedrem & Baranski, 2010). Moreover, genetically improved feed efficiency, by growth or other means, will reduce production costs and reduce the environmental footprint per unit produced (Besson et al., 2016; de Verdal et al., 2011). However, feed efficiency is a complex trait determined by several factors such as feed intake, digestibility, metabolism and differential use of net energy for maintenance, growth, activity (Byerly, 1967; Gjedrem, 2005; Varley, 2009). In addition to growth, other traits

(e.g., digestibility) potentially adding information to the feed efficiency complex thus deserve attention in selective breeding programs.

Increased growth is positively correlated with higher feed intake (r = 0.98) (Kolstad et al., 2004), resulting in more nutrients being available for growth. However, increased feed intake may adversely affect digestibility, as increased feed intake may reduce passage time, potentially reducing the ability of the fish to digest and absorb the nutrients in the feed. If so, a negative genetic correlation may be expected between growth and digestibility.

Improved digestibility, measured as the apparent digestibility coefficient (ADC), of protein in aquafeeds, will have a potential to improve feed efficiency, implying that a larger fraction of the ingested nutrients would be available for physiological processes within the animal, rather than lost through the feces. Apparent digestibility is defined as the fraction of a nutrient eaten that is digested, absorbed, and not excreted with the feces, without correcting for endogenous fecal excretions (Halver & Hardy, 2002). A method to estimate individual apparent digestibility coefficients with an inert marker (e.g., yttrium oxide), based on stripping the fish for feces has been established by Austreng et al. (2000). Using this method on a large number of individual fish enables estimation of heritability of individual digestibility, as well as its genetic correlation to other traits such as growth.

The aim of the study was to estimate the genetic variance and heritability of nitrogen and carbon digestibility parameters and their genetic and phenotypic correlations with growth in juvenile Atlantic salmon using individually recorded phenotypes and genotypes.

2. Materials and Methods

This study used phenotypic data from a family experiment with Atlantic salmon carried out at the fish laboratory at the Norwegian University of Life Sciences (NMBU), Aas, Norway, according to the laws and regulations controlling experiments on live animals in EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 11676). No mortality or sign of disease occurred during the experimental period.

2.1 Fish and housing

A 30-day experiment was performed using 14 full-sib families of Atlantic salmon (*Salmo salar*) of the AquaGen population. The families had clear differences with respect to growth potential. From the eyed-egg stage until the start of the experiment, all families were communally reared in a single tank. When the fish were ~5-10 g, they were pit-tagged with a 2 x 12 mm unique glass tag (RFID Solutions, Hafrsfjord, Norway), and a fin-clip was collected for genotyping. All fish were genotyped using AquaGen's custom Axiom®SNP genotyping array from Thermo Fisher Scientific (San Diego, CA, USA), containing 56,177 single-nucleotide polymorphisms (SNP). Prior to the experiment, the family background of each individual fish was established by the genomic relationship likelihood for parentage assignment (Grashei et al., 2018).

At the age of 10 months, 4-16 pre-smoltified members of 14 different families were individually weighed and transferred into the experimental tank (129 fish in total). The tank, with a 3000-L capacity, was supplied with recirculated fresh water, at a flow rate of 8 L min^{-1}, and the fish were kept under 24 h light regime, with an average temperature of 15 ℃. Dissolved oxygen was measured daily and maintained above 7.5 mg L^{-1} in the outlet water (Handy Delta, OxyGuard® AS, Farum, Denmark).

2.2 Dietary treatment, feeding and sampling

The experimental diet fed during the entire experimental period was a fishmeal-based diet with yttrium oxide (Y_2O_3) as an inert marker, with ingredients known to have no negative effect on health in Atlantic salmon. The analyzed content of the diet was in accordance with the dietary formulation presented in Table 1. The diet was produced at the feed laboratory of the Norwegian University of Life Sciences (Aas, Norway) as explained in Dvergedal et al. (2019). A feed sample of 500 g was taken from the produced batch of feed and stored at 4 ℃ for chemical analyses. Feeding was continuous (24 h/day) by automatic belt feeders. The feeding level equaled 10% in excess, based on the estimated specific growth rate calculated as described in Halver and Hardy (2002). Fish consumed the diet in accordance with the estimated daily growth rate. Once a week throughout the 30-day experimental period, fish were anesthetized with metacaine (MS-222TM; 1 g L^{-1} water), and feces were stripped and collected as explained by Austreng (1978). At the termination of the experiment, fish were anesthetized, killed with a sharp blow to the head, stripped and whole-body weight and length were recorded.

129 2.3 Chemical analyses

130

131 The diet was dried and ground prior to analysis, and results of the chemical analyses are
132 presented as an average of two samples (Table 1). The diet was analyzed for dry matter by
133 drying to a constant weight at 104 ℃, for ash by combustion at 550 ℃, for crude protein by
134 Kjeldahl nitrogen x 6.25 according to Commission Regulation (EC) No 152/2009, and for starch
135 as described in McCleary et al. (1994). Lipid was determined after extraction with petroleum
136 ether and acetone (70/30) on an Accelerated Solvent Extractor (ASE 200) (Dionex Corp,
137 Sunnyvale, CA, USA), while gross energy was established with a PARR 1281 Adiabatic Bomb
138 calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. Amino acids were
139 analyzed according to Commission Regulation (EC) No 152/2009 on a Biochrom 30 Amino
140 Acid Analyzer (Biochrom Ltd,. Cambridge, UK), for all amino acids except tryptophan.
141 Tryptophan analysis was performed according to Commission Regulation (EC) No 152/2009
142 using a Dionex Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany)
143 equipped with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan).
144 Three replicates of the diet were homogenized, and to obtain enough feces per fish the four feces
145 samples per fish were pooled, freeze-dried and homogenized, before analyses of nitrogen (N)
146 and carbon (C) using a CHNS Elemental Analyzer (Vario El Cube elemental analyzer system
147 GmbH, Hanau, Germany). The internal digestibility marker, Yttrium oxide (Y) (Metal Rare
148 Earth Limited, Shenzhen, Guangdong, China), in diets and feces was determined by ICP-MS
149 (Agilent 8800 Triple Quadrupole mass spectrometer, Agilent Technologies Inc., Santa Clara,
150 CA, USA). The samples were decomposed with concentrated ultrapure HNO_3 at 250°C using a
151 Milestone microwave UltraClave III (Milestone Srl, Sorisole, Italy). The Y analyses were

152 validated using certified reference material no. NIM-GBW07603 (National Analysis Centre for
153 Iron & Steel, Beijing, China).
154
155 2.4 Calculation of apparent digestibility coefficients
156
157 Individual ADC was calculated as follows, using Y_2O_3 as the inert marker (Austreng et al., 2000)
158 for both elements nitrogen and carbon:

159 $$ADC_{element}(\%) = \frac{a-b}{a} \times 100,$$

160 where $a = \frac{\%\ element\ in\ feed}{\%\ Y_2O_3\ in\ feed}$, and $b = \frac{\%\ element\ in\ feces}{\%\ Y_2O_3\ in\ feces}$.
161
162 2.5 Phenotypes analyses
163
164 After one week in the experiment, the initial weight of each fish *i* (IW_i, g) was recorded. After
165 the experiment, i.e. at sampling, final weight (FW_i, g) was recorded. From these two variables,
166 individual weight gain (WG_i) and relative weight gain (RG_i) were calculated as follows:

167 $$WG_i = FW_i - IW_i$$

168 $$RG_i = ((FW_i - IW_i) / FW_i)\ 100.$$

169 From the feces samples, the apparent digestibility coefficient for nitrogen (ADC_N_i) and the
170 apparent digestibility coefficient for carbon (ADC_C_i) were available at an individual level.
171
172 2.6 Genetic analyses

Pairwise bivariate analyses of the individual phenotypes for WG, RG, ADC_N, and ADC_C were performed. For each bivariate analysis, the model was:

$$\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{a1}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_{a2}}\end{bmatrix}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} + \begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix},$$

where $\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix}$ is a vector of individual phenotypes for the two traits included in the model, $\mathbf{b_1}$ and $\mathbf{b_2}$ are vectors of fixed effects including the intercept for the two traits, $\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{G_o}\otimes\mathbf{G})$, is a vector of random additive genetic effects for the two traits, and $\begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{R}\otimes\mathbf{I})$, is a vector of random residuals for the two traits. The **X** and **Z** matrices are corresponding incidence matrices, $\mathbf{G_o}$ is an additive genetic (co)variance matrix, **G** is the genomic relationship matrix, and **R** is the residual (co)variance matrix. The genomic relationship matrix was generated according to VanRaden's first method (VanRaden, 2008). The number of phenotyped individuals in this study was rather low (n =129), i.e., there are very few phenotyped fish per family (9.2 ± 3.1). Using a traditional pedigree-based model, where genetic variation is estimated based on between-family variation, estimation of genetic (co)variance components will thus be imprecise. However, by using a genomic relationship matrix all individuals (n = 129) will contribute to the estimation of the genetic parameters. In fact, using genomic relationships genetic parameters can be estimated using one or a few families, even when applied to selectively genotyped data (Ødegård & Meuwissen, 2012). Matrix **G** (129x129) was calculated based on a subset of 51,543 SNPs of high genotype quality, covering all chromosomes.

193 Heritability was calculated as: $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$, where σ_a^2 is the additive genetic variance and σ_e^2 is

194 the residual variance of the trait, for the pooled sample of four stripping's per fish. For each trait,

195 the coefficient of phenotypic as well as a genetic variation (CV_P and CV_G, respectively) were

196 calculated (e.g. Felix et al., 2012).

197

198 The significance of the genetic effect was tested using a likelihood-ratio (LR) test-statics,

199 comparing a single-trait model with genetic effect (H_1) to a model without genetic effects (H_0)

200 with the **G** matrix in H_1. LR was then calculated as:

201 $$LR = 2\left(\left(ln\, L\left|\hat{\theta}_{H_1}\right.\right) - \left(ln\, L\left|\hat{\theta}_{H_0}\right.\right)\right).$$

202 The genetic effect was considered significant if LR < χ^2 ($\alpha = 0.05$, df = 1).

203

204 All genetic analyses were performed using the ASReml4 software package (Gilmour et al.,

205 2015).

206

207 ## 3. Results

208

209 Descriptive statistics of the data are given in Table 2. Initial body weight for fish at the same age

210 ranged 32.6-337.7 g. A large variation in IW, FW, WG, and RG was observed between families

211 (Figure 1a-d). Even if the experimental period was relatively short (30 days) the growth was

212 substantial, averaging 42% (relative to initial body weight).

213

Figure 2a-b shows ADC_N and ADC_C for all families. In Table 3 significant genetic effects on nitrogen (*p = 1.1E-04*) and carbon (*p= 7.4E-07*) digestibility as well as for WG and RG (both < 0.001) are shown. Table 3 also demonstrate high heritability estimates for ADC_N, ADC_C, WG and RG (0.39 ± 0.17, 0.51 ± 0.18, 0.52 ± 0.17 and 0.57 ± 0.17, respectively). Tables 2 and 3, gives CV_P and $CV_{G,}$ respectively, that were generally low for ADC_N and ADC_C, but higher for WG and RG.

Estimated phenotypic and genetic correlations are presented in Table 4. The phenotypic correlations to relative weight gain were -0.40 and -0.56 for nitrogen and carbon digestibility, respectively, as also indicated in Figure 3. Generally, the estimated genetic correlation between ADC_N and ADC_C on one side and WG on the other were negative, i.e., that fast growth seems genetically associated with lower digestibility of both nitrogen and carbon. The estimated genetic correlation between WG and ADC_C was most expressed (-0.85 ± 0.16), followed by that to ADC_N (-0.77 ± 0.24) and RG (0.79 ± 0.11). The estimated genetic correlation between the two digestibility coefficients and RG were also highly negative, -0.84 for both. Moreover, the estimated genetic correlation between ADC_N and ADC_C was positive and very high (fixed at the boundary of 1.0 by the program), indicating that digestibility of nitrogen and carbon are largely the same genetic trait.

4. Discussion

The two growth traits, WG and RG, were as expected highly genetically correlated (Table 4). The same result was obtained for the two digestibility traits, ADC_N and ADC_C (Table 4),

which was expected as protein was the main source of both nitrogen and carbon in the diet. The apparent digestibility of nitrogen and carbon had a strong adverse genetic correlation to growth (-0.77 ± 0.24 to -0.85 ± 0.16; Table 4). The negative genetic correlation might be explained by a high positive correlation between growth rate and feed intake (0.98) in Atlantic salmon (Kolstad et al., 2004). A low feed intake is associated with a reduced gastric evacuation time (Venou et al., 2009), which leads to longer time for digestion and absorption of nutrients, improving digestibility (Aas et al., 2011; Adamidou et al., 2009). On the other hand, given a fixed average slaughter weight, genetically increased growth rate will reduce time to slaughter, reduce the energetic cost for maintenance and thereby improve feed conversion ratio (FCR) (Cook et al., 2000; Gjedrem & Baranski, 2010). In fact, Henryon et al. (2002) obtained a significant favorable genetic correlation between the growth rate and FCR (r_g = *-0.63-0.99*) in rainbow trout (*Oncorhynchus mykiss*). In consequence, the selection for improved feed efficiency through improvement of growth is expected to dominate the genetically negative correlated effect on digestibility. However, assuming the genetic parameters estimated in this study, the digestibility is expected to decrease by 0.65 percentage points per genetic standard deviation improvement in weight gain, which is noticeable. Thus, in the future, given that genetic variation in digestibility exists, one should consider the potential of including digestibility in the selection scheme for fish. Still, as the estimated genetic correlations are strongly unfavorable (-0.77 to -0.85), combined selection for improved growth rate and improved digestibility will be challenging.

The average ADCs values obtained in this experiment (Table 2) were in accordance with earlier studies performed on a fishmeal-based diet (Espe et al., 2006; Storebakken et al., 2000). Highly significant heritabilities were found for all traits, for ADC_N (0.39 ± 0.17) and ADC_C (0.51 ±

0.18) (Table 3). These results corresponded with Austreng and Refstie (1979) who reported the existence of genetic variation in apparent digestibility for protein in rainbow trout. Both measures of digestibility showed much lower coefficients of genetic variance than the growth traits (Table 3). This implies that growth capacity has a larger potential for genetic change, compared with digestibility.

The stripping method used to calculate ADCs has been evaluated to be suitable by Percival et al. (2001). However, the amount of feces is restricted for fish smaller than one kg, and repeated stripping is needed to perform the analyses. Stone et al. (2008) investigated the effect of repeated fecal collections in rainbow trout and found an induced cortisol stress response and indications of pro-inflammatory cytokine responses. Nevertheless, Stone et al. (2008) found no pathological or histological alterations in the distal intestine. Moreover, for protein, their ADCs remained unaffected by the repeated fecal collection procedures. However, this experiment did not register any health parameters, but no mortality or sign of disease were observed. Fish were healthy and grew continuously throughout the experiment confirming that repeated fecal collections by stripping did not have any major detrimental impact on the performance of the fish.

In this study, a fishmeal-based diet was used, but commercial diets are now mostly plant-based for which a reduction in both protein and lipid digestibility has been observed (Krogdahl et al., 2003; Refstie et al., 1998). Interestingly, a substantial genetic variation in utilizing plant-based diets for growth has been observed in rainbow trout (Pierce et al., 2008). To what extent the diet affects the genetic variation in digestibility is, however, not known. Moreover, Atlantic salmon in the freshwater phase was studied, although feed utilization during the saltwater phase is, by

far, much more important. In saltwater, the relative weight gain (for a given time unit) is expected to be smaller (Davidson et al., 2014; Gjedrem & Gunnes, 1978; Santosh, 1999), and this might somewhat affect the association between growth rate and digestibility. In spite of this, the estimated genetic correlations in this study give an indication of an unfavorable relationship between growth rate and digestibility that deserves more study.

5. Conclusion

Significant genetic differences in digestibility of nitrogen and carbon were found, and estimated heritabilities were high (0.39 ± 0.16 and 0.51 ± 0.18, respectively). The estimated genetic correlations between digestibility and growth traits were strongly unfavorable with moderate standard errors, indicating that genetic selection for increased growth might lead to reduced digestibility.

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Declaration of interest

The authors declare that they have none.

Submission declaration

All authors read and approved the final manuscript for submission. The content of the manuscript has not been published or submitted for publication elsewhere.

Authors' contributions

H.D., J.Ø., M.Ø., L.T.M., and G.K. designed the experiment. H.D. was responsible for feed production and carried out the experiment. H.D. and J.Ø. conducted the statistical analyses. H.D. wrote the first draft of the manuscript, improved by H.D., J.Ø., M.Ø., L.T.M. and G.K.

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Table 1.

Formulation and analyzed content of experimental diet

Formulation and content	Diet†
Formulation, g kg^{-1}	
Fish meal‡	460.0
Gelatinized potato starch§	130.0
Wheat gluten¶	129.6
Spirulina††	20.0
L-Lysine‡‡	2.0
Fish oil§§	170.0
Gelatin¶¶	80.0
Premix fish†††	6.3
Monocalcium phosphate‡‡‡	2.0
Y_2O_3§§§	0.1
Analyzed content, kg^{-1}	
Dry matter, g	876.9
Crude protein, g	496.1
Lipid, g	173.1
Starch, g	120.7
Ash, g	71.1
Gross energy, MJ	21.3
Essential amino acids, g kg^{-1}	
Arginine	29.8
Histidine	8.5
Isoleucine	17.1
Leucine	32.6
Lysine	28.1
Methionine	10.6
Phenylalanine	19.2
Threonine	18.4
Valine	19.6
Tryptophan	4.0
Non-essential amino acids, g kg^{-1}	
Alanine	30.1
Aspartic acid	35.3
Glycine	41.1
Glutamic acid	93.3
Cysteine	4.3
Tyrosine	11.7
Proline	35.5
Serine	23.2
Total Amino Acids	462.5

†The analyses were a mean of duplicates.

‡Norse LT 16-001, Norsildmel, Egersund Sildoljefabrikk AS, Egersund, Norway.

§Lygel F 60, Lyckeby Culinar, Fjälkinge, Sweden.

¶Vital Wheat Gluten, Amilina, Panevezys, Lithuania.

††CIL-NLM-8401 Spirulina Whole cells (U-^{15}N, 98%+), Cambridge Isotope Laboratories, Larodan, Solna, Sweden.

‡‡CIL-NLM-143 L-Lysine*2HCl (alfa-^{15}N, 95-99%), Cambridge Isotope Laboratories, Larodan, Solna, Sweden.

§§NorSalmOil, Norsildmel, Bergen, Norway.

¶¶Rousselot® 250 PS, Rousselot SAS, Courbevoie, France.

†††Farmix, Trouw Nutrition, LA Putten, The Netherlands. Per kg feed; retinol 2500.0 IU, cholecalciferol 32400.0 IU, α-tocopherol SD 0.2 IU, menadione 40.000 mg, thiamine 15.0 mg, riboflavin 25.0 mg, d-Ca-pantothenate 40.002 mg, niacin 150.003 mg, biotin 3000.0 mg, cyanocobalamin 20.0 mg, folic acid 5.0 mg, pyridoxine 15.0 mg, ascorbate polyphosphate 0.098 g, Cu: Cu sulfate $5H_2O$ 11.998 mg, Zn: Zn sulfate 89.992 mg, Mn: Mn(II) sulfate 34.993 mg, I: K-iodine 1.999 mg, Se: Na-selenite 0.200 mg, Cd Max. 0.0003 mg, Pd Max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g.

‡‡‡Bolifor®MCP-F.KPP Oy, Animal Nutrition, Helsingborg, Sweden.

§§§Yttrium oxide (Y2O3), Metal Rare Earth Limited, Shenzhen, Guangdong, China.

Table 2.

Descriptive statistics of individual trait variables; initial and final weights (IW and FW, respectively), weight gain (WG), relative weight gain (RG), and apparent digestibility coefficient for nitrogen (ADC_N) and carbon (ADC_C), respectively.

	n[†]	Mean	Min	Max	SD	CV_P[‡]
IW, g	129	194.4	32.6	337.7	52.7	27.0
FW, g	128	275.9	49.1	498.4	84.8	30.6
WG, g	128	82.6	6.9	201.5	39.3	47.4
RG, %	128	28.6	7.4	50.2	7.79	27.1
ADC_N, %	125	90.0	86.9	92.8	1.19	1.3
ADC_C, %	125	87.0	81.3	91.1	1.86	2.1

[†]One fish lacked recording of final weight, and four fish had a too small amount of feces material for apparent digestibility determination.

[‡] Coefficient of phenotypic variation in percentage.

Table 3.

Estimated† genetic (σ_a^2) and residual variance (σ_e^2) components, heritability (h^2) of weight gain (WG), relative weight gain (RG), apparent digestibility coefficients for nitrogen (ADC_N) and carbon (ADC_C), respectively, all with standard errors. The χ^2 - test statistics for the additive genetic family effect with the corresponding level of significance (p), and the coefficient of genotypic variance (CV_G, %) is given.

	σ_a^2	σ_e^2	h^2	χ^2	p	CV_G
WG	712.7 ± 311.2	632.5 ± 185.03	0.52 ± 0.17	39.9	2.7E-10	32.3
RG	35.6 ± 14.7	24.9 ± 8.4	0.57 ± 0.17	27.5	1.6E-07	20.9
ADC_N	0.60 ± 0.31	0.81 ± 0.21	0.39 ± 0.17	15.0	1.1E-04	0.86
ADC_C	2.01 ± 0.88	1.48 ± 0.50	0.51 ± 0.18	24.5	7.4E-07	1.63

† Estimates based on a pooled sample of four feces stripping's per fish.

Table 4.

Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between weight gain (WG), relative weight gain (RG) and apparent digestibility coefficients for nitrogen (ADC_N) and carbon (ADC_C), respectively, with their standard errors.

Trait	WG	RG	ADC_N	ADC_C
WG		0.79 ± 0.11	-0.77 ± 0.24	-0.85 ± 0.16
RG	0.81 ± 0.04		-0.84 ± 0.19	-0.84 ± 0.14
ADC_N	-0.38 ± 0.09	-0.40 ± 0.09		1.00[†]
ADC_C	-0.51 ± 0.08	-0.56 ± 0.07	0.89 ± 0.02	

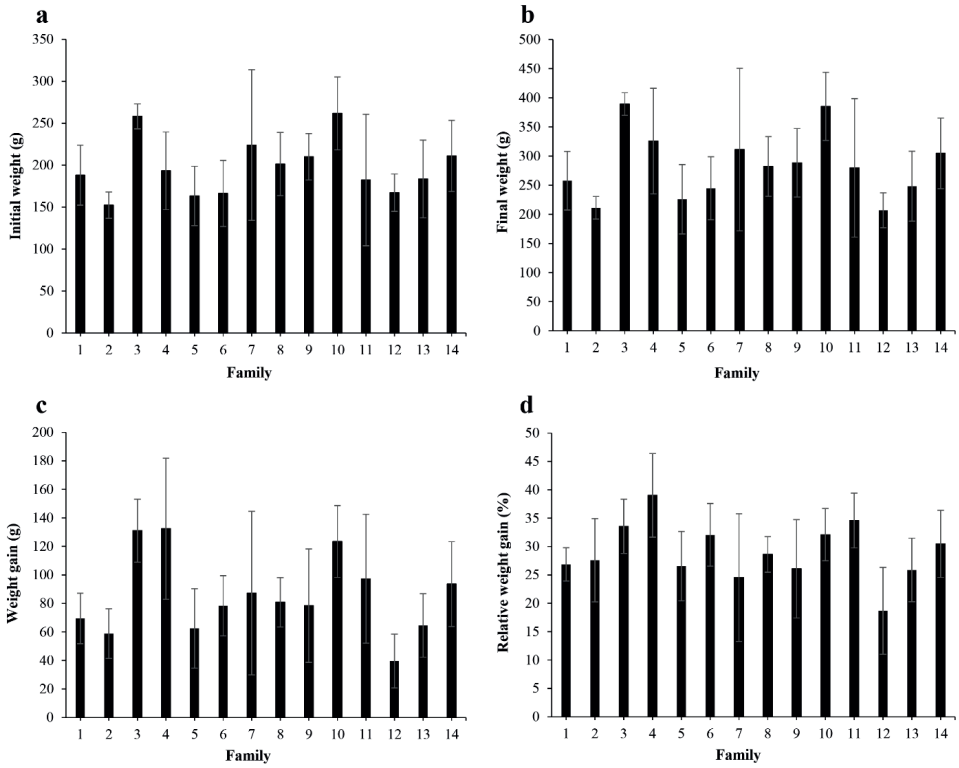
[†]Fixed at the boundary by the program.

Figure 1. Averages per family for (a) initial weight (IW), (b) final weight (FW), (c) weight gain (WG) and (d) relative weight gain (RG), with standard deviations represented by vertical bars.

Figure 2. Averages per family for apparent digestibility coefficients of (a) nitrogen (ADC_N) and (b) carbon (ADC_C), with standard deviations represented by vertical bars.

Figure 3. A plot of the negative linear relationship between relative weight gain and apparent digestibility of (a) nitrogen (ADC_N) and (b) carbon (ADC_C). The estimated regression lines were: (a) $\mathbf{y} = -0.06x + 91.77$, with $R^2 = 0.15$ and (b) $y = -0.14x + 91.02$, with $R^2 = 0.32$.

504 **Figure 1.**



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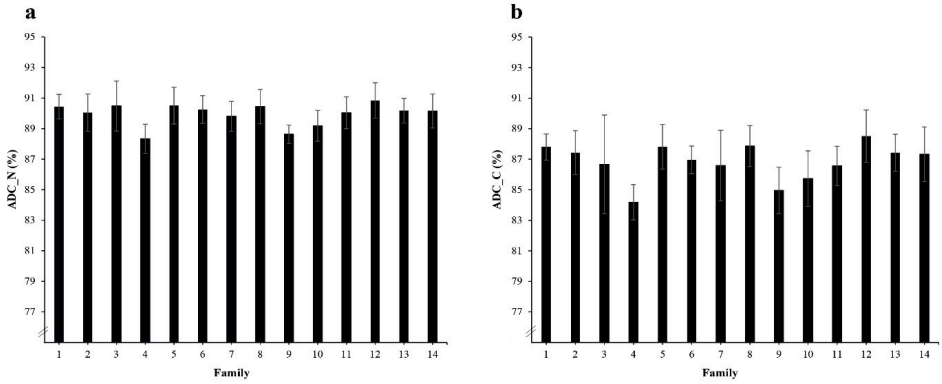
513 **Figure 2.**



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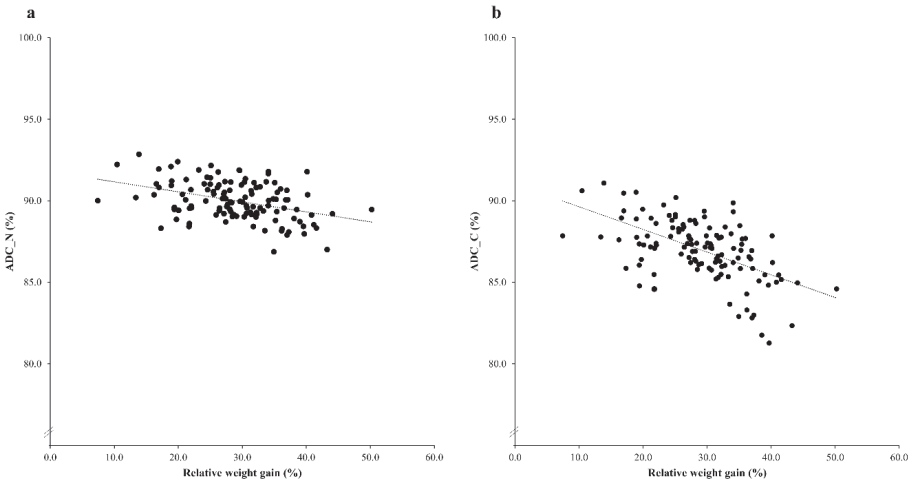
526 **Figure 3.**



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Paper II

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ORIGINAL ARTICLE

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Stable isotope profiling for large-scale evaluation of feed efficiency in Atlantic salmon (*Salmo salar*)

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Abstract

In growing animals, individual variation in feed efficiency may arise from individual differences in growth rate and protein metabolism. Over a period of time, these factors will affect the ratio between 'new' vs. 'old' protein, which can be quantified using isotope profiling. The aim of this study was to investigate the relationship between relative weight gain and atom percentage excess ^{15}N in the muscle, liver and mid-intestine. A 50-day experiment was conducted with a total of 375 fish initially fed a standard diet, subsequently replaced by one out of five experimental diets, enriched with ^{15}N. In general, fast-growing fish are expected to have a better feed efficiency, and the results show that this is captured by isotope profiling in liver and muscle tissues. Furthermore, individual variation in isotope content, that is relative fraction of 'new' protein, among fish with comparable growth rates was observed, most expressed around ~50% isotope saturation, indicating differences in protein degradation and replacement not attributed to growth. The results suggest that isotope profiles can be used as individual indicator traits for feed efficiency and that inclusion levels of stable isotopes of 1%–2% gave the most reliable results.

KEYWORDS
Atlantic salmon, growth, maintenance requirements, protein metabolism, protein turnover, selective breeding

1 | INTRODUCTION

Advanced selective breeding programmes for Atlantic salmon (*Salmo salar*, Salmonidae) have been carried out since the early 1970s (Gjedrem, Gjøen, & Gjerde, 1991), with growth as the pivotal trait. After five generations of selection, Thodesen, Grisdale-Helland, Helland, and Gjerde (1999) compared selected salmon to wild salmon, reporting a 25% improvement in feed efficiency, defined as growth per unit of feed. Recording of individual feed efficiency involves recording of the individual feed intake as well as individual weight gain. While the latter is relatively easy to obtain, individual feed intake is not easily attainable in large-scale aquaculture systems. Research have shown that feed intake cannot fully explain individual variation in growth, due to different individual growth responses when consuming the same amount of feed (Carter, Houlihan, Buchanan, & Mitchell, 1993b; Houlihan, Carter, & McCarthy, 1995). Although genetic variation in feed efficiency obviously exists, the underlying mechanisms are unknown, but can be assumed partly due to individual variation in protein metabolism.

Traditionally, the individual feed efficiency in fish has been improved through selection for increased growth rate, assuming a favourable genetic correlation to feed efficiency (Thodesen, Gjerde, Grisdale-Helland, & Storebakken, 2001). A fast-growing animal will likely be more feed efficient by using a relatively larger fraction of the feed for growth and less for maintenance of existing body tissue. On a family level, Thodesen et al. (2001) estimated a favourable

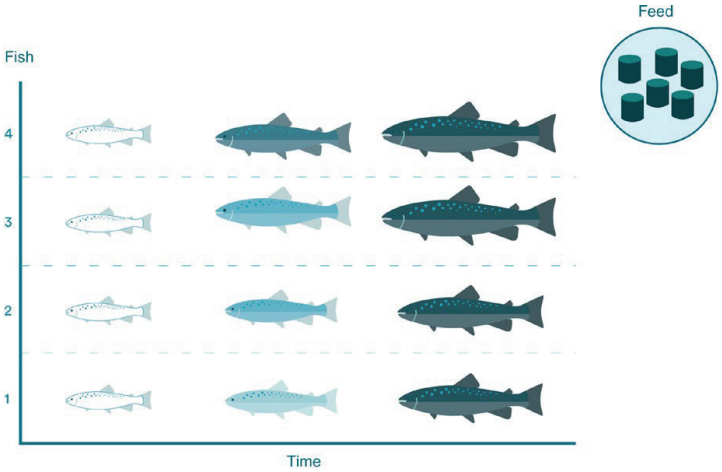

FIGURE 1 A schematic drawing illustrating the overall reflection of the regulation of growth over time, synthesized on a new diet, with different coloured fish resembling the metabolic breakdown of tissues that were synthesized during feeding on the previous diet, and their subsequent replacement and growth of tissues synthesized on a new diet

correlation of 0.8 between feed efficiency and growth rate. They stated an apparently decreasing response in feed efficiency with increasing growth rates, suggesting that direct selection for improved feed efficiency would be beneficial. Hence, it is timely to address other indicator traits related to feed efficiency as a tool to improve selective breeding for feed efficiency in aquatic species.

Diets with distinct stable isotope ratios, for example $^{15}N/^{14}N$ and $^{13}C/^{12}C$, can be used to trace metabolism of nutrients (Houlihan et al., 1995). Feeding diets with enriched levels of specific isotopes will result in an isotopic change in the organism (Le Vay & Gamboa-Delgado, 2011). Changes in the isotopic composition of tissues after a change in diet occur through two processes (Fellerhoff, 2002; Jardine, McGeachy, Paton, Savoie, & Cunjak, 2003). First, the process involves the metabolic breakdown of tissues that were synthesized during feeding on the previous diet, and their subsequent replacement with tissues synthesized on a new diet. Second, the growth of new tissue that occurs after a diet switch will reflect the isotopic composition of the current diet, and will contribute to the overall reflection of that diet in the isotopic composition of the fish (Figure 1). Growth rate is a result of the net difference between absorption of new nutrients from feed and loss of nutrient components in faeces, while efficiency depends on the ratio between them. Hence, growth rate may not explain all variation in efficiency (i.e. a slow- or moderately growing individual may still be efficient if the degradation losses are sufficiently low). By combining growth rate and change in isotope profile, the efficiency can be more accurately assessed than by using relative increase in growth alone. The change in isotope ratio can be used to determine the rate of protein metabolism in various tissues, as a result of metabolic activity, nutritional state, partitioning of nutrients, physical activity and tissue growth (Bloomfield, Elsdon, Walther, Gier, & Gillanders, 2011).

The current experiment is part of a study aiming at investigating the use of stable isotope in feed to assess feed efficiency in Atlantic salmon. The current study was a pilot with five inclusion levels of ^{15}N in feed and sampling fish at seven time points with the aim to study the relationship between relative weight gain and atom percentage excess (APE) ^{15}N in the muscle, liver and mid-intestine. From this, one objective was to draw inference as to how phenotyping could be carried out and yet another to discuss the potential of the indicator traits in selective breeding for improved feed efficiency in aquaculture species.

2 | MATERIALS AND METHODS

2.1 | Fish, housing and health

The experiment was carried out at the fish laboratory at the Norwegian University of Life Sciences (NMBU), Ås, Norway, following the laws and regulations for experiments on live animals in EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 9484). A total of 510 Atlantic salmon with an average initial body weight of 21 g were randomly distributed into 15 tanks (34 fish per tank). Prior to start of the 50-day experimental period, all fish were pit-tagged with a 2 x 12 mm unique glass tag (RFID Solutions, Hafrsfjord, Norway), and the initial length and weight were recorded. The tanks, each with a 270-L capacity, were supplied with recirculated fresh water. Water flow rate was 7–8 L/min, and the fish were kept under 12 hr/12 hr light/dark regime, with temperature in range of 15–16°C. Dissolved oxygen was measured daily and kept above 8 mg/L in the outlet water (Handy Delta, OxyGuard® AS, Farum, Denmark). There were no mortality or sign of disease during the experimental period.

2.2 | Dietary treatments and feeding

The dietary treatments consisted of four ^{15}N-marked diets with different inclusion levels of ^{15}N. Diets SP1 and SP2 contained 1% and 2% ^{15}N marked *Spirulina* whole cells (Larodan, Sweden), while diets L0.1 and L0.2 had 0.1% and 0.2% ^{15}N marked L-Lysine (Sigma, Norway). A control diet was formulated with no added (0%) ^{15}N. All diets were formulated to meet requirements for Atlantic salmon

for protein (NRC, 2011), and their composition are given in Table 1. The feed ingredients were mixed at the feed laboratory at NMBU, Ås, Norway. All dry ingredients, except gelatine, were mixed in a Forberg mixer (vacuum coater) (Forberg AS, Hegdal, Larvik, Norway). Gelatine was dissolved in cold water and then heated to 55°C in a microwave oven. Fish oil, dissolved gelatine, water and the dietary ingredients were mixed with a Moretti Foreni kneading machine (Spiry 25, Mondolfo, Italy). This resulted in a firm dough that was cold pelleted using an Italgi pasta extruder (P35 A, Carasco, Italy) equipped with a 2.5-mm die. Feed was cooled to room temperature and dried at 45–60°C to about 95% dry matter in a batch dryer with a DANIA 9 kW fan (Inelco A7S, Fjerntslev, Denmark). The feed was stored at 4°C until feeding. The diets were fed to triplicate groups of fish for a period of 1 hour, twice a day (08:00 and 17:00) by automatic belt feeders. The feeding level equalled 1.5% of body weight the first day and then 10% in excess, based on the level of uneaten feed. Uneaten feed was collected from the water outlet, and feed intake was calculated as described by Helland, Grisdale-Helland, and Nerland (1996).

TABLE 1 Formulated composition of experimental diets

	Dietary treatments				
Formulation, g/kg	**Control**	**SP1**	**SP2**	**L0.1**	**L0.2**
Fish meal[a]	460	460	460	460	460
Gelatinized potato starch[b]	130	130	130	130	130
Wheat gluten[c]	129.6	129.6	129.6	129.6	129.6
Spirulina[d]	20.0	10.0	0.0	20.0	20.0
Spirulina ^{15}N[e]	0.0	10.0	20.0	0.0	0.0
L-Lysine[f]	2.0	2.0	2.0	1.0	0.0
L-Lysine ^{15}N[g]	0.0	0.0	0.0	1.0	2.0
Fish oil[h]	170.0	170.0	170.0	170.0	170.0
Gelatine[i]	80.0	80.0	80.0	80.0	80.0
Premix fish[j]	6.3	6.3	6.3	6.3	6.3
Monocalcium phosphate[k]	2.0	2.0	2.0	2.0	2.0
Y_2O_3[l]	0.1	0.1	0.1	0.1	0.1

[a]Norse LT 16-001, Norsildmel, Egersund Sildoljefabrikk AS, Egersund, Norway. [b]Lygel F 60, Lyckeby Culinar, Fjälkinge, Sweden. [c]Vital Wheat Gluten, Amilina, Panevezys, Lithuania. [d]CIL-ULM-8453 Spirulina Whole cells (unlabelled), Cambridge Isotope Laboratories, Larodan, Solna, Sweden. [e]CIL-NLM-8401 Spirulina Whole cells (U-^{15}N, 98%+), Cambridge Isotope Laboratories, Larodan, Solna, Sweden. [f]L5751 L-Lysine dihydrochloride (98% unlabelled), Sigma-Aldrich, St. Louis, USA. [g]CIL-NLM-143 L-Lysine*2HCl (alfa-^{15}N, 95%–99%), Cambridge Isotope Laboratories, Larodan, Solna, Sweden. [h]NorSalmOil, Norsildmel, Bergen, Norway. [i]Rousselot® 250 PS, Rousselot SAS, Courbevoie, France. [j]Farmix, Trouw Nutrition, LA Putten, The Netherlands. Per kg feed; retinol 2,500.0 IU, cholecalciferol 32,400.0 IU, α-tocopherol *SD* 0.2 IU, menadione 40.000 mg, thiamine 15.0 mg, riboflavin 25.0 mg, d-Ca-pantothenate 40.002 mg, niacin 150.003 mg, biotin 3,000.0 mg, cyanocobalamin 20.0 mg, folic acid 5.0 mg, pyridoxine 15.0 mg, ascorbate polyphosphate 0.098 g, Cu: Cu sulphate 5H2O 11.998 mg, Zn: Zn sulphate 89.992 mg, Mn: Mn(II) sulphate 34.993 mg, I: K-iodine 1.999 mg, Se: Na-selenite 0.200 mg, Cd Max. 0.0003 mg, Pd max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g. [k]Bolifor®MCP-F.KPP Oy, Animal Nutrition, Helsingborg, Sweden. [l]Yttrium oxide (Y_2O_3), Metal Rare Earth Limited, Shenzhen, Guangdong, China.

2.3 | Chemical analysis

The diets were ground and analyses were performed in duplicates. Diets were analysed for dry matter by drying to constant weight at 104°C, ash by combustion at 550°C, crude protein by Kjeldahl nitrogen x 6.25 according to Commission regulation (EC) No 152/2009 and starch as described in McCleary, Solah, and Gibson (1994). Lipid was analysed after extraction with petroleum ether and acetone (70/30) on an Accelerated Solvent Extractor (ASE 200) (Dionex Corp, Sunnyvale, CA, USA). Gross energy was determined with a PARR 1281 Adiabatic Bomb calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. Amino acids except tryptophan were analysed according to Commission regulation (EC) No 152/2009 on a Biochrom 30 Amino Acid Analyser (Biochrom Ltd., Cambridge, UK). For tryptophan, a Dionex Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) equipped with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan) was used, and the analysis was carried out according to Commission regulation (EC) No 152/2009. The chemical composition of the diets is given in Table 2.

2.4 | Sampling

Four fish from each tank were sampled at different time points (2, 4, 8, 16, 32 and 50 days). The remaining fish (n = 150) were kept for another experiment. Fish were anaesthetized with metacaine (MS-222™; 1 g/L water) and killed with a sharp blow to the head prior to dissection. Slaughter weight and length were recorded for all fish, and tissue samples (muscle, liver and mid-intestine) were collected in cryotubes, snap-frozen in liquid nitrogen and kept in a freezer at −80°C until freeze-drying, grinding and stable isotope analysis. Prior to the experiment, tissue samples from 15 fish were collected to determine the initial isotopic atom percentage (IA%). Tissue sampling was standardized; the muscle was sampled in front of the dorsal fin (1 x 1 cm cube), the whole liver was sampled and the mid-intestine from the end of pyloric ceca to distal intestine.

2.5 | Stable isotope analysis

Tissue samples were freeze-dried and homogenized with two stainless steel beads 5 mm (Qiagen, Retsch GmbH, Haan, Germany) in a TissueLyser (Qiagen, Retsch GmbH, Haan, Germany) for 2 minutes at 20 Hertz. Diets and ingredients were ground in an Ultra Centrifugal Mill ZM 100 (Retsch GmbH, Haan, Germany) to a homogeneous powder. Molecules containing ^{14}N and ^{15}N differ in mass, and the ratio of these isotopes can be detected with an element analysis isotope ratio mass spectrometry (EA-IRMS). First, the sample was subjected to element analysis by being dropped into a heated reactor which contains an oxidant such as copper or chromium oxide. Samples were combusted at 1,000°C to produce N_2, NOx, H_2O, O_2 and CO_2. The abundance of the isotopes in the sample was then determined by

TABLE 2 Analysed chemical composition of experimental diets

Chemical composition	Dietary treatments[a]				
	Control	SP1	SP2	L0.1	L0.2
Analysed content per kg					
Dry matter, g	910.6	910.6	911.2	922.0	924.3
Ash, g	76.4	75.8	76.5	76.2	76.6
Crude protein, g	503.6	507.2	503.3	506.6	509.8
Starch, g	121.0	119.0	121.0	124.0	125.0
Lipid, g	170.4	170.6	174.7	183.4	182.1
Gross energy, MJ	22.3	22.2	22.2	22.5	22.6
Analysed content, %					
^{15}N[b]	0.003	0.964	1.974	0.081	0.151
Essential amino acids, g/kg					
Arginine	32.3	32.2	30.4	32.4	31.4
Histidine	9.7	9.5	8.8	9.6	9.0
Isoleucine	20.3	19.9	18.3	19.9	18.5
Leucine	35.0	34.6	32.2	34.7	33.2
Lysine	31.3	30.5	28.4	30.8	29.3
Methionine	11.2	10.9	10.2	11.1	10.4
Phenylalanine	20.8	20.6	19.3	20.6	19.4
Threonine	19.1	18.9	17.8	19.2	18.2
Valine	23.2	22.8	21.3	22.9	21.5
Tryptophan	4.7	4.6	4.2	4.6	4.6
Non-essential amino acids, g/kg					
Alanine	28.7	28.7	27.3	28.9	28.0
Aspartic acid	40.3	39.7	37.4	40.2	37.3
Glycine	36.3	36.8	35.6	36.6	35.8
Glutamic acid	95.3	95.2	88.6	93.6	89.3
Cysteine	5.5	5.4	5.1	5.3	5.4
Tyrosine	11.7	11.7	10.7	11.8	10.9
Proline	38.1	38.4	36.5	37.4	35.5
Serine	22.3	22.3	21.3	22.5	21.9
Sum amino acids	485.7	482.9	453.2	482.2	459.7

[a]All analyses was a mean of duplicates except atom percentage excess ^{15}N being an average of triplicates. [b]Atom percentage excess ^{15}N.

mass spectrometry (Iso-analytical, 2018). Samples of approximately 1 mg were weighed into small tin capsules (8 x 5 mm, Elemental Microanalysis, Devon, UK). Samples were analysed for N-isotope composition using a Nu Horizon isotope-ratio mass spectrometer (IRMS) (Nu Instruments, Wrexham, UK) coupled to an Eurovector element analyser (EA) 3,028 (Eurovector S.p.A, Redavalle, Italy) at the Institute for Energy Technology (Kjeller, Norway). Analysed content of ^{15}N in the diets is given in Table 2.

Isotopic signatures were reported as $\delta^{15}N$ values, and converted to atom percentage excess (APE) as follows (Fry, 2006):

$$APE^{15}N = \left(\left(\frac{\left(\delta^{15}N_{Sample} + 1000\right)}{\left(\delta^{15}N_{Sample} + 1000 + \left(\frac{1000}{\delta^{15}N_{Standard}}\right)\right)}\right)100\right) - IA\%$$

where $\delta^{15}N_{Sample}$ and $\delta^{15}N_{Standard}$ are the proportion of ^{15}N in the ratios $^{15}N/^{14}N$ in the sample and in the reference standard (atmospheric N_2; $\delta^{15}N_{Standard}$= 0.003663 IAEA (International Atomic Energy Agency) 305) and *IA%* is the initial atom percentage in $N_{standard}$. The APE ^{15}N after feeding with enriched feed will be proportional to the fraction of newly deposited amino acids in the tissue, resulting from both tissue growth and replacement of previously deposited protein, denoted as protein metabolism. Atom percentage excess ^{15}N is the total atom percentage ^{15}N in the sample adjusted for the IA%. When enrichment of ^{15}N was low (~0.5% ^{15}N) calibration of ^{15}N was performed against the international standards IAEA 305B and IAEA N-1, while when enrichment of ^{15}N was high (2% ^{15}N), calibration standards IAEA 311 and IAEA N-1 were used. Three samples of the internal reference material (IFE Trout) was analysed in the beginning, middle

and end of a sequence (75–78 samples per sequence) and for every sixth tissue sample. The average $\delta^{15}N$ in IFE Trout was 11.60‰ with a standard deviation of 0.20. The corresponding $\delta^{15}N$ values for samples analysed according to IAEA 305B and IAEA 311 were 375.3 ± 0.96‰ and 4,693 ± 4.49‰ respectively. The content of ^{15}N before feeding with enriched feed was expected very low. For 15 fish, the IA% was on average 0.370 with a standard deviation of 0.0001 respectively.

2.6 | Calculations and statistics

Feed conversion ratio (FCR) was calculated on a tank level as follows:

$$FCR = FI\ (FW - IW)^{-1}$$

where FI is the feed intake (g dry matter), and FW and IW are the final and initial weights (g) of fish respectively. An average FCR over three tanks given the same diet was calculated. Furthermore, relative weight gain for an individual, that is growth relative to final body weight, was calculated as follows:

$$\text{relative weight gain} = \left((FW - IW)\ FW^{-1}\right) 100$$

An effect of diet on averages of recorded variables was tested by use of the following univariate model:

$$\bar{y}_{ij} = \mu + diet_i + \varepsilon_{ij}$$

where $\bar{y}_{ij}$ is the average feed intake, initial and final weights, weight gain, relative weight gain and FCR in tank j (j = 1…15), μ is the overall mean, diet is the fixed effect of i^{th} diet (i = 1…5) and ε_{ij} is a random residual.

Simple linear regression as well as Pearson correlations between relative weight gain and APE ^{15}N in the muscle, liver and mid-intestine were calculated per diet and sampling time points. All statistical analyses above were carried out by use of SAS® software, V.9.4 (SAS Inst. Inc., Cary, NC).

3 | RESULTS

All diets contained the same level of *Spirulina* and L-Lysine, but with different inclusion levels of their ^{15}N marked counterparts (Table 1). Chemical analysis of the diets (Table 2) showed that there were some minor differences in total amino acid content between diets, being somewhat lower for SP2 and L0.2. The enrichment percentage of ^{15}N was slightly under the formulated values in all diets, but all diets were isotopically distinct.

All diets were consumed as expected. Overall average and standard deviation for start and final weights per fish were 21 ± 2.5 g and 36.2 ± 14.1 g, respectively, with a relative weight gain over the 50 days of 35.6 ± 19.0%. The corresponding average feed intake over the 15 tanks throughout the 50-day period was 489.4 ± 42.4 g, and the average FCR was 0.68 ± 0.013. The model showed no significant (p < 0.05) effect of diet on any of the traits tested, as expected due to the same chemical composition. Descriptive statistics of the different traits by diet are therefore given in Table 3.

Table 4 shows the correlations between APE ^{15}N and relative weight gain in the muscle, liver or mid-intestine over time in diets with added ^{15}N. Correlations were generally positive (one exception), ranging 0.12–0.98, −0.09–0.94 and 0.03–0.90 in the muscle, liver and mid-intestine respectively (Table 4). The relationship between relative weight gain and APE ^{15}N in the muscle for all diets over time is shown in Figure 2a-e. For diets enriched with ^{15}N, there was a strong positive relationship between relative weight gain and APE ^{15}N in the muscle (Figure 2b-e), which is expected due to the fact that protein growth is necessarily based on deposition of newly consumed and thus enriched protein. As expected, a stable ^{15}N concentration over time was observed for fish given the control diet (Figure 2a). The same tendency was also evident for liver and mid-intestine (data not shown). The main differences between the muscle, liver and mid-intestine were that the liver and mid-intestine were nearly in equilibrium with the diets around day 50, due to faster metabolic rate in these tissues.

Among inclusion levels, the SP2 diet (2% inclusion level of ^{15}N) was the only diet estimated with significant correlations (p < 0.05) between APE ^{15}N and relative weight gain both in the muscle and in the liver at all time points (r = 0.82–0.97 and r = 0.59–0.94, respectively). These, correlations were slightly lower and less stable in liver than in muscle tissue. For the mid-intestine, many of the correlations between APE ^{15}N and relative weight gain were not significant.

Figure 3 shows that the level of isotope in muscle tissue for fish fed the SP2 diet over time asymptotes towards an equilibrium atom

TABLE 3 Means and standard deviations of diets for initial and final weights as well as for weight gain, relative weight gain, feed intake and feed conversion ratio, over the 50 day test period

	Dietary treatments				
	Control	SP1	SP2	L0.1	L0.2
Initial weight, g	711.1 ± 10.3	717.2 ± 6.0	713.0 ± 18.6	699.5 ± 3.4	709.6 ± 14.4
Final weight, g	1,478.9 ± 57.6	1,446.6 ± 49.5	1,364.1 ± 28.2	1,456.1 ± 66.0	1,412.8 ± 20.5
Weight gain, g	767.8 ± 64.6	729.4 ± 54.6	651.1 ± 41.9	756.6 ± 64.1	703.1 ± 30.0
Relative weight gain, %	36.3 ± 2.4	35.7 ± 1.3	33.9 ± 1.84	35.7 ± 1.48	36.5 ± 1.9
Feed intake, g	520.7 ± 41.6	498.8 ± 41.1	437.9 ± 9.4	516.7 ± 39.2	473.0 ± 20.2
Feed conversion ratio	0.68 ± 0.003	0.68 ± 0.008	0.67 ± 0.03	0.68 ± 0.007	0.67 ± 0.005

TABLE 4 Pearson correlation coefficients (r) between atom percentage excess (APE) ^{15}N in the muscle (M), liver (L) and mid-intestine (MI), and relative weight gain over time (n = 12, at 2, 4, 8, 16, 32 and 50 days) in four ^{15}N-enriched diets

	Days																	
	2			4			8			16			32			50		
	Trait																	
Diet	M	L	MI	M	L	MI	M	L	MI	M	L	MI	M	L	MI	M	L	MI
SP1	0.87*	0.66*	0.56	0.31	0.76*	0.65*	0.51	0.60*	0.49	0.92*	0.72*	0.75*	0.83*	0.62*	0.78*	0.98*	0.90*	0.86*
SP2	0.89*	0.88*	0.22	0.82*	0.72*	0.63*	0.97*	0.94*	0.90*	0.92*	0.91*	0.70*	0.85*	0.59*	0.25	0.95*	0.83*	0.54
L0.1	0.77*	0.39	0.41	0.81*	0.48	0.68*	0.53	0.51	0.24	0.72*	0.16	0.47	0.67*	0.78*	0.90*	0.75*	−0.09	0.43
L0.2	0.55	0.73*	0.59*	0.12	0.23	0.03	0.82*	0.49	0.78*	0.25	0.40	0.70*	0.46	0.68*	0.83*	0.92*	0.34	0.36

Note. Significant correlations (p < 0.05) are indicated with asterisks (*).

percentage. Moreover, Figure 3 indicates that the individual variation in isotope level was low in the beginning of the 50-day experimental period and increased in accordance with the individual relative weight gain before it approaches equilibrium. Similar shape of curves was, in fact, obtained for the other diets (data not shown). This implies that for all the diets, the individual variation in APE ^{15}N is likely highest when the tissue is, on average, 50% saturated with the isotope in the feed, leaving room for individual variation around the value. For the SP2 diet this occurred for an APE ^{15}N in body tissue being 1% (Figure 3).

4 | DISCUSSION

The basic idea of the current project was to develop a method for use of isotope-enriched feed to assess individual feed efficiency in fish, without recording their individual feed intake. By switching from normal to isotope-enriched feed, the isotope profile of the fish will change accordingly. The change in the nitrogen isotope profile can be explained by protein metabolism that is growth dilution in addition to replacement of existing body tissue (losses). Here, Atlantic salmon in the freshwater phase were fed diets with different inclusion levels, and the corresponding changes in relative weight gain and APE ^{15}N of tissues were monitored over time. Generally, the largest positive relationship between the two variables was found in the muscle with APE ^{15}N approaching an asymptote over time for all diets. This implies that diets with variable inclusion of isotopes, either by enrichment or natural variation in isotope content, may be used for assessing feed efficiency. It is recommended to test until ~50% saturation with the isotope level in the feed.

The finding that the body content of the feed-enriched isotope increases with body growth is hardly surprising. Figure 2b–e show that fish of similar relative weight gain, but different growth rates (i.e. reaching the same relative weight gain at different time points), had clearly different isotope contents, with the fastest-growing fish having the lowest content of enriched isotope. This cannot be attributed to growth dilution (as relative weight gain is similar), implying that fast growth results in reduced replacement of existing body tissue, and better FCR, likely due to less maintenance costs from a shorter growth period. The experiment indicates that individual differences in FCR are likely to be captured by individual differences in isotope profiles. Furthermore, within each time point, Figure 2, there is individual variation in protein metabolism between fish of similar relative weight gain. This can partly be explained by measurement errors, but potentially by variation in protein degradation between fish at the same relative weight gain. Variation in levels of APE ^{15}N (i.e. variable replacement of body nitrogen) may be explained by variation in maintenance requirements (per unit body protein and day), and relates to individual differences in protein efficiency. Likewise, Figure 2 shows individual variation in relative weight gain for fish at the same APE ^{15}N, again pointing to potential differences in

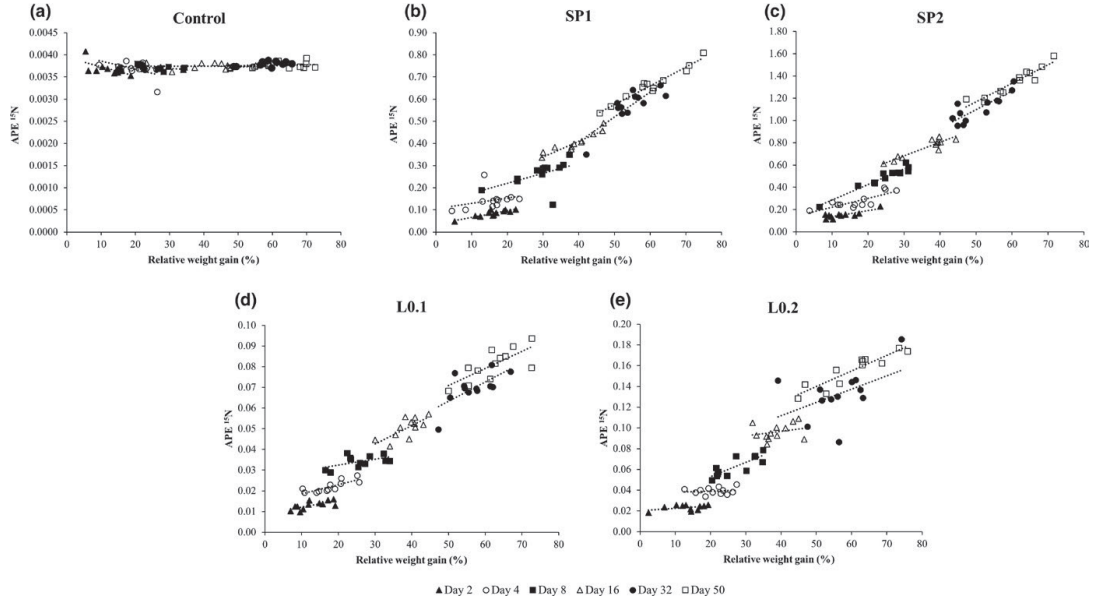

FIGURE 2 Regression lines for the relationship between atom percentage excess (APE) ^{15}N in muscle and individual relative weight gain over time (n = 12 at 2, 4, 8, 16, 32 and 50 days), for the (a) control (0% inclusion of ^{15}N), (b) SP1 (1% inclusion of ^{15}N from Spirulina), (c) SP2 (2% inclusion of ^{15}N from Spirulina), (d) L0.1 (0.1% inclusion of ^{15}N from L-Lysine) and (e) L0.2 (0.2% inclusion of ^{15}N from L-Lysine) diets

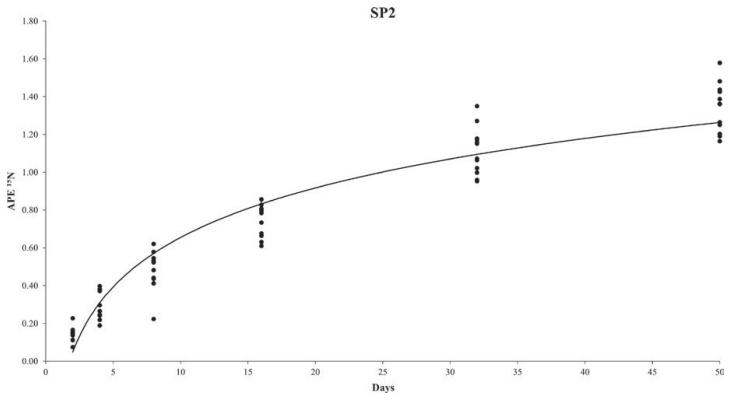

FIGURE 3 Atom percentage excess (APE) ^{15}N and individual isotopic variation in muscle over time (n = 12 at 0, 2, 4, 8, 16, 32 and 50 days) in fish fed the SP2 (2% inclusion of ^{15}N) diet. The estimated regression line was: y = 0.38 ln(X) – 0.22, with R^2 = 0.93

maintenance requirements. It remains to explore whether some of this variance has a genetic component.

Stable isotope analysis is a well-established method to obtain measurements of protein metabolism in fish. Protein efficiency, where amount of ingested protein is accreted as growth in muscle has been shown to vary between groups of individuals, where efficient fish obtain a higher growth rate for a reduced protein degradation (Carter, Houlihan, Buchanan et al., 1993b; McCarthy, Houlihan, & Carter, 1994; Morgan, McCarthy, & Metcalfe, 2000). These studies have all been based on the flooding dose method (Garlick, McNurlan, & Preedy, 1980), injecting radioactive [^{3}H] phenylalanine in the caudal vein, with subsequent tissue measurements over a shorter period of time (1–6 hr). The isotope profiling in this study, however, is based on adding stable isotopes to feed and considers cumulative individual measures over longer period of time, herein up to 3 weeks dependent on the growth rate of the fish. Another advantage of labelling the feed, is the ability to trace the nutrient deposition and loss without disturbing the fish. Labelling the feed allows to measure the change in isotope profile for a large number of individuals, making the indicator trait more relevant in a selective breeding context. As for the flooding dose method, the footprint of isotopes is measured in tissues, herein muscle, liver

and mid-intestine. In fish, the muscle alone accounts for as much as 40%–60% of the total body weight and is the main protein accretion site (Verri, Terova, Dabrowski, & Saroglia, 2011), directly reflecting the whole-body growth (Peragon, Barroso, Garcıa-Salguero, de la Higuera, & Lupianez, 1999). Moreover, it has been proposed that protein metabolism in the white muscle of fish could be used as a biochemical index for protein accretion and growth (Fauconneau, Gray & Houlihant, 1995), which is confirmed in this study. In addition, significant correlations between APE ^{15}N and relative weight gain in the liver and mid-intestine existed for all diets (Table 4). The liver as a highly metabolic tissue with high protein metabolism, as well as epithelial renewal in the mid-intestine can explain the correlation between APE ^{15}N and relative weigh gain in these tissues. Correlations between the mid-intestine APE ^{15}N and relative weight gain throughout the experiment were unstable, and many were non-significant (Table 4), likely because the tissue was difficult to rinse properly. In summary, our results show that isotope profile changes in the muscle, liver and mid-intestine can be effectively traced by feed labelling. The clear association between individual relative weight gain and individual APE ^{15}N in different tissues over time demonstrates that isotope profiling from labelling the feed accurately assess deposition of new protein especially in the muscle, but also in the liver.

The EA-IRMS technique is an accepted method for analysis of ^{15}N/^{14}N ratios, the analysis can be used for samples with low ^{15}N-concentrations (Grassineau, 2006) and has very good precision (<0.1% relative standard deviation) (Matthews & Hayes, 1978). Thus, enrichments of ^{15}N with an APE of 0.004 can be detected (Matthews & Hayes, 1978). In this experiment, the SP2 diet with an enrichment as high as 1.974 APE ^{15}N gave the most stable correlations throughout the 50-day sampling period (Table 4). This stability was considered important because the purpose of this experiment was to investigate the potential of using the change in isotope profile in a relation to relative weight gain with a consequence for individual feed efficiency. In addition, an increasing isotopic variance was observed (Figure 3) throughout the experiment, which can be explained by individual differences in growth and tissue replacement. However, the relationship between average level and time was nonlinear, and the level asymptotes when the body tissues approach equilibrium with ^{15}N in the diet. At equilibrium, the isotope profile is fully dictated by the diet, and no variation among fish is expected (as observed prior to the feed trial). In contrast, the variation between fish will be highest when, on average, ~50% saturation is reached (i.e. saturation varies among fish). Due to high growth rates, fish reached ~50% saturation at day 25 for the SP2 diet. Relative growth rates vary considerably among species and life-stages within species, and the experiment should be adapted to each specific case.

Growth can be explained by increased protein synthesis, reduction in protein degradation and high accretion rate of proteins to the skeletal muscle (Carter, Houlihan, Brechin, & McCarthy, 1993a; McCarthy et al., 1994; Morgan et al., 2000). The strong relationship between muscle protein metabolism and relative weight gain can be explained by the fact that 80% of the synthesized proteins in white muscle are retained as net protein accretion (Houlihan & Laurent, 1987). McCarthy et al. (1994) compared groups (n = 6) of protein 'efficient' and 'inefficient' rainbow trout (*Oncorhynchus mykiss*) where faster growing and more efficient fish showed reduced degradation rates, and differences in growth correlated to protein metabolism differences. Differences in protein degradation rates were important determinants of their reported variation of growth efficiencies. Our individual differences corresponded well with the results of McCarthy et al. (1994): fast-growing fish showed lower protein degradation in muscle, when compared at the same relative weight gain, as explained above. Correspondingly, Hawkins, Day, Rusin, and Worrall (1989) have reported genotype-dependent differences in protein metabolism in mussels (*Mytilus edulis*). Results in the current study indicate that efficient fish are characterized by a high ratio between relative weight gain and change in isotope profile, meaning high gain for low cost, by individual variation in feed efficiency.

According to literature (e.g. Thodesen et al., 2001), a favourable correlation is expected between feed efficiency and growth rate. Given that isotope profiling can be used to assess feed efficiency on an individual level, this should be observed as a lower content of APE ^{15}N in faster growing (more efficient) fish, which should be evident from the isotopic profiles. The findings in this study support this. The results indicate that individual isotope profiles can be used as an indicator trait of individual feed efficiency in fish.

5 | CONCLUSIONS

The results show that the stable isotope ^{15}N added to feed and accumulated over time in the muscle and liver has the potential to be a promising biomarker for revealing insight into individual feed efficiency of fish. The results show that fast growth results in reduced replacement of existing body tissue and improved FCR, interpreted as reduced degradation rates that is reduced maintenance requirement. Efficient fish are characterized by a high ratio between relative weight gain and change in isotope profile, meaning high gain for low cost or improved feed efficiency. Adding stable isotopes to feed can be done for a considerable number of fish and has therefore a potential in a breeding context. It is recommended to feed with stable isotopes to reach 50% saturation.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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Paper III

Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling

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Abstract

Background

We used stable isotope profiling (^{15}N and ^{13}C) to obtain indicator phenotypes for feed efficiency in aquaculture. Our objectives were to (1) examine whether atom percent of stable isotopes of nitrogen and carbon can explain more of the variation in feed conversion ratio than growth alone, and (2) estimate the heritabilities of and genetic correlations between feed efficiency, growth and indicator traits as functions of nitrogen and carbon metabolism in various tissues. A 12-day experiment was conducted with 2281 Atlantic salmon parr, with an average initial weight of 21.8 g, from 23 full-sib families that were allocated to 46 family tanks and fed an experimental diet enriched with ^{15}N and ^{13}C.

Results

Using leave-one-out cross-validation, as much as 79% of the between-tank variation in feed conversion ratio was explained by growth, indicator traits, and sampling day, compared to 62% that was explained by growth and sampling day alone. The ratio of tissue metabolism, estimated by a change in isotope fractions relative to body growth, was used as an individual indicator for feed efficiency. For these indicator ratio traits, the estimated genetic correlation to feed conversion ratio approached unity but their heritabilities were low (0.06 to 0.11). These results indicate that feed-efficient fish are characterized by allocating a high fraction of their metabolism to growth. Among the isotope indicator traits, carbon metabolism in the liver had the closest estimated genetic correlation with feed conversion ratio on a tank level (-0.9) but a low estimated genetic correlation with individually recorded feed efficiency indicator ratio traits. The underlying determinants of these correlations are largely unknown.

Conclusions

Our findings show that the use of indicator ratio traits to assess individual feed efficiency in Atlantic salmon has great prospects in selection programs. Given that large quantities of feeds with contrasting isotope profiles of carbon and/or nitrogen can be produced cost-effectively, the use of stable isotopes to monitor nitrogen and carbon metabolism in various tissues has potential for large-scale recording of individual feed efficiency traits, without requiring individual feed intake to be recorded.

Background

The steadily growing human population increases the demand for protein resources from both the livestock and aquaculture industries. In 2050, the number of mouths to feed is expected to reach ~9 billion [1]. In the near future, livestock and aquaculture production will be in competition with direct human consumption for many of the same protein resources and, therefore, efficiency must be increased. Selective breeding is, and has for several decades, been an important tool to improve feed efficiency in both livestock and farmed fish [2-6].

Feed efficiency can be defined as feed conversion ratio (FCR), which is the amount of feed consumed per unit growth, or alternatively, by its inverse, the feed efficiency ratio (FER), i.e., growth per unit of feed consumed [7]. Selective breeding for improved feed efficiency assumes that both individual growth and individual feed intake can be routinely recorded on a large number of individuals. In aquaculture, recording of individual growth rate is easily attainable, and it has been the major trait in breeding schemes of Atlantic salmon since the 1970s [8]. Various methods for recording individual feed intake have been proposed such as X-radiography, where generally radio-opaque ballotini glass beads are mixed into the feed, fish are

x-rayed, and the number of pellets eaten is counted [9-12]. Video recording is another method for feed intake recording [13-14], with manual feeding of pellets one by one and retrospective identification of individual fish from video analysis. However, since sib-testing of Atlantic salmon is carried out in large sea-cage units and since fish are communally fed with feed dispersed into the water, large-scale recording of individual feed intake with these methods is difficult to implement in selective breeding programs of Atlantic salmon. Hence, the first option in selective breeding for improved FCR has been to rely on selection for traits such as growth rate [15-16], which has been shown to improve feed retention ratio and FCR [3, 16-19] because of the generally accepted high genetic correlation between FCR and growth rate, ranging from 0.63 to 0.99 in rainbow trout (*Oncorhynchus mykiss*) [20]. The effect of increased growth rate on feed efficiency is through reducing maintenance requirements per unit of growth produced, mainly by reducing time to slaughter. Kause et al. [12] proposed to add information from indicator traits such as the percentage of muscle lipid to enhance the genetic progress in feed efficiency, which could be an alternative to recording feed intake.

In our study, we examined the potential use of stable isotopes to assess feed efficiency traits in Atlantic salmon, with the objective to establish indicator phenotypes that explain more of the genetic variation in feed efficiency than growth alone. McCarthy et al. [9] identified individual variation in protein metabolism, with feed efficient fish having a lower protein degradation for the same level of feed eaten than inefficient fish. The potential use of feeding stable isotope such as ^{15}N to fish to assess individual protein metabolism was investigated in a previous study [21]. In this study, fish were fed a standard diet (low in ^{15}N) followed by a ^{15}N-enriched diet with various inclusion levels, which resulted in isotope profile changes of body nitrogen (protein metabolism), which is closely related to body growth. Using protein-bound ^{15}N enrichment,

significant correlations between relative weight gain and protein metabolism were found in muscle (r = 0.31-0.98) and in liver (r = 0.59-0.94) [21]. This study also found that not all individual variation in protein metabolism was explained by growth. Isotope profiles can be recorded individually, in contrast to the challenge of recording feed intake and feed efficiency at the individual level. If feed efficiency can be accurately predicted by atom percentages (atom %) of nitrogen and carbon stable isotopes, individual isotope profiles could be used for more direct selection for improved feed efficiency. However, first it is necessary to validate the method in an experiment in which both isotope profiles and feed efficiency are recorded and estimate associated genetic parameters, i.e., in family material. This requires a large-scale experiment, in which families are kept in separate (replicate) tanks, and feed consumption and growth are monitored at the tank level. Dvergedal et al. [21] reported a curvilinear increase in the level of isotopes in tissue over time, with the atom % reaching an asymptote when fish were fed until saturation, i.e., all fish will eventually approach equilibrium isotopic levels, reflecting that of the feed. This implies that length of the experiment is crucial for recording individual variation in metabolism, since individual variation in nitrogen and carbon metabolism can be detected only prior to the point when the fish are expected to be in equilibrium with the isotopic level in the feed.

In this paper, we report the results of a large-scale experiment, in which families were kept separate in replicate tanks, growth and isotope profiles were recorded at the individual level, and feed consumption and FCR were recorded at the tank level. Feed was labelled with both ^{15}N and ^{13}C stable isotopes. One objective was to examine whether the atom % of stable isotopes of nitrogen and carbon can explain more of the variation in FCR than growth alone, i.e. to explore the potential of using indicator traits in selective breeding for improved feed efficiency in

Atlantic salmon. Another objective was to estimate the heritabilities of and genetic correlations between feed efficiency, growth and indicator traits, as functions of nitrogen and carbon metabolism in various tissues.

Methods

Fish and housing

The experiment included 23 full-sib families (offspring of 23 dams and 22 sires) of Atlantic salmon (*Salmo salar*) from AquaGen's breeding population. To ensure clearly contrasted family groups with respect to growth potential and, potentially, feed efficiency, the parents of the families were selected for high/low estimated breeding values for growth in seawater, although the experiment was conducted in freshwater.

From the eyed egg stage until the start of the experiment, all families were communally reared in a single tank. Before pit-tagging, 15 fish were individually weighed to establish whether they were ready for tagging. The fish were pit-tagged with a 2x12 mm unique glass tag (RFID Solutions, Hafrsfjord, Norway) and a fin-clip was collected for genotyping. All fish were genotyped using AquaGen's custom Axiom®SNP genotyping array from Thermo Fisher Scientific (San Diego, CA, USA), which includes 56,177 single-nucleotide polymorphisms (SNPs). Prior to the experiment, the parentage of each individual fish was established using genomic relationship likelihood for parentage assignment [22].

Based on parentage assignment, 100 family members were identified for each of the 23 families used in the experiment. These fish were randomly allocated to family tanks with 50 fish per tank and two tanks per family, except for nine tanks in which the number of fish varied between 42 and 54, due to some mortality prior to the start of the experiment and to a larger number in one

tank because of a counting mistake. A single fish was allocated to an incorrect family tank but it was later identified. In total, 2281 fish were included in the experiment. The tanks, each with a 270-L capacity, were supplied with recirculated fresh water, at a flow rate of 7 to 8 $L.min^{-1}$, and the fish were kept under 24 h light regime, with an average temperature of 14.5°C. Dissolved oxygen was measured daily and maintained above 8 $mg.L^{-1}$ in the outlet water (Handy Delta, OxyGuard® AS, Farum, Denmark).

Dietary treatment and feeding

A labelled diet with the stable isotopes ^{15}N and ^{13}C, with inclusion levels of 2% and 1% respectively, was fed during the experimental period of 12 days. Due to the large variation in growth rate and thus in the rate of inclusion of new nutrients among families, a pre-defined period of 12 days was set to feed the labelled feed, such that an equilibrium was not reached in any of the families. Termination of the experiment and tissue sampling were done over a 5-day period with different tanks being sampled each day, i.e., the dietary switch was done according to the pre-defined termination day of the tank. The formulation and analysed chemical composition of the diet are in Table 1. The diet was produced at the feed laboratory of the Norwegian University of Life Sciences, Aas, Norway, as explained by Dvergedal et al. [21]. The fish were fed twice daily (07:00 and 15:00) for a period of one hour, by automatic belt feeders. The feeding level equalled 10% in excess, based on the level of uneaten feed. Registrations of uneaten feed and calculations of feed intake were performed according to Helland et al. [23]. The daily feed intake per tank was calculated by first collecting the waste feed on a wedge wire screen [24] and correcting the total waste feed for leasing losses. As explained by Shomorin et al. [24], the wedge wire is placed at an inclined position in the outlet water column of the tank. The design of the screen ensures efficient drainage so that uneaten feed that is trapped on the screen

is exposed minimally to water. Then, the difference between total fed feed and total uneaten feed was calculated as g dry matter intake, after drying the uneaten feed at 105 ℃ overnight.

Sampling

Sampling was carried out over five days, about 10 tanks were sampled each day, i.e. ~500 fish daily. Fish were anesthetized with metacaine (MS-222TM; 1 $g.L^{-1}$ water) and killed with a sharp blow to the head prior to dissection. Whole body weight and length were recorded for all fish, and tissue samples from muscle, liver, and adipose were collected in a cryotube, snap-frozen in liquid nitrogen and stored at -20°C until stable isotope analysis. Tissue sampling was standardized; muscle was sampled in the front area of the dorsal fin (1x1 cm cube), the liver was divided into four small pieces, and adipose tissue was sampled from the fat that was deposited around the gut between the pyloric ceca and the distal intestine.

Chemical analysis

The feed was dried and ground prior to analysis, and analyses were performed in duplicate for dry matter by drying to a constant weight at 104°C, for ash by combustion at 550°C, for crude protein by Kjeldahl nitrogen x 6.25 according to Commission Regulation (EC) No 152/2009, and for starch as described in McCleary et al. [25]. Lipid was determined after extraction with petroleum ether and acetone (70/30) on an accelerated solvent extractor (ASE 200) (Dionex Corp, Sunnyvale, CA, USA), while gross energy was established with a PARR 1281 Adiabatic bomb calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. Amino acids were analysed according to Commission Regulation (EC) No 152/2009, for all amino acids except tryptophan, on a Biochrom 30 amino acid analyser (Biochrom Ltd,. Cambridge, UK). Tryptophan was analysed according to Commission Regulation (EC) No 152/2009 with a Dionex

Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) and a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan).

Stable isotope analysis

Tissue samples were freeze-dried and homogenized, and samples of approximately 1 mg were weighed into small tin capsules (8x5 mm, Elemental Microanalysis, Devon, UK). Samples were analysed for N- and C-isotope compositions using a Nu Horizon isotope-ratio mass spectrometer (IRMS) (Nu Instruments, Wrexham, UK) coupled to a Eurovector element analyser (EA) 3028 (Eurovector S.p.A, Redavalle, Italy) at the Institute for Energy Technology (Kjeller, Norway). Analysed contents of ^{15}N and ^{13}C in the diet are in Table 1.

Isotopic signatures were reported as δ values, and Atom % was calculated as follows (taking ^{15}N as an example) [26]:

$$\text{Atom }\%^{15}\text{N} = \left(\frac{(\delta^{15}\text{N}_{Sample} + 1000)}{\left(\delta^{15}\text{N}_{Sample} + 1000 + \left(\frac{1000}{\delta^{15}\text{N}_{Standard}}\right)\right)} \right) 100,$$

where $\delta^{15}\text{N}_{Sample}$ ($\delta^{13}\text{C}_{Sample}$) and $\delta^{15}\text{N}_{Standard}$ ($\delta^{13}\text{C}_{Standard}$) are the proportion of ^{15}N divided by the proportion of ^{14}N in the sample and in the reference standard (air for nitrogen; $\delta^{15}\text{N}_{Standard}$ = 0.003676 [27], and Vienna Pee Dee Belemnite for carbon (VPDB); $\delta^{13}\text{C}_{Standard}$= 0.0112372 [28]). The atom % ^{15}N and ^{13}C in excess (APE) after feeding with enriched feed is proportional to the fraction of newly deposited amino acids in the tissue, resulting from both tissue growth and replacement of previously deposited nitrogen and carbon, denoted as metabolism. Atom % ^{15}N (^{13}C) in excess is the total atom % ^{15}N (^{13}C) in the sample adjusted for the initial isotope percentage in the sample (IA %). Initial isotope profile was

accounted for in the calculations of individual feed conversion ratio (IFCR) and of individual feed efficiency ratio (IFER) (described in the next paragraph). Prior to the experiment initial Atom % was assessed by using 20 randomly sampled fish from the experimental population. The ^{15}N average and standard deviations were 0.370 ± 0.0001 in muscle and 0.370 ± 0.0003 in liver. Corresponding values for ^{13}C in muscle, liver, and adipose tissue were 1.087 ± 0.0005, 1.086 ± 0.0007 and 1.082 ± 0.0003, respectively.

Calibration of ^{15}N and ^{13}C was performed against international certified reference materials and internal standards. The internal standard IFE Trout and USGS-41 were analysed as unknowns, and certified standards such as USGS-41 (certified value), IAEA (International Atomic Energy Agency) N-1, USGS-24, Isolife P10501 and IAEA 311 were used to define the calibration curve. Three calibration standards (USGS-41, USGS-24, and Isolife P10501) were analysed in each sequence, with ~60 samples per sequence. In addition, IAEA 303B ($\delta^{13}C_{VPDB}$: 466 ± 3) was analysed on multiple occasions to verify the linearity of $\delta^{13}C_{VPDB}$ measurements above the Isolife P10501 standard. The $\delta^{15}N$ composition of IFE trout was calibrated using a two-point calibration curve using IAEA 311 and IAEA-N-1 standards. The $\delta^{13}C$ composition of IFE trout was calibrated against the USGS-24 standard. The average $\delta^{15}N$ in IFE trout was 11.60‰ with a standard deviation of 0.20 and, correspondingly, for $\delta^{13}C$ the average was -20.22‰ with a standard deviation of 0.19. The corresponding $\delta^{15}N$ values for samples analysed according to IAEA 311 were 4693 ± < 5.0‰, and for $\delta^{13}C$ values according to USGS-24 the values were -16.05 ± < 0.25‰.

Phenotypes analysed

When entering the tank, the initial weight of each fish i (IW_i, g) was recorded. After the experiment, i.e. at sampling, final weight (FW_i, g) was recorded. From these two variables, individual weight gain (WG_i) and relative weight gain (RG_i) were calculated as follows:

$$WG_i = FW_i - IW_i,$$

$$RG_i = ((FW_i - IW_i)/FW_i) \times 100.$$

A total of 32 fish (1.4% of the total) were set to missing for these two variables, with four fish having either missing initial or final weights. Furthermore, missing was imposed for fish with an extremely low growth rate ($N = 21$) (relative weight gain less than 6.4%, corresponding to a growth rate of less than 1.3 g) or an extremely high growth rate ($N = 7$) (relative weight gain higher than 49%, not accompanied by a corresponding change in the isotope profile), indicating abnormal development and phenotyping error, respectively.

From the tissue samples, the following Atom % variables were available at the individual level: Atom % for ^{13}C in muscle (AMC_i), ^{15}N in muscle (AMN_i), ^{13}C in liver (ALC_i), ^{15}N in liver (ALN_i) and ^{13}C in adipose tissue (AAC_i). Lack of tissue sample resulted in nine fish with missing records for Atom % variables; AAC (5), AMC (1), AMN (1), ALC (1) and ALN (1).

From feed recording at the tank level ($t = 1…46$), tank feed intake (FI_t, g dry matter) was obtained, as well as the feed conversion ratio (FCR_t), which calculated as follows:

$$FCR_t = \frac{FI_t}{WG_t},$$

where WG_t is the total WG in tank t. As mentioned above, 32 fish had missing phenotypes for weight gain and thus were not included in the FCR calculation. Some of these fish had a low or even negative growth indicating that their contribution to the total tank feed intake was likely rather small. In any case, the fraction of fish that lacked growth records was low (< 1.4%), which implies that the potential bias in FCR is limited.

From the individual levels of Atom % ^{13}C (AMC_i) and Atom % ^{15}N (AMN_i) in muscle, individual isotope-based indicator ratio traits for feed conversion ratio ($IFCR$) and feed efficiency ratio ($IFER$); $IFCR\_AMC_i$, $IFCR\_AMN_i$, $IFER\_AMC_i$, and $IFER\_AMN_i$, were defined as follows (taking ^{15}N as an example):

$$IFCR\_AMN_i = \frac{FW_i * APE_{Ni}}{FW_i - IW_i},$$

$$IFER\_AMN_i = \frac{FW_i - IW_i}{FW_i * APE_{Ni}},$$

where $APE_{Ni} = (AMN_i - IA\ \%)$ with IA % equal to 0.370% for ^{15}N and 1.087% for ^{13}C. After diet switching, the APE of a stable isotope in muscle tissue is expected to be proportional to the fraction of newly synthesized nutrients in the muscle, and the product of APE and final weight is expected to be proportional to the mass of new nutrients in body tissue. Because the $IFCR$ ratio is expected to be proportional to the amount of newly deposited body nutrients per g increase in body weight, fish that exchange a larger fraction of the body mass per unit of growth will be less feed-efficient. Exchange of body tissue is traceable with stable-isotope profiling and is related to the feed intake of the individual, while the denominator of the ratio is the weight gain, and the ratio between these two variables is equal to $IFCR$ or, alternatively, the inverse is equal to $IFER$.

Statistical analysis of FCR

At the tank level, first we examined to what degree tank averages for $\overline{WG}$ and $\overline{RG}$, in addition to the tank average isotope content, could explain variation in FCR between tanks by using the following multiple regression model:

$$FCR_{td} = \mu + \beta d + bX_t + e_t,$$

where FCR_{td} is the observed FCR in tank t on sampling-day d, the latter taking values 1 to 5 and was included as a covariate, since this gave better predictive ability, β is the corresponding regression coefficient, X_t is the covariate value for tank t based on one of the following covariates at a time: FI, $\overline{WG}$, $\overline{RG}$, $\overline{AMC}$, $\overline{AMN}$, $\overline{ALC}$, $\overline{ALN}$ and $\overline{AAC}$, b is the corresponding regression coefficient, and e_t is the tank residual. The final model was chosen by including the covariates: FI, $\overline{RG}$, $\overline{AMC}$, $\overline{AMN}$, $\overline{ALC}$, $\overline{ALN}$ and $\overline{AAC}$ ($k = 7$) simultaneously using the following model:

$$FCR_{td} = \mu + \beta d + \sum_{j=1}^{k} b_j X_{jt} + e_t.$$

Backward elimination with leave-one-out cross-validation was used to identify the model with the lowest predicted residual error sum of squares (PRESS). The analyses were conducted using PROC REG in SAS®.

For all regression models, the bias of the model was calculated as the average difference between the observed phenotypes and predicted values obtained by PROC GLM in SAS®. Moreover, the coefficient of determination of prediction was computed as:

$$\hat{R}^2 = 1 - \frac{PRESS}{SS_{tot}},$$

where $PRESS = \sum(y_t - \hat{y}_t)^2$ and $\hat{y}_t$ is the predicted FCR phenotype for tank t, using data from all other tanks in the analysis and SS_{tot} is the total sums of squares. The $\hat{R}^2$ is an estimate of the fraction of variance in FCR explained by the model in the prediction of missing observations.

Genetic analysis

Genetic analysis of traits was performed using the ASReml4 software package [29]. Bivariate analyses were conducted between FCR and FI and of FCR and FI with each of the following traits: $\overline{RG}$, $\overline{WG}$, $\overline{AMC}$, $\overline{AMN}$, $\overline{ALC}$, $\overline{ALN}$, $\overline{AAC}$, $\overline{IFCR\_AMC}$, $\overline{IFCR\_AMN}$, $\overline{IFER\_AMC}$ and $\overline{IFER\_AMN}$. For each bivariate analysis, the model was:

$$\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{a1}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_{a2}}\end{bmatrix}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} + \begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix}, \quad (1)$$

where $\mathbf{y_1}$ is a vector of tank level phenotypes for FCR or FI, $\mathbf{y_2}$ is a vector of (tank) phenotypes for one of the other traits; $\overline{RG}$, $\overline{WG}$, $\overline{AMC}$, $\overline{AMN}$, $\overline{ALC}$, $\overline{ALN}$, $\overline{AAC}$, $\overline{IFCR\_AMC}$, $\overline{IFCR\_AMN}$, $\overline{IFER\_AMC}$ or $\overline{IFER\_AMN}$, $\mathbf{b_1}$and $\mathbf{b_2}$ are vectors of fixed effects, including trait-specific intercepts and effects of sampling day, $\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{T_0} \otimes \mathbf{G_T})$ is a vector of random additive genetic tank effects for the two traits, $\begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{R} \otimes \mathbf{I})$ is a vector of random tank residuals for the two traits. The $\mathbf{X}$ and $\mathbf{Z}$ matrices are appropriate incidence matrices, $\mathbf{T_0}$ is an additive genetic (co)variance matrix between traits at the tank level, $\mathbf{G_T}$ is an (46×46) additive genetic relationship matrix that describes the average genomic relationships between fish in different tanks and $\mathbf{R}$ is the tank residual (co)variance matrix, which was diagonal. Matrix $\mathbf{G_T}$ was calculated based on a subset of 51,543 SNPs of high genotype quality, covering all chromosomes and is defined as:

$\mathbf{G_T} = \mathbf{TT}'$,

where element tj in $\mathbf{T}$ (tank t, locus j) is: $T_{tj} = \frac{1}{n_t}\sum_{i=1}^{n_t}\left(M_{ij} - 2P_j\right)$, M_{ij} is the genotype of individual i within tank t at locus j, P_j is the allele frequency at locus j, and n_t is the number of individuals in tank t. Finally, the elements of $\mathbf{G_T}$ were scaled such that the average of the diagonal elements in $\mathbf{G_T}$ equalled 1.0. Narrow-sense heritability cannot be estimated for traits that are modelled at the tank level, i.e. FCR and FI. Instead h_t^2, which quantifies the fraction of the between-tank variance explained by genetics, was estimated as $h_t^2 = \frac{\sigma_{a_t}^2}{\sigma_{a_t}^2 + \sigma_{e_t}^2}$, where $\sigma_{a_t}^2$ and $\sigma_{e_t}^2$ are the estimates at the tank level of additive genetic and residual variance, respectively, of the trait.

The individual phenotypes for RG, WG, AMC, AMN, ALC, ALN, AAC, $IFCR\_AMC$, $IFCR\_AMN$, $IFER\_AMC$, and $IFER\_AMN$ were also analysed using bivariate models. For each bivariate analysis, the model was:

$$\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & \mathbf{0}\\ \mathbf{0} & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{a1}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_{a2}}\end{bmatrix}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{t1}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_{t2}}\end{bmatrix}\begin{bmatrix}\mathbf{t_1}\\ \mathbf{t_2}\end{bmatrix} + \begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix}, \quad (2)$$

where $\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix}$ is a vector of individual phenotypes for the two traits analysed, $\mathbf{b_1}$ and $\mathbf{b_2}$ are vectors of fixed effects for the two traits as described above, $\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{G_0}\otimes\mathbf{G})$ is a vector of random additive genetic effects for the two traits, $\begin{bmatrix}\mathbf{t_1}\\ \mathbf{t_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{T}\otimes\mathbf{I})$ is a vector of random tank effects for the two traits, and $\begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{R}\otimes\mathbf{I})$ is a vector of random residuals. The $\mathbf{X}$ and $\mathbf{Z}$ matrices are corresponding incidence matrices, $\mathbf{G_0}$ is an additive genetic (co)variance matrix, $\mathbf{G}$ is the

genomic relationship matrix, **T** is the tank (co)variance matrix, and **R** is the residual (co)variance matrix. The genomic relationship matrix was generated according to VanRaden's first method [30] and was used to account for stratification of the individuals by selection of families based on fast and slow growth rates (in seawater). Matrix **G** was calculated based on the same subset of SNPs as defined for $\mathbf{G_T}$ above.

Heritabilities of individual traits were estimated as: $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_t^2 + \sigma_e^2}$, where σ_a^2, σ_t^2 , and σ_e^2 are the estimates of the individual additive genetic, tank environmental, and individual residual variance, respectively, of the trait. The fraction of variance explained by tank was estimated as: $c^2 = \frac{\sigma_t^2}{\sigma_a^2 + \sigma_t^2 + \sigma_e^2}$. Significance of the genetic effect was tested using a likelihood-ratio (LR) test-statistic, comparing a single-trait model with genetic effects (H_1) to a model without genetic effects (H_0) with the **G** matrices ($\mathbf{G_T}$ and **G**, respectively) in H_1:

$$LR = 2\left(\left(log\, L \left| \hat{\theta}_{H_1}\right.\right) - \left(log\, L \left| \hat{\theta}_{H_0}\right.\right)\right).$$

The genetic effect was considered significant if $LR < \chi^2_{(\alpha=0.05;\, df=1)}$.

Results

The diet was formulated for increased ^{15}N and ^{13}C isotope levels, by using 2% and 1% of ^{15}N- and ^{13}C-labelled spirulina, respectively, which resulted in an Atom % of 2.7 and 2.0 of ^{15}N and ^{13}C, respectively, in the diet (Table 1). All fish were healthy throughout the experiment and tanks were fed the diet at 10% in excess of uneaten feed. Table 2 shows the descriptive statistics of the data. The mean Atom % of ^{15}N and ^{13}C in muscle, liver, and adipose tissue ranged from 1.01 to 1.64% and from 1.17 to 1.59%, respectively. These results confirm that none of the tissues was

in equilibrium with the diet that contained 2.7 and 2.0% ^{15}N and ^{13}C, respectively. Thus, variation in the Atom % of ^{15}N and ^{13}C could be determined between individuals. For the individually recorded traits, large differences in WG and RG were observed between families (Figs. 1a and b). However, for the tank-recorded traits, i.e. FI and FCR, larger differences were observed between families for FI than for FCR (Figs. 1c and d), which was reflected in the larger coefficient of variation for FI than for FCR (Table 2). This is logical because the coefficient of variation for FCR contains the standard deviation for WG, which was calculated from individual observations. Figures 2a, b, c, d, and e show the Atom % of ^{15}N and ^{13}C in muscle, liver, and adipose tissue for all families, showing considerable differences between families.

Table 3 shows that $\overline{RG}$ explained the largest fraction of variance in FCR as a single variable (in addition to day) (R^2 = 62% and $\hat{R}^2$= 55%), followed by $\overline{ALC}$ (R^2 = 57% and $\hat{R}^2$ = 52%) and $\overline{WG}$ (R^2 = 53% and $\hat{R}^2$ = 46%). When simultaneously regressing all the explanatory variables on FCR and using backward elimination, the preferred model with the lowest PRESS value had an R^2 of 79% (Table 3). This implies that the variables included in the model explained a major part of the variation between tanks with respect to FCR. The variables retained were Day, $\overline{RG}$, $\overline{AMN}$, $\overline{ALC}$ and $\overline{AAC}$. Using leave-one-out cross-validation, the coefficient of determination of the predicted tank averages was $\hat{R}^2$ = 73%, i.e., even when predicting missing observations, the model explained most of the tank variation in FCR, while the bias was negligible. Moreover, when including interactions between indicator variables in the backward elimination process (data not shown), PRESS was reduced to 0.0118 in the preferred model, which had an R^2 of 88%, while $\hat{R}^2$ was 77% under prediction.

The results obtained for traits recorded at the tank level and analysed with model (1) showed that genetic background (family) explained 52 and 92% of the between-tank variation for FCR (p = 0.0002) and FI ($p = 9.3\times10^{-16}$), respectively (Table 4), i.e., the corresponding correlations between the average family phenotypes in different tanks were 0.72 and 0.96 for FCR and FI, respectively. For the individually recorded traits, significant ($p < 0.05$) heritabilities were estimated for all traits. The estimated heritability for WG was high (0.45), whereas heritabilities were moderate for RG, AMC, AMN, ALC, ALN and AAC (0.28, 0.18, 0.28, 0.15, 0.26 and 0.18, respectively), and relatively low for IFCR_AMC, IFCR_AMN, IFER_AMC and IFER_AMN (0.09, 0.06, 0.11 and 0.08, respectively). Non-genetic tank effects were generally low and explained 2 to 13% of the total phenotypic variance for individual traits.

Genetic correlations between FCR/FI and all the other traits were estimated with model (1) and those between the remaining traits were estimated with model (2) (Table 5). Generally, estimates of the genetic correlation between FCR, measured at the tank level, with each other trait were negative, while those for the IFCR were positive, as expected. This means that FI, growth (WG and RG), and the indicator traits (i.e. the fraction of newly deposited tissue) all had favourable genetically correlations with FCR. For the indicator traits measured directly (excluding the indicator ratio traits), the closest genetic correlation with FCR was estimated for ALC (-0.90 ± 0.11), followed by RG (-0.82 ± 0.10), WG (-0.74 ± 0.17), AMN (-0.73 ± 0.14), AMC (-0.69 ± 0.17), ALN (-0.63 ± 0.19), FI (-0.61 ± 0.21), and AAC (-0.43 ± 0.28). In addition, a perfect genetic correlation was estimated between the indicator ratio traits IFCR_AMC, IFCR_AMN and IFER_AMN and FCR (1.0, 1.0 and -1.0), except for IFER_AMC, which had a lower genetic correlation estimate with FCR (-0.63 ± 0.30), albeit not significantly different from 1. Internally, IFCR and IFER variables had high estimated genetic correlations (-0.71 to -0.99). In general,

estimated genetic correlations of the isotope content of the various tissues with growth (in particular RG) and FI were positive. Among the indicator traits, ALC had the lowest genetic correlation with the other isotope indicator traits (0.04-0.38) and with RG (0.12). AMN and ALN were closely genetically correlated to each other (0.89), which indicates that nitrogen metabolism in liver and in muscle are largely the same genetic trait. Estimates of the genetic correlation of AMN and ALN with RG were high (0.98 and 0.89, respectively). Likewise, AMC and AAC were closely genetically correlated with each other (0.73), with ALN and AMN (0.69 to 0.96) and with RG (0.78 to 0.92). FI was also closely genetically correlated with WG (0.98). For individual traits, phenotypic and genetic correlations were generally similar. Among the traits evaluated, ALC, IFCR (for both nitrogen and carbon) and IFER (for nitrogen) stood out as individual indicator traits for FCR. Estimates of the genetic correlation of ALC with the indicator ratio traits IFCR and IFER were low for both nitrogen and carbon (-0.27 to 0.11).

Discussion

In aquaculture, feed constitutes about half of the total production costs in the grow-out phase at sea [31]. Genetic improvement of feed efficiency will reduce production costs and, at the same time, have a favourable environmental impact by maximizing resource utilization and reducing nutrient load (e.g., nitrogen) to the environment. Protein metabolism is a major determinant of the conversion of feed into growth. Consequently, minimizing the energetic cost of protein metabolism is a strategic goal for enhancing fish growth and feed efficiency. Because Atom % of nitrogen and carbon, and functions thereof, can be individually recorded, these traits could be used as indicator traits for individual feed conversion ratio in growing fish.

The observed between-family difference in nitrogen and carbon metabolism (Figs. 2a-e) have the potential to affect feed efficiency. To evaluate whether ^{15}N and ^{13}C stable isotopes can be used to capture variation in feed efficiency in Atlantic salmon, the observed tank level FCR was best predicted using a multiple regression model that included $\overline{RG}$, $\overline{AMN}$, $\overline{ALC}$ and $\overline{AAC}$ as covariates, in addition to sampling day. This prediction model explained 73% of the variation in masked FCR records (Table 3; $\hat{R}^2$). The single most important isotope variable for prediction was $\overline{ALC}$, which together with sampling day explained 57% (R^2) of the variation in FCR (Table 3). In comparison, for similar models using $\overline{WG}$ or $\overline{RG}$, the proportions of variance in FCR explained were 53 and 62%, respectively (Table 3). Hence, by including information on isotope profiles, prediction of FCR data was substantially improved, compared to what was obtained by growth data alone (Table 3). This indicates that stable isotopes can be used to improve the prediction of individual FCR, which is of considerable value to fish breeding. The regression analyses revealed that, after adjusting for growth, improved feed efficiency was associated with reduced metabolism of nitrogen in muscle (AMN, $\beta = 0.31 \pm 0.17$, results not shown) and reduced carbon metabolism in adipose tissue (AAC, $\beta = 0.90 \pm 0.41$), but with elevated carbon metabolism in liver (ALC, $\beta = -0.75 \pm 0.18$). In fish, the main source of nitrogen in both liver and muscle is protein [7]. Similarly, the main sources of carbon in muscle is protein, but in liver the main sources are protein, fat and glycogen [32]. In adipose tissue, the main source of carbon is lipids, but they can originate from lipid biosynthesis from protein through oxidative degradation and deamination of amino acids, or from carbohydrates through acetyl-CoA formed in the mitochondria [33].

Fish are highly efficient in converting dietary protein into body protein [34]. This requires regulation of the flux of amino acids into metabolic fates such as oxidation, gluconeogenesis, and

lipogenesis. Because salmonids are carnivores, they depend highly on glucose synthesis from non-carbohydrate sources. The surplus of amino acids has a major role in energy metabolism as oxidative substrates in many tissues. Fish with efficient growth seem to use a low-protein metabolism strategy [9, 35-36]. It has also been reported that a reduced capacity for body lipid deposition is favourably associated with high protein growth efficiency [12]. Furthermore, low proteasome activity, i.e., reduced proteolysis in the liver has been linked to higher growth efficiency [37]. In this experiment, adjusted for growth, efficient fish were characterized by older nitrogen (i.e. protein) profiles of muscle tissues, which confirms earlier findings, since reduced proteolysis of body protein will preserve more of the old protein. These results suggest that efficient fish had newer liver carbon profiles (i.e., glycogen, fat, and protein, combined), which might be linked to the origin of the glycogen in the liver; efficient fish possibly synthesize relatively more of their glycogen through gluconeogenesis or lipogenesis in the liver and thus from nutrients that come directly from digestion of feed (new nutrients) and relatively less from proteolysis of older body protein. Our findings indicate that fast growth combined with reduced degradation rates of existing body tissues, especially in the muscle, is favourable, and that individual differences in these traits can be captured by nitrogen and carbon isotope profiling of the various tissues. The underlying biological mechanisms are likely complex and further studies are needed to elucidate the underlying factors relevant to feed efficiency.

The prediction equation for individual feed efficiency shows that indicator traits add information to the prediction of feed efficiency beyond growth. The limitation of the prediction equation developed is that all variables are averages at the tank level because FCR was only recorded per tank. If this prediction equation was to be used to predict individual FCR, this would imply that the phenotypic and genetic correlations are assumed to be the same at both the individual and

group levels, which may not be realistic. In addition, the prediction equation was estimated in freshwater during a phase of high growth and needs to be validated or re-estimated for larger fish in seawater, but this would require recording of feed intake in the sea. A prediction equation estimated during the freshwater phase may not predict the feed efficiency performance in the grow-out phase in the sea very well. During grow-out, other metabolic pathways such as lipid metabolism may explain more of the variation in feed efficiency in Atlantic salmon, since the relative weight gain decreases, which may leave more room for other factors than growth to contribute to feed efficiency, as previously demonstrated for large rainbow trout [12]. Hence, it is considered more effective to use individually measured phenotypes that are highly genetically correlated to feed efficiency to improve the feed efficiency indirectly. Indicator traits that are more highly correlated to feed efficiency in later life-stages could, therefore, be of high value.

Estimates of genetic correlations (Table 5) revealed, as expected, that fast growth (WG and RG) is favourably associated with improved feed efficiency (r = -0.74 and -0.82, respectively). The indicator traits AMN, AMC, and ALN were estimated to be highly genetically correlated with the growth traits and feed efficiency, as expected, since body growth depends on the deposition of new nutrients from enriched feed, which increased isotope levels in tissues. The estimate of the genetic correlation of carbon metabolism in adipose tissue with FCR (-0.43) was moderate. The link between lipid deposition and FCR should, however, not be disregarded, since it is known to affect feed efficiency later in the life cycle of salmonids because lipid deposition is at its maximum first during the grow-out phase in the sea [12]. ALC had the closest estimated genetic correlation with FCR (-0.90), but had lower genetic correlation estimates with the other indicator traits, which suggests that ALC might explain additional variation in the feed efficiency complex among the indicator traits considered here. As explained above, protein is likely the

main source of nitrogen and carbon in muscle and nitrogen (but not necessarily carbon) in liver. This might explain the high genetic and phenotypic correlations of nitrogen and carbon metabolism in the muscle and nitrogen metabolism in the liver, since they all likely reflect protein metabolism. Compared with muscle, carbon metabolism in the liver (ALC) is affected by fat and glycogen to a larger extent and, thus, is expected to relate less to the other indicator traits.

The IFCR and IFER variables for nitrogen and carbon in muscle are expected to be proportional to the mass of newly deposited nutrients in muscle and, as such, relate directly to the efficiency complex. Buchheister and Latour [38] proposed a ratio between specific growth rate and total metabolism, estimated from isotope profiling, as an indicator trait. A preliminary analysis showed that the trait definition of Buchheister and Latour was close to perfectly genetically correlated with the IFER indicators used in this study (results not shown). In our study, the estimate of the genetic correlation of IFCR with the observed FCR was very high, to the extent that the estimate was fixed at the border of the parameter space ($r_g \sim 1.0$) for both nitrogen and carbon metabolism in muscle, with a phenotypic (tank-level) correlation with observed FCR of 0.72 and 0.58, respectively. The IFER_AMN variable, being the inverse of IFCR_AMN, and correspondingly IFER_AMC were estimated with, respectively a highly negative genetic correlation (-1.0) and a moderately negative, albeit highly uncertain, genetic correlation (-0.63 ± 0.30) to FCR. These results indicate that the mass of new nutrients in the muscle is closely genetically associated with FCR at the tank level. Since the indicator ratio traits (IFCR/IFER) can be measured on individual fish, they are promising indicator traits for individual phenotyping of feed efficiency. However, the estimates of heritability of the indicator ratio traits were lower (0.06 to 0.11) than the estimates of heritability for the remaining traits. In addition, estimates of the genetic correlation of the indicator ratio traits IFCR_AMC, IFCR_AMN, IFER_AMC, and

IFER_AMN with ALC were low. However, estimates of the genetic correlation of ALC and the indicator ratio traits with tank-FCR were high, which indicates that ALC explained individual variation in feed efficiency that was not explained by growth. The indicator ratio traits IFCR and IFER are intuitively appealing and can be easily interpreted biologically, compared to ALC, for which the underlying determinants are largely unknown. The efficiency of metabolization and allocation of nutrients for growth is closely related to the feed efficiency complex; using body tissue as fuel for, e.g., maintenance, is less efficient than using nutrients absorbed and metabolized from feed directly. However, there is some variation between individuals in the extent to which body tissues are used for maintenance [10, 39-41]. A lower exchange of body tissue components would result in more efficient use of protein and thus reduced feed costs [40]. The IFCR and IFER variables allow for direct measurement of nitrogen and carbon fluxes by using stable-isotope profiling to trace the contribution and allocation of nutrients from feed to growth in animal tissue [40-42] and are expected to have a universal relationship with FCR and could be useful independently of life-stage and species.

The standard errors of the estimates of the genetic correlations were rather low in spite of the limited number of families in the study. However, the standard errors of the genetic correlations between our traits and FCR could be made smaller by increasing the size of the family dataset and could thus be used to validate our approach. Our experimental design made it possible to keep all individuals in one common environment until the start of the experiment, which strengthens our results by reducing the environmental variation between families. Our results indicate that the total variation between tanks was, to a large extent, explained by genetics, 52% for FCR and 92% for feed intake.

Phenotyping of stable isotopes at the individual level requires liver and muscle samples, which normally implies that the fish are sacrificed. However, the isotope profile in muscle can be obtained from a muscle biopsy on live animals, which would allow these indicator ratio traits to be recorded even on selection candidates. Alternatively, if test fish have to be sacrificed through sib-testing, information on the full-sibs can be used to predict breeding values on the untested selection candidates. Genomic selection methods that use individual phenotypes and genotypes on training animals for selection among genotyped candidates are expected to be much more effective than traditional pedigree-based selection methods [43-45]. Hence, individual phenotyping is still very important, even for traits that cannot be recorded on the selection candidates. Thus, in full-sib testing an indicator trait is efficient if the estimated breeding value for the indicator ratio trait is estimated with high accuracy (which requires a considerable number of full-sibs), the indicator trait has a high genetic correlation with feed efficiency (as estimated for the IFCR phenotype), and feed efficiency has significant genetic variance (considered considerable, with 3% point standard deviation for FCR). A slaughter test using full-sibs of the breeding candidates is currently part of the breeding program and, thus, implementation of the indicator ratio traits can be carried out in the existing test under field conditions.

Conclusions

Given that isotope-enriched feed can be produced at an acceptable cost, this study presents indicator ratio traits for individual FCR that might be recorded on a massive scale and used for selection, without requiring individual feed intake recording. This requires that the indicator ratio traits, IFCR and IFER, which have a strong genetic relationship to FCR (as reported here in freshwater) are also shown to have such a genetic relationship in the grow-out phase.

Declarations

Ethics approval

The experiment used phenotypic data, which were collected from a family experiment with Atlantic salmon carried out at the fish laboratory, Norwegian University of Life Sciences (NMBU), Aas, Norway, following the laws and regulations for experiments on live animals in EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 11676).

Consent for publication

Not applicable.

Availability of data and material

The genotypic data are owned by AquaGen AS, used under license for this study, and not publicly available. Phenotypic data can be made available on request.

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Competing interests

The authors declare that they have no competing interest.

Authors' contributions

HD, JØ, MØ, LTM, and GK designed the experiment. HD was responsible for feed production and carried out the experiment. All authors contributed during sampling. HD prepared samples for stable isotope analysis. HD and JØ conducted the statistical analysis. HD, JØ and GK developed the methods, variables, and approaches, wrote and finalized the manuscript. MØ and LTM read and corrected language. All authors read and approved the final manuscript.

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Figures

Figure 1 Averages per family for (a) weight gain (WG), (b) relative weight gain (RG), (c) feed intake (FI), and (d) feed conversion ratio (FCR = FI/WG).

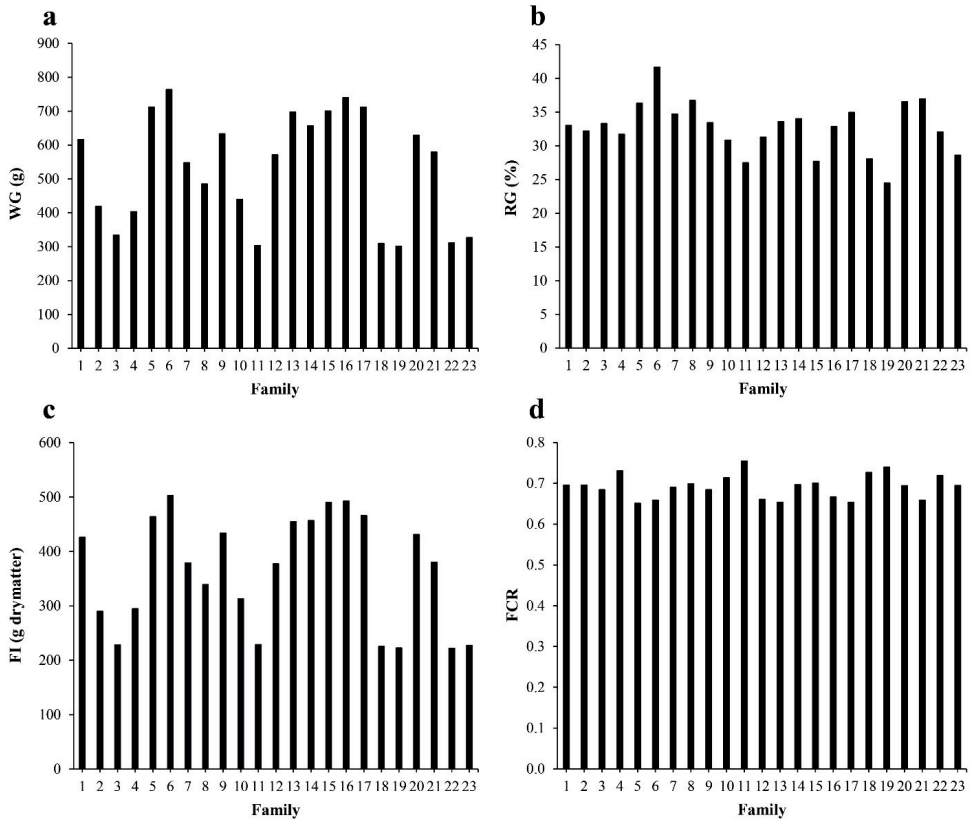
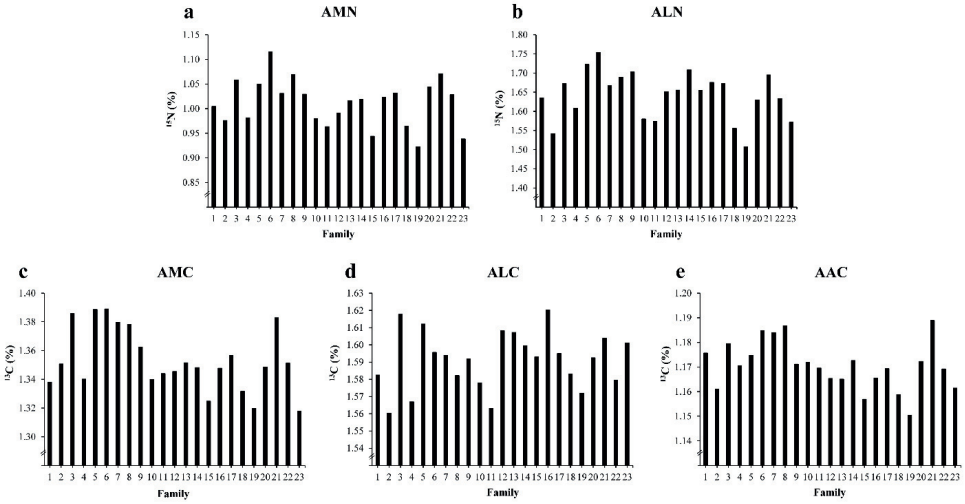


Figure 2 Averages per family for (a) Atom % ^{15}N in muscle (AMN), (b) Atom % ^{15}N in liver (ALN), (c) Atom % ^{13}C in muscle (AMC), (d) Atom % ^{13}C in liver (ALC), and (e) Atom % ^{13}C in adipose tissue (AAC).



Tables

Table 1 Formulation and analysed content[a] of the experimental diet

	Content
Formulation, g kg^{-1}	
Fish meal [b]	455.8
Gelatinized potato starch [c]	105.9
Wheat gluten [d]	150.0
Spirulina ^{15}N-labelled [e]	20.0
Spirulina ^{13}C-labelled [f]	10.0
Fish oil [g]	170.0
Gelatine [h]	80.0
Premix fish [i]	6.3
Monocalcium phosphate [j]	2.0
Analysed content, kg^{-1}	
Dry matter, g	912.5
Crude protein, g	512.7
Lipid, g	187.3
Starch, g	103.7
Ash, g	75.6
Gross energy, MJ	22.2
Analysed content, %	
Atom ^{15}N	2.7 [k]
Atom ^{13}C	2.0 [l]
Essential amino acids, g kg^{-1}	
Arginine	30.3
Histidine	8.8
Isoleucine	19.6
Leucine	34.6
Lysine	28.2
Methionine	11.2
Phenylalanine	20.0
Threonine	19.2
Valine	23.0
Tryptophan	4.1
Non-essential amino acids, g kg^{-1}	
Alanine	31.6
Aspartic acid	39.2
Glycine	43.7
Glutamic acid	99.4
Cysteine	4.8
Tyrosine	11.9
Proline	39.9
Serine	24.2
Total amino acids	493.7

[a]Analysis performed in duplicates
[b]Norse LT 16-001, Norsildmel, Egersund Sildoljefabrikk AS, Egersund, Norway
[c]Lygel F 60, Lyckeby Culinar, Fjälkinge, Sweden
[d]Vital Wheat Gluten, Amilina, Panevezys, Lithuania
[e]CIL-NLM-8401 Spirulina Whole cells (U-^{15}N, 98%+), Cambridge Isotope Laboratories, Larodan, Solna, Sweden
[f]CIL-CLM-8400 Spirulina Whole cells (U-^{13}C, 98%+), Cambridge Isotope Laboratories, Larodan, Solna, Sweden

[g]NorSalmOil, Norsildmel, Bergen, Norway
[h]Rousselot® 250 PS, Rousselot SAS, Courbevoie, France
[i]Farmix, Trouw Nutrition, LA Putten, the Netherlands. Per kg feed; retinol 2500.0 IU, cholecalciferol 32400.0 IU, α-tocopherol SD 0.2 IU, menadione 40.000 mg, thiamine 15.0 mg, riboflavin 25.0 mg, d-Ca-pantothenate 40.002 mg, niacin 150.003 mg, biotin 3000.0 mg, cyanocobalamin 20.0 mg, folic acid 5.0 mg, pyridoxine 15.0 mg, ascorbate polyphosphate 0.098 g, Cu: Cu sulfate $5H_2O$ 11.998 mg, Zn: Zn sulfate 89.992 mg, Mn: Mn(II) sulfate 34.993 mg, I: K-iodine 1.999 mg, Se: Na-selenite 0.200 mg, Cd Max. 0.0003 mg, Pd Max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g
[j]Bolifor®MCP-F.KPP Oy, Animal Nutrition, Helsingborg, Sweden
[k]SE = 0.1
[l]SE = 0.02

Table 2 Descriptive statistics of recorded trait phenotypes

Trait name	Abbreviation	Mean	Min	Max	SD	CV
Individual traits (N = 2281)						
Initial weight (g)	IW	21.8	1.7	52.4	8.0	36.8
Final weight (g)	FW	32.6	4.9	70.3	11.3	34.8
Weight gain: $(FW-IW)$ (g)	WG	10.8	0.3	30.6	4.5	41.9
Relative weight gain: $(((FW-IW)/FW)\times 100)$ (%)	RG	32.8	1.8	64.3	8.1	24.6
Atom % ^{13}C in muscle (%)	AMC	1.35	1.14	1.62	0.05	3.8
Atom % ^{15}N in muscle (%)	AMN	1.01	0.54	1.76	0.12	11.8
Atom % ^{13}C in liver (%)	ALC	1.59	1.27	1.77	0.04	2.4
Atom % ^{15}N in liver (%)	ALN	1.64	0.77	2.00	0.13	7.9
Atom % ^{13}C in adipose tissue (%)	AAC	1.17	1.09	1.55	0.02	2.1
Tank traits (N = 46)						
Feed intake (g dry matter)[a]	FI	363	163	556	110	30.0
Feed conversion ratio: $(FI/FW-IW)$	FCR	0.69	0.64	0.78	0.03	4.8

[a]Calculated according to Helland et al. [23]

Table 3 Results of regression analysis of tank level feed conversion rate on sampling day and each indicator trait, one by one, or when regressing on all[a] experimental variables, following backward elimination

Indicator trait(s)	R^2	Adjusted-R^2	$\widehat{R}^{2b}$	Bias	PRESS[c]
FI + Day	0.42	0.39	0.32	$4.3x10^{-10}$	0.035
$\overline{WG}$ + Day	0.53	0.51	0.46	$6.5x10^{-10}$	0.028
$\overline{RG}$ + Day	0.62	0.60	0.55	$2.2x10^{-10}$	0.023
$\overline{AMC}$ + Day	0.31	0.28	0.21	$-4.4x10^{-10}$	0.041
$\overline{AMN}$ + Day	0.42	0.40	0.34	$-4.4x10^{-10}$	0.034
$\overline{ALC}$ + Day	0.57	0.55	0.52	$-4.4x10^{-10}$	0.025
$\overline{ALN}$ + Day	0.49	0.46	0.40	$-4.4x10^{-10}$	0.031
$\overline{AAC}$ + Day	0.16	0.12	0.03	$-4.4x10^{-10}$	0.050
$\overline{RG}$ + $\overline{AMN}$ + $\overline{ALC}$ + $\overline{AAC}$ + Day[d]	0.79	0.77	0.73	0.00	0.014

[a]Except weight gain
[b]$\widehat{R}^2$ = The coefficient of determination (R^2)
[c]PRESS = Predicted residual error sums of squares
[d]All variables left in the model are significant at the 0.10 level

Table 4 Estimates with standard errors of genetic and residual variance components (σ_a^2 and σ_e^2, respectively), fraction of phenotypic variance explained by environmental tank effect (c^2), heritability (h^2), fraction of between-tank variance explained by genetics (h_t^2), as well as the χ^2 statistics for the additive genetic family effect, with the corresponding level of significance (p)

	$\sigma_a^{2\,a}$	$\sigma_e^{2\,a}$	c^2	h^2	h_t^2	χ^2	p
FCR	5.48 ± 2.69	4.63 ± 1.49	-	-	0.52 ± 0.17	14.0	0.0002
FI	50.99 ± 16.50	4.68 ± 1.87	-	-	0.92 ± 0.04	64.6	9.3×10^{-16}
WG	5.82 ± 0.67	6.44 ± 0.26	0.06 ± 0.02	0.45 ± 0.04	-	222.6	2.4×10^{-50}
RG	14.42 ± 2.36	36.35 ± 1.34	0.03 ± 0.01	0.28 ± 0.04	-	106.7	5.1×10^{-25}
AMC	4.62 ± 0.97	19.43 ± 0.68	0.05 ± 0.02	0.18 ± 0.03	-	47.5	5.4×10^{-12}
AMN	39.23 ± 6.38	97.32 ± 3.58	0.02 ± 0.01	0.28 ± 0.04	-	101.2	8.1×10^{-24}
ALC	1.90 ± 0.44	9.14 ± 0.32	0.13 ± 0.04	0.15 ± 0.03	-	43.1	5.2×10^{-11}
ALN	40.99 ± 7.09	110.20 ± 4.04	0.05 ± 0.02	0.26 ± 0.04	-	77.0	1.7×10^{-18}
AAC	0.99 ± 0.21	4.21 ± 0.15	0.05 ± 0.02	0.18 ± 0.03	-	46.0	1.2×10^{-11}
IFCR_AMC	28.88 ± 8.83	275.38 ± 9.18	0.03 ± 0.01	0.09 ± 0.03	-	24.7	6.6×10^{-07}
IFCR_AMN	86.92 ± 35.33	1270.90 ± 41.67	0.04 ± 0.02	0.06 ± 0.02	-	13.0	0.0003
IFER_AMC	45.75 ± 12.92	364.42 ± 12.28	0.05 ± 0.02	0.11 ± 0.03	-	29.4	5.9×10^{-08}
IFER_AMN	3.59 ± 1.22	39.72 ± 1.32	0.04 ± 0.02	0.08 ± 0.02	-	21.3	4.0×10^{-06}

[a]Variance components and standard error estimates have been multiplied with 10^4, except WG and RG
[b]Chi-square statistic for genetic effect and its level of significance

Table 5 Estimates of genetic (above diagonal) and phenotypic (below diagonal) correlations between traits, with standard errors

Traits	FCR [a]	FI	WG	RG	AMC	AMN	ALC	ALN	AAC	IFCR_AMC	IFCR_AMN	IFER_AMC	IFER_AMN
FCR		-0.61 ± 0.21	-0.74 ± 0.17	-0.82 ± 0.10	-0.69 ± 0.17	-0.73 ± 0.14	-0.90 ± 0.11	-0.63 ± 0.19	-0.43 ± 0.28	1.0 [b]	1.0[b]	-0.63 ± 0.30	-1.0[b]
FI	-0.52 ± 0.13		0.98 ± 0.01	0.44 ± 0.20	0.13 ± 0.24	0.16 ± 0.23	0.31 ± 0.25	0.40 ± 0.21	0.13 ± 0.26	-0.79 ± 0.17	-0.91 ± 0.21	0.76 ± 0.22	0.84 ± 0.16
WG	-0.65 ± 0.10	0.97 ± 0.01		0.46 ± 0.07	0.19 ± 0.11	0.28 ± 0.09	0.16 ± 0.12	0.56 ± 0.07	0.44 ± 0.04	-0.76 ± 0.1	-0.83 ± 0.15	0.75 ± 0.09	0.74 ± 0.12
RG	-0.79 ± 0.07	0.45 ± 0.16	0.54 ± 0.02		0.92 ± 0.04	0.98 ± 0.01	0.12 ± 0.14	0.89 ± 0.03	0.78 ± 0.07	-0.83 ± 0.08	-0.80 ± 0.11	0.76 ± 0.09	0.74 ± 0.1
AMC	-0.61 ± 0.12	0.17 ± 0.19	0.29 ± 0.03	0.71 ± 0.01		0.96 ± 0.02	0.38 ± 0.14	0.88 ± 0.04	0.73 ± 0.09	-0.61 ± 0.17	-0.65 ± 0.16	0.47 ± 0.18	0.57 ± 0.16
AMN	-0.70 ± 0.1	0.22 ± 0.19	0.35 ± 0.03	0.82 ± 0.009	0.86 ± 0.008		0.20 ± 0.14	0.89 ± 0.03	0.71 ± 0.08	-0.72 ± 0.12	-0.72 ± 0.15	0.63 ± 0.13	0.63 ± 0.15
ALC	-0.73 ± 0.08	0.25 ± 0.18	0.09 ± 0.03	0.19 ± 0.03	0.20 ± 0.03	0.15 ± 0.03		0.34 ± 0.12	0.04 ± 0.16	0.10 ± 0.20	-0.27 ± 0.22	-0.26 ± 0.18	0.11 ± 0.20
ALN	-0.63 ± 0.12	0.39 ± 0.17	0.42 ± 0.03	0.74 ± 0.01	0.63 ± 0.02	0.70 ± 0.01	0.49 ± 0.02		0.69 ± 0.08	-0.70 ± 0.11	-0.77 ± 0.12	0.58 ± 0.13	0.66 ± 0.13
AAC	-0.40 ± 0.16	0.17 ± 0.19	0.30 ± 0.03	0.60 ± 0.02	0.52 ± 0.02	0.56 ± 0.02	0.24 ± 0.03	0.54 ± 0.02		-0.58 ± 0.14	-0.63 ± 0.15	0.53 ± 0.14	0.62 ± 0.14
IFCR_AMC	0.58 ± 0.11	-0.56 ± 0.12	-0.41 ± 0.02	-0.58 ± 0.02	0.06 ± 0.03	-0.19 ± 0.03	-0.12 ± 0.03	-0.37 ± 0.02	-0.25 ± 0.02		0.90 ± 0.006	-0.96 ± 0.03	-0.89 ± 0.07
IFCR_AMN	0.72 ± 0.09	-0.54 ± 0.11	-0.41 ± 0.02	-0.57 ± 0.02	0.10 ± 0.03	-0.10 ± 0.03	-0.21 ± 0.03	-0.36 ± 0.02	-0.26 ± 0.02	0.87 ± 0.007		-0.71 ± 0.13	-0.99 ± 0.03
IFER_AMC	-0.46 ± 0.14	0.50 ± 0.13	0.42 ± 0.02	0.58 ± 0.02	-0.14 ± 0.03	0.21 ± 0.03	0.07 ± 0.03	0.33 ± 0.02	0.24 ± 0.03	-0.87 ± 0.006	-0.66 ± 0.01		0.82 ± 0.09
IFER_AMN	-0.74 ± 0.08	0.57 ± 0.11	0.45 ± 0.02	0.62 ± 0.02	-0.10 ± 0.03	0.09 ± 0.03	0.16 ± 0.03	0.34 ± 0.02	0.28 ± 0.02	-0.77 ± 0.01	-0.88 ± 0.005	0.74 ± 0.01	

[a]Genetic analysis with FCR and FI was conducted on a tank basis, due to lack of individual recording for FCR and FI ($N = 46$)
[b]Restricted on boundary of parameter space

Paper IV

Genome-wide association study for indicator traits of feed efficiency in Atlantic salmon (*Salmo salar*)

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Running title: Feed efficiency in Atlantic salmon

Abstract

The objective was to search for putative quantitative trait loci affecting the following indicator phenotypes; relative weight gain, weight gain, atom % ^{13}C in muscle, atom % ^{15}N in muscle, atom % ^{13}C in liver, atom % ^{15}N in liver, atom % ^{13}C in adipose tissue and indicator ratio traits of feed conversion and efficiency ratios for atom % ^{15}N and ^{13}C in muscle. The material was a family experiment performed in the freshwater-phase, encompassing 2281 individuals from 23 full-sib families. Eggs from each family were hatched and families kept separately until start feeding. At start feeding 120 fry were randomly sampled from each family and reared together in a single tank until the start of the feed conversion test. During the 12-day feed conversion test, families were randomly allocated to family tanks (50 fish per tank and 2 tanks per family), and feed conversion ratio was registered on a family group level. Families were fed a fishmeal-based diet labeled with the stable isotopes ^{15}N and ^{13}C, with inclusion levels of 2% and 1%, respectively. For genotyping, a custom 56K single-nucleotide polymorphism array was used. Using a linear mixed-model algorithm, several significant associated single-nucleotide polymorphisms related to growth, and nitrogen and carbon metabolism in muscle and liver were identified on chromosomes 3, 5, 9, 11, 12, 15 and 20. The most important results from this study are the finding of a quantitative trait locus for growth on chromosome 9. Yet another finding was a quantitative trait locus for carbon metabolism in liver on chromosome 12, a trait closely related to feed conversion ratio on a tank level. However, the peak was broad, likely due to the few and numerous families in this study. For the indicator feed efficiency ratio traits, derived from the ratios between the fraction of stable isotopes (^{15}N and ^{13}C) in muscle and growth, no convincing quantitative trait locus was obtained.

Introduction

Sustainable aquaculture production depends on efficient conversion of feed resources into high-quality products (Frankic & Hershner, 2003). In Norway, the feed costs made up ~50% of total production costs in 2017, adding up to a total of approximately 2.2 billion euros (Directorate of Fisheries, 2018): Feed conversion ratio (FCR) was improved from 2010 to 2012, but not for the last five years. Feed efficiency is a complex trait where a genetic variation exists for digestibility (Dvergedal et al., 2019b) and nitrogen and carbon metabolism (Dvergedal et al., 2019c). The genes controlling the trait is, however, unknown.

Using feed enriched with certain isotopes (i.e., with altered ratios of $^{13}C/^{12}C$ and/or $^{14}N/^{13}N$) and monitoring the subsequent rate of change in isotope profile of different tissues, the relative contribution of the nutrients to protein growth can be assessed (Houlihan et al., 1995; Le Vay & Gamboa-Delgado, 2011; MacAvoy et al., 2005). Nitrogen and carbon isotopes are the most relevant when assessing feed efficiency; by definition, all organic compounds contain carbon, while nitrogen is common to all amino acids. Molecules containing ^{14}N and ^{15}N differ in mass, and the ratio of these isotopes can be detected with an element analysis isotope ratio mass spectrometry. In an earlier study the genetic components of nitrogen and carbon metabolism, monitored by measuring the rate of change in isotope profile in different tissues, was elucidated by Dvergedal et al. (2019c). Results showed high genetic correlations between tank-FCR and indicator ratio traits for feed efficiency (IFCR/IFER), based on nitrogen and carbon metabolism in muscle tissue measured with stable isotopes (^{15}N and ^{13}C) (r_g ~1.0), and also between feed

efficiency and carbon metabolism in liver (ALC) (0.9). These results were in accordance with Hawkins et al. (1989), who proposed that differences in protein metabolism between individuals are genotype-dependent. Efficient fish were characterized by high protein growth and reduced protein degradation in muscle at the same relative growth rates (Dvergedal et al., 2019a). In addition, by predicting the observed tank-FCR using multiple regression, growth, isotope-based indicator traits and sampling day jointly explained 73% of the observed variance in masked tank-FCR records, compared to 53-63% by growth and sampling day alone (Dvergedal et al., 2019c). Hence, by including nitrogen and carbon metabolism traits in different tissues, the prediction of FCR data was improved substantially. If the genetic basis of individual differences in feed utilization is established, it has the potential to have major implications for the selection of future breeding candidates in aquaculture breeding programs. Moreover, genetic improvement of feed efficiency by growth or other means will reduce production costs and reduce the environmental footprint per unit produced (Besson et al., 2016; de Verdal et al., 2011).

To date, no genome-wide association study (GWAS) have reported quantitative trait loci (QTL) related to feed efficiency in Atlantic salmon (Laghari et al., 2014; Yue, 2014). However, several studies in beef cattle (Lu et al., 2013; Rolf et al., 2012), chicken (Mignon-Grasteau et al., 2015; van Kaam et al., 1999; Wolc et al., 2013), pigs (Do et al., 2014; Sahana et al., 2013) and in some fish species (Pang et al., 2017; Wang et al., 2012) have reported potential QTLs related to feed efficiency traits (i.e., feed conversion efficiency or residual feed intake). The difficulty of obtaining individual phenotypic records has made it difficult to assess feed efficiency in aquatic species. If chromosomal regions and genes related to feed efficiency could be identified it would make it possible to select breeding candidates carrying the favorable allele(s) (Goddard & Hayes,

2009; Vallejo et al., 2017). With indicator phenotypes for nitrogen and carbon metabolism, feed efficiency can now be obtained at an individual level. With individual phenotypes, a GWAS could be carried out (Goddard & Hayes, 2009). Identified QTL could be used in marker-assisted selection so that selective breeding could be carried out even when breeding candidates have no records of their own. Improved feed efficiency would lead to reduced feed costs, for which an improvement of feed efficiency by 1% has a present value of ~ € 23 million in Norway (Directorate of Fisheries, 2018). Hence, it is time to address feed efficiency, which has the potential to improve profitability and sustainability in aquaculture production.

This study is based on a large-scale family experiment, where families were kept separate in replicate tanks, with individual recordings of growth and isotope profiles after feeding with ^{15}N and ^{13}C-enriched feed. The objective was to search for putative QTLs affecting relevant indicator phenotypes; relative weight gain (RG), weight gain (WG), atom % ^{13}C in muscle (AMC), atom % ^{15}N in muscle (AMN), atom % ^{13}C in liver (ALC), atom % ^{15}N in liver (ALN), atom % ^{13}C in adipose tissue (AAC), indicator ratio trait of FCR for AMC (IFCR_AMC), indicator ratio trait of FCR for AMN (IFCR_AMN), indicator ratio trait of feed efficiency ratio (FER) for AMC (IFER_AMC) and indicator ratio trait of FER for AMN (IFER_AMN) (Dvergedal et al., 2019c). By these means, one aimed at improving the understanding of the relationship between feed efficiency, nitrogen, and carbon metabolism, and growth.

Materials and methods

Phenotypic data were collected from a family experiment with Atlantic salmon carried out at the fish laboratory, Norwegian University of Life Sciences (NMBU), Aas, Norway, according to the laws and regulations controlling experiments on live animals in EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761). The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 11676).

Phenotypic data

Broodstock from AquaGen's breeding population (19 males and 23 females) were used to generate 23 families of Atlantic salmon (*Salmo salar*). To ensure clearly contrasted family groups with respect to growth potential and thus most likely feed efficiency, the families were selected based on (high/low) estimated breeding values (EBVs) for growth in seawater.

Eggs from each family were hatched and families kept separately until start feeding. At start feeding 120 fry were randomly sampled from each family and reared together in a single tank until the start of the feed conversion test. *A priori* to the 12-day feed conversion test, families were allocated to tanks (50 fish per tank and 2 tanks per family, except for nine tanks in which the number of fish varied between 42 and 54, due to some mortality prior to the start of the experiment and to one tank with a larger number of fish due to a counting mistake. The total number of fish was 2281), and feed conversion was registered on a family group level. Families were fed a fishmeal-based diet labeled with the stable isotopes ^{15}N and ^{13}C, with inclusion levels of 2% and 1%, respectively, as described in Dvergedal et al. (2019c).

Phenotypic data were registered individually for RG, WG, AMC, AMN, ALC, ALN and AAC, as described by Dvergedal et al. (2019c). For the various variables, phenotypes were available for 2249-2280 fish. From the individual (*i*) levels of AMC and AMN, individual isotope-based indicator ratio traits for feed conversion ratio (IFCR) and feed efficiency ratio (IFER); $IFCR\_AMC_i$, $IFCR\_AMN_i$, $IFER\_AMC_i$, and $IFER\_AMN_i$, were defined as follows (taking ^{15}N as an example):

$$IFCR\_AMN_i = \frac{FW_i * APE_{Ni}}{FW_i - IW_i},$$

$$IFER\_AMN_i = \frac{FW_i - IW_i}{FW_i * APE_{Ni}},$$

where $APE_{Ni} = (AMN_i - IA\ \%)$ with IA % equal to 0.370% for ^{15}N and 1.087% for ^{13}C. After a diet switch, the APE of a stable isotope in muscle tissue is expected to be proportional to the fraction of newly synthesized nutrients in the muscle, and the product of APE and final weight is expected to be proportional to the mass of new nutrients in body tissue. Because the $IFCR$ ratio is expected to be proportional to the amount of newly deposited body nutrients per g increase in body weight, fish exchanging a larger fraction of the body mass per unit of growth will be less feed-efficient. Exchange of body tissue is traceable with stable-isotope profiling and is related to the feed intake of the individual, while the denominator of the ratio is the weight gain, and the ratio between these two variables equals $IFCR$ or, alternatively, the inverse equals $IFER$.

Muscle, liver and adipose samples from each individual were collected in a cryotube and snap frozen in liquid nitrogen for stable isotope analysis. The sampling procedure and determination

158 of atom % ^{15}N and ^{13}C in the samples are explained in detail in Dvergedal et al. (2019c). The
159 stable isotope analysis was carried out at the Institute for Energy Technology (Kjeller, Norway).
160
161 *Genotypic data*
162
163 When the fish reached 5-10 g, they were pit-tagged with a 2 x 12 mm unique glass tag (RFID
164 Solutions, Hafrsfjord, Norway), and a fin-clip was collected for DNA-extraction and genotyping
165 of a total of 2300 fish. Fin clips (20 mg) were incubated in lysis buffer and treated with
166 proteinase K (20µg/ml) at 56°C overnight. The following day, DNA was isolated from the lysate
167 using the sbeadex livestock kit (LGC Genomics) according to the manufacturer's protocol
168 (Thermo Fisher Scientific) at Biobank AS (Hamar, Norway). The DNA concentration was
169 measured using a Nanodrop 8000 (Thermo Fisher Scientific). All fish were genotyped using
170 AquaGen's custom Axiom®SNP (single-nucleotide polymorphism) genotyping array from
171 Thermo Fisher Scientific (former Affymetrix) (San Diego, CA, USA). This SNP-chip contains
172 56,177 SNPs which were originally identified based on Illumina HiSeq reads (10-15x coverage)
173 from 29 individuals from AquaGen's breeding population. Genotyping was done at CIGENE
174 (Aas, Norway). Genotypes were called from the raw data using the Axiom Power Tools software
175 from Affymetrix. Individuals having a Dish-QC score below 0.82, and/or a call-rate below 0.97
176 were deleted from further analyses.
177
178 *Association analysis*
179

The phenotypes related to nitrogen and carbon metabolism (AMC, AMN, ALC, ALN, and AAC), growth (RG, WG) and indicator ratio traits for feed efficiency (IFCR_AMC, IFCR_AMN, IFER_AMC, and IFER_AMN) were used in this study. Association between each SNP and the phenotypes was tested by use of a linear mixed-model algorithm implemented in a genome-wide complex trait analysis (GCTA) (Yang et al., 2014), with the leave one chromosome out option (*--mlm-loco*). With this option, the chromosome harboring the SNP tested for was left out when building the genetic relationship matrix (GRM). The linear mixed model can be written:

$$Y_i = a + bx + g_i^- + \varepsilon_i,$$

where Y_i is one of the phenotypes; RG, WG, AMC, AMN, ALC, ALN, AAC, IFCR_AMC, IFCR_AMN, IFER_AMC or IFER_AMN of individual *i*, *a* is the intercept, *b* is the fixed regression of the candidate SNP to be tested for association, *x* is the SNP genotype indicator variable coded as 0, 1 or 2, g_i^- is the random polygenic effect for individual $i \sim N(0, \mathbf{G}\sigma_g^2)$ where **G** is the GRM and σ_g^2 is the variance component for the polygenic effect, and ε_i is the random residual. In this algorithm, the variance component σ_g^2 is re-estimated each time a chromosome is left out from the calculation of the GRM. The dataset was filtered according to the following criteria: individuals with < 10% missing genotypes (n = 2279), SNPs with minor allele frequency (MAF) >= 1% and SNPs with a missing call rate < 10%. After filtering 54,200 SNPs were included in the analysis. The level of significance was evaluated with a built-in likelihood-ratio test, and the threshold value for genome-wide significance was calculated by use of Bonferroni correction (0.05/54200) = 9.23 x 10^{-7}, corresponding to a $-\log_{10} p\text{-}value\ (p)$ of 6.03. The actual number of SNPs at each chromosome was utilized to calculate the chromosome-wide

significance level. The Bonferroni correction is used to account for multiple comparisons. However, Bonferroni correction is known to be overly conservative especially when applied to correlated SNP data i.e., SNPs in linkage disequilibrium, which could produce false negative results (Duggal et al., 2008). To visualize the $-log_{10}(p)$ of SNPs over the chromosomes (n = 29) Manhattan plots were used (Figure 1 and 2), while QQ-plots showed the distribution of observed versus expected genome-wide $-\log_{10}(p)$ (Figure 3 and 4).

Results and discussion

The data was generated in a family experiment with Atlantic salmon in the freshwater-phase, using 23 full-sib families (Dvergedal et al., 2019c). Families were selected based on EBVs for growth rate (high/low growth) in the sea, in order to maximize the expected differences in feed efficiency between families. Using AquaGen's 56K Atlantic salmon SNP array, 2300 fish were genotyped. In the experiment, each family was allocated to two tanks, with 50 fish per tank (except for some tanks), and a diet enriched with ^{15}N and ^{13}C was fed over a 12-day pre-defined period. Growth and isotope profiles in muscle, liver and adipose tissue were recorded individually. With isotope profiling, nutrient allocation in the body from ingestion to deposition in tissue is traceable. Change in isotope profile over time relates to degradation and re-synthesis of previously deposited protein, originating from the previous diet, and growth based on proteins from the new diet. Efficient fish will minimize the loss of deposited nutrients per unit growth, which is expected to affect the rate of change in the observed isotope profile. Using isotope data individual phenotyping for feed efficiency is possible in Atlantic salmon even without obtaining registrations of individual feed intake. Heritability, genetic and phenotypic correlations among

the studied traits and FCR were reported in Dvergedal et al. (2019c). The results indicated that the indicator ratio traits IFCR/IFER in muscle is closely genetically associated with FCR on a tank level (r_g ~ 1.0). However, ALC showed a close genetic correlation to FCR on a tank level (r_g = -0.90), but correlated less to the growth-related traits than IFCR/IFER. Consequently, ALC may explain individual variation in feed efficiency that is not related to growth. To test whether phenotypes for feed efficiency such as ALC and IFCR/IFER variables are associated with SNPs, a GWAS was performed with a linear mixed-model algorithm, using indicator traits related to nitrogen and carbon metabolism, growth and indicator ratio traits for feed efficiency as phenotypes (RG, WG, AMC, AMN, ALC, ALN, AAC, IFCR_AMC, IFCR_AMN, IFER_AMC and IFER_AMN).

To our knowledge, this is the first GWAS applied to indicator ratio traits of feed efficiency and metabolism in muscle, liver and adipose tissues of Atlantic salmon. The results from the association analyses were visualized through Manhattan plots (Figures 1 and 2) and QQ plots (Figures 3 and 4). Figure 1 illustrates that there are significant associations between SNPs and traits of interest. The Manhattan plots for RG, WG, AMC, AMN and ALN (Figures 1a, b, c, d and f), indicates that the significant association on Ssa09 was in two peaks, the first between 13 Mbp and 31 Mbp and the second ranging from 45 to 106 Mbp, respectively. Significant associations corresponding to a $-\log_{10}(p) > 8$ between indicator phenotypes and SNPs are presented in Table 1.

According to Table 1, most of the significant SNPs were shared between traits (indicated as bold in Table 1), at Ssa09 with 4-10 SNPs in common between the traits RG, WG, AMC, AMN, and

ALN. In addition, two SNPs at Ssa03, four SNPs at Ssa05 and 12 at Ssa20 were found with -$\log_{10}$ (p) > 6.03 to WG (Figure 1b). Correspondingly, one SNP at Ssa11, Ssa12, and Ssa15 associated with AMN (Figure 1d) and one SNP at Ssa20 was associated with ALN (Figure 1f). No significant associations between AAC and SNPs were found at this stage of life (~ 10 months), which might be explained by the fact that lipid deposition is at its maximum later, during the grow-out phase in the sea (~1.5-4 kg). Therefore, we cannot rule out a possible link between lipid deposition and FCR that might occur at a later life-stage in salmonids (Azevedo et al., 2004; Einen & Roem, 1997; Kause et al., 2016).

SNPs genome-wide significantly associated with growth-related traits such as RG, WG, AMC, AMN, and ALN were mainly located at Ssa09. Gutierrez et al. (2012) who mapped QTLs related to body weight in Atlantic salmon at different life stages, reported genome-wide significant SNPs (QTL) at Ssa09 in Atlantic salmon at the same age as in this study (~ 10 months). They also reported chromosome-wide significant SNPs at Ssa20, but they could not find any relationship between their findings at different stages. Baranski et al. (2010) argued that the large number of different QTLs which are acting at different life-stages imply that body weight can be considered a polygenic trait in Atlantic salmon. However, as growth in large occurs in saltwater, the commercial interest of a QTL for body weight in the freshwater-phase is most likely limited.

Genome-wide SNPs were also found on a chromosomal region of Ssa12 affecting ALC (Figure 1e), with 19 SNPs at Ssa12 with -$\log_{10}$ *(p)* > 8. Again, this confirms that ALC is genetically distinct from growth (RG and WG) as well as AMC, AMN, and ALN. A QTL improving feed efficiency without increasing growth would be highly relevant from an economic point of view

271 and would add valuable information that cannot be captured by recording growth of the
272 individuals. However, the SNPs of interest were spread over a region of 40 Mbp and a total of
273 128 genes (NCBI search).
274
275 Three genome-wide significant associations were found between SNPs and the indicator ratio
276 trait IFER_AMN, at Ssa06, Ssa23, and Ssa27 (Figure 2d), while no genome-wide significant
277 associations between SNPs and IFCR_AMC, IFCR_AMN, and IFER_AMC were identified
278 (Figure 2a-c). At a chromosome level, a consistent pattern was obtained with all the IFCR/IFER
279 phenotypes having common significant SNPs at Ssa03, Ssa06, Ssa21, Ssa23 and Ssa27 (Table
280 2). Dvergedal et al. (2019c) found low heritability estimates for these indicator ratio traits, and
281 families were few, meaning that if a "true" QTL exist in the population it might not be
282 represented in the 23 families, pointing towards the need for more families. With more families,
283 more haplotypes will also be represented in the data, increasing the possibility to more accurately
284 pinpoint the position of a QTL. With strong family structures, long stretches of the same
285 haplotype, being identical by descent (Sahana et al., 2013) are likely to occur, which can result in
286 the wide peaks, most expressed for ALC in Ssa12. This might have reduced the probability of
287 finding significant SNPs for the indicator ratio traits IFCR/IFER in these data. Thus, QTLs might
288 still exist for IFCR/IFER traits although no convincing findings were obtained in this study. The
289 priority should, therefore, be to do a following up study with more adequate material. In such a
290 study, one should aim at increasing the marker density in the chromosomal region of interest by
291 genotype imputation (Li et al., 2009; Marchini & Howie, 2010) supplied with utilization of a
292 haplotype approach that could have reduced the number of tests to be carried out, leading to a
293 less stringent genome-wide threshold.

With related families, it is not strange that the QQ-plots (Figure 3a-f) indicate confounding effects, due to the substructure in the data material creating stratification. This is less visible for AAC (Figure 3g) and IFCR/IFER traits (Figure 4a-d). With more and less related families, recombination will leave shorter stretches of the genome associated with the trait (Yue, 2014), increasing the probability of finding significant associations. Further, the SNP effects were obtained in the freshwater-stage. At later stages in the life-cycle, other SNPs might explain individual variation, as has been observed for growth (Gutierrez et al., 2012). In this context, the phenotypes that relate to individually recorded feed efficiency is of special interest. This is worth exploring in a larger and more adequate material, with the potential to increase our understanding of the genetics of feed efficiency in Atlantic salmon.

Conclusion

The most important results from this study are the finding of a QTL for growth on Ssa09. Yet another finding was a QTL for carbon metabolism in liver on Ssa12, closely related to FCR on a tank level, but the peak was broad, likely due to the few and related families in this study. For the IFCR/IFER phenotypes, derived from the ratios between the fraction of stable isotopes (^{15}N and ^{13}C) in muscle and growth, no convincing QTLs were obtained.

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324

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Table 1. Single-nucleotide polymorphisms (SNP) with *p*-values > 10^{-8} (for which they are ranked), corresponding to $-\log_{10}(p) > 8$ (all genome-wide significant), for relative weight gain (RG), weight gain (WG), atom % ^{13}C muscle (AMC), atom % ^{15}N muscle (AMN), atom % ^{13}C liver (ALC) and atom % ^{15}N liver (ALN). Significant associations common between traits are indicated in bold.

Trait	Chr	SNP	bp	A1	A2	Freq	b	se	p
RG	9	**ctg7180001820745_5080_SAG**	23240272	G	A	0.202	2.191	0.337	7.98E-11
	9	**ctg7180001604256_10823_SAG**	23113694	G	A	0.156	2.406	0.380	2.39E-10
	9	**GCR_cBin45958_Ctg1_101**	19444985	G	A	0.386	2.035	0.339	1.99E-09
	9	**ctg7180001789610_1630_SCT**	25039628	C	T	0.349	-1.669	0.288	7.03E-09
	9	**ctg7180001841302_7054_SGT**	21739717	G	T	0.184	2.112	0.367	8.33E-09
	9	**ctg7180001841302_7076_SGT**	21739695	T	G	0.191	2.046	0.365	2.02E-08
	9	ctg7180001809374_3372_SCT	19466507	C	T	0.373	1.900	0.343	3.03E-08
	9	**ctg7180001847789_6042_SAG**	16428841	A	G	0.263	2.034	0.367	3.07E-08
	9	**ctg7180001857693_2711_SAG**	85086045	G	A	0.278	1.814	0.329	3.60E-08
	9	**ctg7180001545661_2981_SGT**	16416138	G	T	0.402	1.704	0.310	3.68E-08
	9	ctg7180001468960_5703_SCT	14756989	C	T	0.226	2.035	0.370	3.83E-08
	9	ctg7180001931759_8478_SAG	16329499	G	A	0.217	2.046	0.376	5.14E-08
	9	**ctg7180001898949_10269_SAG**	22638682	A	G	0.482	-1.584	0.292	5.86E-08
WG	5	ctg7180001180119_4244_SCT	33597277	C	T	0.141	1.063	0.190	2.28E-08
	5	ctg7180001923117_1435_SAG	31819838	A	G	0.097	1.153	0.215	7.63E-08
	9	ctg7180001197157_4756_SAG	89517134	G	A	0.356	-1.016	0.166	9.54E-10
	9	ctg7180001818540_14626_SGT	96172057	G	T	0.233	0.991	0.163	1.23E-09
	9	ctg7180001818540_11784_SCT	96174899	C	T	0.234	0.976	0.163	1.98E-09
	9	ctg7180001664612_2468_SCT	91842480	C	T	0.234	0.967	0.163	3.00E-09
	9	ctg7180001664612_1619_SAG	91841631	G	A	0.234	0.958	0.163	4.19E-09
	9	**ctg7180001545661_2981_SGT**	16416138	G	T	0.402	0.819	0.140	4.53E-09
	9	ctg7180001832507_11515_SCG	75470675	G	C	0.448	-0.847	0.145	5.35E-09
	9	ctg7180001868348_9058_SAG	15945100	G	A	0.492	-0.781	0.137	1.18E-08
	9	**ctg7180001628780_1051_SAG**	17214390	G	A	0.414	-0.871	0.153	1.20E-08
	9	ctg7180001197157_4700_SAC	89517078	A	C	0.311	0.859	0.151	1.40E-08
	9	ctg7180001894494_11001_SAG	89556217	G	A	0.310	0.851	0.152	2.08E-08
	9	**ctg7180001911598_32299_SCT**	17106888	C	T	0.436	-0.808	0.146	2.88E-08
	9	ctg7180001802227_6890_SAC	78211162	A	C	0.216	0.972	0.176	3.34E-08
	9	ctg7180001806806_477_SAC	78242149	A	C	0.216	0.969	0.176	3.67E-08
	9	ctg7180001588841_1060_SGT	86047894	G	T	0.234	0.855	0.155	3.77E-08
	9	ctg7180001926947_6570_SGT	81306559	T	G	0.247	0.885	0.161	3.86E-08
	9	ctg7180001380355_4100_SGT	96979010	G	T	0.422	0.721	0.133	5.50E-08
	9	ctg7180001921692_473_SGT	95413530	G	T	0.212	0.946	0.175	6.46E-08
	9	ctg7180001898405_11116	82286805	A	C	0.312	0.818	0.152	7.65E-08
	9	ctg7180001678561_512_SGT	90982168	T	G	0.335	0.769	0.143	7.99E-08
	9	**GCR_cBin45958_Ctg1_101**	19444985	G	A	0.386	0.825	0.154	8.16E-08
	9	ctg7180001859612_1950_SCT	106163425	T	C	0.426	0.837	0.157	9.45E-08
	20	ctg7180001900661_2996_SAG	29391087	A	G	0.472	0.651	0.116	1.97E-08
	20	ctg7180001900661_8312_SAC	29385772	A	C	0.472	0.632	0.116	4.75E-08
	20	ctg7180001403181_749_SGT	32398670	T	G	0.413	0.701	0.131	7.97E-08
AMC	9	**ctg7180001628780_1051_SAG**	17214390	G	A	0.415	-0.013	0.002	1.04E-09
	9	**ctg7180001820745_5080_SAG**	23240272	G	A	0.202	0.013	0.002	2.45E-09
	9	**ctg7180001789610_1630_SCT**	25039628	C	T	0.349	-0.011	0.002	5.22E-09
	9	ctg7180001763729_3905_SAG	15474718	A	G	0.283	0.013	0.002	8.27E-09
	9	ctg7180001763729_4055_SGT	15474568	T	G	0.283	0.013	0.002	1.14E-08
	9	ctg7180001872184_4046_SAC	59521565	A	C	0.267	-0.012	0.002	1.69E-08
	9	**ctg7180001847789_6042_SAG**	16428841	A	G	0.261	0.013	0.002	1.91E-08
	9	ctg7180001903467_551_SGT	30327474	T	G	0.175	-0.013	0.002	2.06E-08
	9	ctg7180001700380_482_SGT	15707203	G	T	0.282	0.013	0.002	2.08E-08
	9	**GCR_cBin45958_Ctg1_101**	19444985	G	A	0.385	0.012	0.002	3.47E-08
	9	**ctg7180001911598_32299_SCT**	17106888	C	T	0.436	-0.011	0.002	3.78E-08
	9	ctg7180001903534_19011_SGT	13431163	T	G	0.283	-0.013	0.002	3.98E-08
	9	ctg7180001872184_453_SCT	59517972	T	C	0.265	-0.012	0.002	4.40E-08
	9	**ctg7180001604256_10823_SAG**	23113694	G	A	0.157	0.013	0.002	4.58E-08
	9	**ctg7180001343223_1775_SCT**	67759885	T	C	0.411	0.011	0.002	5.85E-08
	9	**ctg7180001545661_2981_SGT**	16416138	G	T	0.401	0.011	0.002	6.44E-08
	9	ctg7180001833924_2266_SCT	24557694	T	C	0.429	0.011	0.002	6.65E-08
	9	ctg7180001794986_4059_SAC	20044519	C	A	0.262	-0.012	0.002	6.93E-08
	9	**ctg7180001898949_10269_SAG**	22638682	A	G	0.481	-0.010	0.002	7.33E-08

Table 1 continued

Table 1 cont.

Trait	Chr	SNP	bp	A1	A2	Freq	b	se	p
AMN	9	**ctg7180001820745_5080_SAG**	23240272	G	A	0.202	0.034	0.005	3.24E-11
	9	**ctg7180001841302_7054_SGT**	21739717	G	T	0.184	0.036	0.006	1.47E-10
	9	**ctg7180001604256_10823_SAG**	23113694	G	A	0.157	0.037	0.006	1.87E-10
	9	**ctg7180001841302_7076_SGT**	21739695	T	G	0.192	0.033	0.006	1.33E-09
	9	**ctg7180001898949_10269_SAG**	22638682	A	G	0.481	-0.027	0.004	1.51E-09
	9	ctg7180001909530_3368_SAC	30671958	A	C	0.289	0.032	0.006	1.69E-08
	9	**ctg7180001857693_2711_SAG**	85086045	G	A	0.278	0.028	0.005	2.38E-08
	9	**ctg7180001628780_1051_SAG**	17214390	G	A	0.415	-0.028	0.005	2.78E-08
	9	ctg7180001912930_10973_SAC	59822403	C	A	0.446	-0.026	0.005	4.30E-08
	9	**ctg7180001343223_1775_SCT**	67759885	T	C	0.411	0.026	0.005	4.62E-08
	9	**ctg7180001911598_32299_SCT**	17106888	C	T	0.436	-0.026	0.005	8.19E-08
	9	ctg7180001254975_135_SCT	30005989	T	C	0.328	0.017	0.003	9.72E-08
	11	ctg7180001912112_756_SAG	11950571	A	G	0.352	-0.019	0.003	5.12E-08
ALC	12	ctg7180001233434_1518_SCT	45935004	C	T	0.418	-0.009	0.001	1.79E-10
	12	ctg7180001878331_16006_SAG	67415693	A	G	0.411	-0.008	0.001	3.95E-10
	12	ctg7180001589944_2780_SAC	67420787	C	A	0.410	-0.008	0.001	5.82E-10
	12	ctg7180001917752_6118_SAG	68229784	A	G	0.342	-0.008	0.001	6.01E-09
	12	ctg7180001926810_6994_SGT	59975663	T	G	0.445	-0.008	0.001	8.58E-09
	12	ctg7180001924417_6623_SAC	66355729	A	C	0.178	-0.010	0.002	1.38E-08
	12	ctg7180001863800_134_SAC	54013376	C	A	0.495	-0.008	0.001	1.71E-08
	12	ctg7180001930970_12364_SCT	34715679	C	T	0.460	0.007	0.001	1.86E-08
	12	ctg7180001787629_3714_SGT	63703424	G	T	0.441	-0.008	0.001	1.92E-08
	12	ctg7180001926810_5584_SCT	59974253	C	T	0.435	-0.008	0.001	2.17E-08
	12	ctg7180001759831_1827_SCT	45916216	C	T	0.364	-0.007	0.001	2.33E-08
	12	ctg7180001481690_187_SAG	73548289	G	A	0.438	-0.008	0.001	3.22E-08
	12	ctg7180001912956_2486_SGT	36748230	T	G	0.416	-0.007	0.001	3.25E-08
	12	ctg7180001926810_6801_SAG	59975470	A	G	0.444	-0.007	0.001	3.28E-08
	12	ctg7180001899463_4736_SCT	45925520	C	T	0.365	-0.007	0.001	3.78E-08
	12	ctg7180001874153_6984_SAC	59968424	C	A	0.444	-0.007	0.001	3.82E-08
	12	ctg7180001903261_15275_SCT	36741726	T	C	0.419	-0.007	0.001	4.80E-08
	12	ctg7180001802518_8127_SAG	38630722	G	A	0.264	-0.008	0.002	5.60E-08
	12	ctg7180001895532_9980_SAC	52536172	C	A	0.150	-0.010	0.002	8.47E-08
ALN	9	**ctg7180001820745_5080_SAG**	23240272	G	A	0.202	0.034	0.005	1.71E-10
	9	**ctg7180001604256_10823_SAG**	23113694	G	A	0.157	0.037	0.006	3.95E-10
	9	ctg7180001902776_3165	44544043	A	G	0.030	-0.082	0.013	1.00E-09
	9	ctg7180001297112_1053_SAC	44743511	C	A	0.029	-0.079	0.013	3.51E-09
	9	ctg7180001846444_1581_SAG	27624708	G	A	0.050	-0.060	0.010	3.64E-09
	9	**ctg7180001898949_10269_SAG**	22638682	A	G	0.481	-0.027	0.005	6.84E-09
	9	ctg7180001841823_6182_SGT	27830435	G	T	0.050	-0.059	0.010	8.19E-09
	9	ctg7180001841823_8622_SAG	27832875	A	G	0.051	-0.058	0.010	9.34E-09
	9	ctg7180001897675_6237_SCG	26358698	C	G	0.050	-0.058	0.010	1.04E-08
	9	**ctg7180001841302_7076_SGT**	21739695	T	G	0.192	0.033	0.006	1.28E-08
	9	**ctg7180001841302_7054_SGT**	21739717	G	T	0.184	0.033	0.006	1.39E-08
	9	ctg7180001516979_6848_SCT	26634813	T	C	0.050	-0.058	0.010	1.54E-08
	9	ctg7180001905112_13597_SAC	25462494	C	A	0.061	-0.052	0.009	1.57E-08
	9	ctg7180001516979_7200_SCT	26635165	C	T	0.050	-0.057	0.010	2.04E-08
	9	GCR_cBin3500_Ctg1_117	27426856	C	G	0.081	-0.044	0.008	3.60E-08
	9	ctg7180001927229_6536	84462126	A	G	0.448	0.027	0.005	4.58E-08
	9	ctg7180001905111_1804_SAC	25477875	C	A	0.062	-0.050	0.009	4.77E-08
	9	ctg7180001322796_3617_SGT	50798901	G	T	0.113	-0.037	0.007	6.77E-08
	9	ctg7180001820745_5080_SAG	23240272	G	A	0.202	0.034	0.005	1.71E-10

Table 2. Single-nucleotide polymorphisms (SNP) associated with the indicator ratio trait of feed conversion ratio for atom % ^{13}C muscle (IFCR_AMC), indicator ratio trait of feed conversion ratio for atom % ^{15}N muscle (IFCR_AMN), indicator ratio trait of feed efficiency ratio for atom % ^{13}C muscle (IFER_AMC) and indicator ratio trait of feed efficiency ratio for atom % ^{15}N muscle (IFER_AMN). Genome significant associations are indicated in bold, while the other SNPs listed were significant at a chromosome level.

Trait	Chr	SNP	bp	A1	A2	Freq	b	se	p
IFCR_AMC	3	ctg7180001842722_6916_SAG	63459464	G	A	0.374	0.036	0.008	3.99E-06
	21	ctg7180001323703_1378_SAG	39211688	A	G	0.069	0.062	0.014	1.54E-05
IFCR_AMN	3	ctg7180001842722_6916_SAG	63459464	G	A	0.374	0.070	0.016	1.67E-05
	13	ctg7180001860456_1459_SAG	98906101	G	A	0.241	0.090	0.019	1.68E-06
	13	ctg7180001811621_8607_SAG	93005594	G	A	0.197	0.091	0.019	2.06E-06
	21	ctg7180001323703_1378_SAG	39211688	A	G	0.069	0.127	0.030	2.83E-05
	23	ctg7180001890689_21525_SCT	16362087	C	T	0.266	-0.078	0.018	1.44E-05
	27	ctg7180001799855_1941_SAC	39176828	A	C	0.165	-0.089	0.021	1.40E-05
IFER_AMC	3	ctg7180001842722_6916_SAG	63459464	G	A	0.374	-0.044	0.009	2.43E-06
	3	ctg7180001886980_6671_SAC	30582174	A	C	0.344	0.039	0.009	5.47E-06
	3	ctg7180001916773_2082_SAC	63507157	C	A	0.424	-0.039	0.009	1.68E-05
	3	ESTV_13699_319	14509743	G	C	0.231	-0.043	0.010	2.13E-05
	3	ctg7180001886980_16100_SCT	30572745	C	T	0.313	0.037	0.009	2.20E-05
	6	ctg7180001654841_454_SAC	44396098	A	C	0.484	-0.037	0.008	1.45E-05
	21	ctg7180001323703_1378_SAG	39211688	A	G	0.069	-0.079	0.017	5.18E-06
	21	ctg7180001914103_1960_SGT	44889338	T	G	0.407	-0.044	0.010	7.85E-06
IFER_AMN	3	ctg7180001842722_6916_SAG	63459464	G	A	0.197	0.016	0.003	8.71E-07
	3	ctg7180001655621_4059_SGT	66167785	G	T	0.266	0.017	0.003	5.07E-07
	6	ctg7180001317004_3705_SAG	55946829	G	A	0.165	0.019	0.004	5.99E-07
	6	**ctg7180001807394_15612_SAC**	**56168860**	**A**	**C**	**0.374**	**-0.013**	**0.003**	**7.81E-06**
	21	ctg7180001323703_1378_SAG	39211688	A	G	0.301	0.013	0.003	2.57E-05
	21	ctg7180001914103_1960_SGT	44889338	T	G	0.251	0.014	0.003	1.45E-05
	23	**ctg7180001890689_21525_SCT**	**16362087**	**C**	**T**	**0.069**	**-0.025**	**0.006**	**9.87E-06**
	23	ctg7180001822028_30009_SAG	29524513	G	A	0.407	-0.014	0.003	2.03E-05
	27	**ctg7180001799855_1941_SAC**	**39176828**	**A**	**C**	**0.296**	**0.012**	**0.003**	**2.62E-05**

Figure 1. Genome-wide Manhattan plot for the different traits; (a) relative weight gain (RG) (b) weight gain (WG) (c) atom % ^{13}C muscle (AMC) (d) atom % ^{15}N muscle (AMN) (e) atom % ^{13}C liver (ALC) (f) atom % ^{15}N liver (ALN) and (g) atom % ^{13}C adipose tissue (AAC). The horizontal line represents the genome-wide Bonferroni $-\log_{10}(p) = 6.03$ threshold.

Figure 2. Genome-wide Manhattan plot for the different traits; (a) indicator ratio trait of feed conversion ratio for atom % ^{13}C muscle (IFCR_AMC), (b) indicator ratio trait of feed conversion ratio for atom % ^{15}N muscle (IFCR_AMN), (c) indicator ratio trait of feed efficiency ratio for atom % ^{13}C muscle (IFER_AMC) and (d) indicator ratio trait of feed efficiency ratio for atom % ^{15}N muscle (IFER_AMN). The horizontal line represents the genome-wide Bonferroni $-\log_{10}(p) = 6.03$ threshold.

Figure 3. Q-Q plots from association analyses of (a) relative weight gain (RG) (b) weight gain (WG) (c) atom % ^{13}C muscle (AMC) (d) atom % ^{15}N muscle (AMN) (e) atom % ^{13}C liver (ALC) (f) atom % ^{15}N liver (ALN) and (g) atom % ^{13}C adipose tissue (AAC).

Figure 4. Q-Q plots from association analyses of (a) indicator ratio trait of feed conversion ratio for atom % ^{13}C muscle (IFCR_AMC), (b) indicator ratio trait of feed conversion ratio for atom % ^{15}N muscle (IFCR_AMN), (c) indicator ratio trait of feed efficiency ratio for atom % ^{13}C muscle (IFER_AMC) and (d) indicator ratio trait of feed efficiency ratio for atom % ^{15}N muscle (IFER_AMN).

Figure 1.

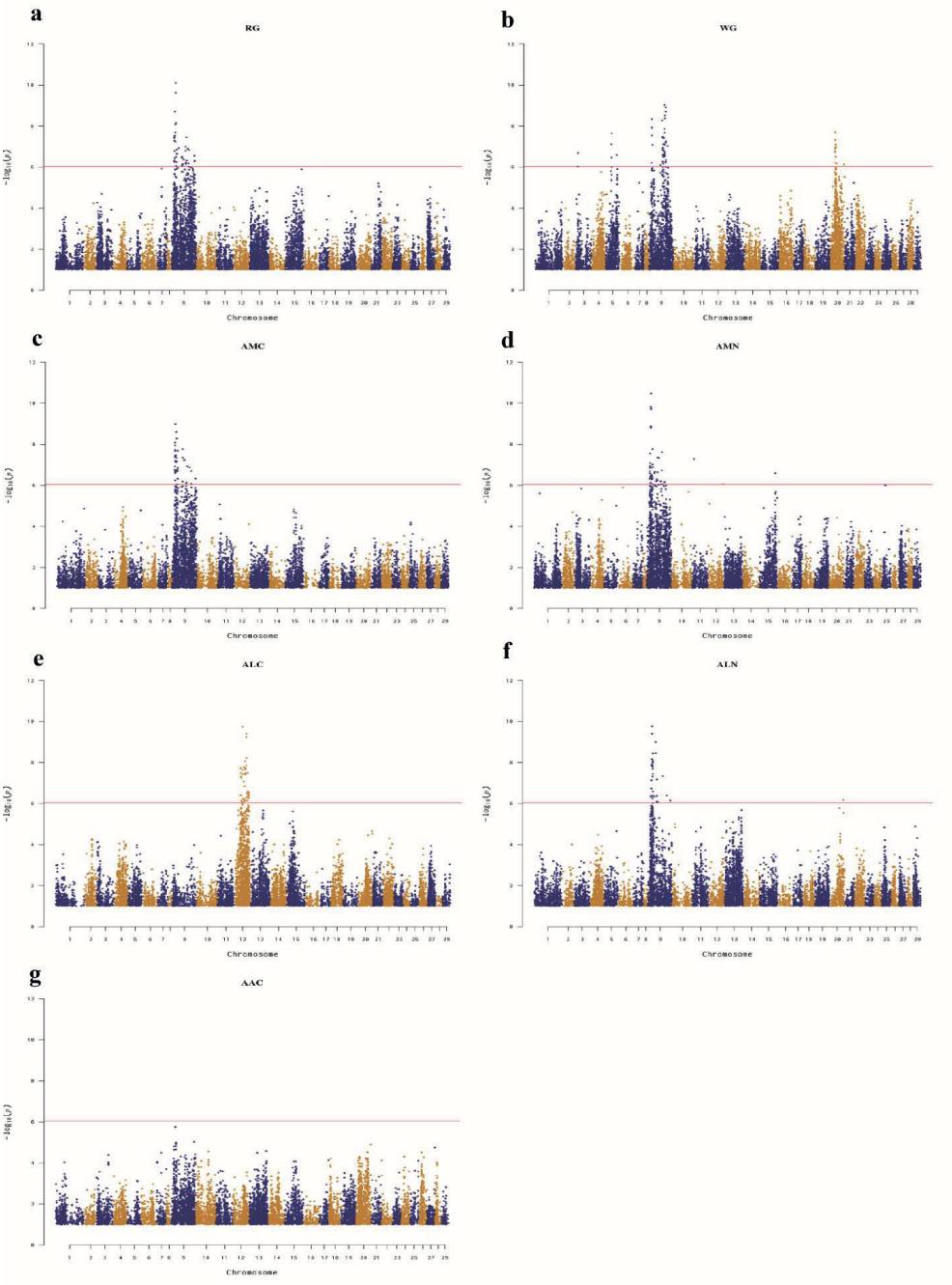


Figure 2.

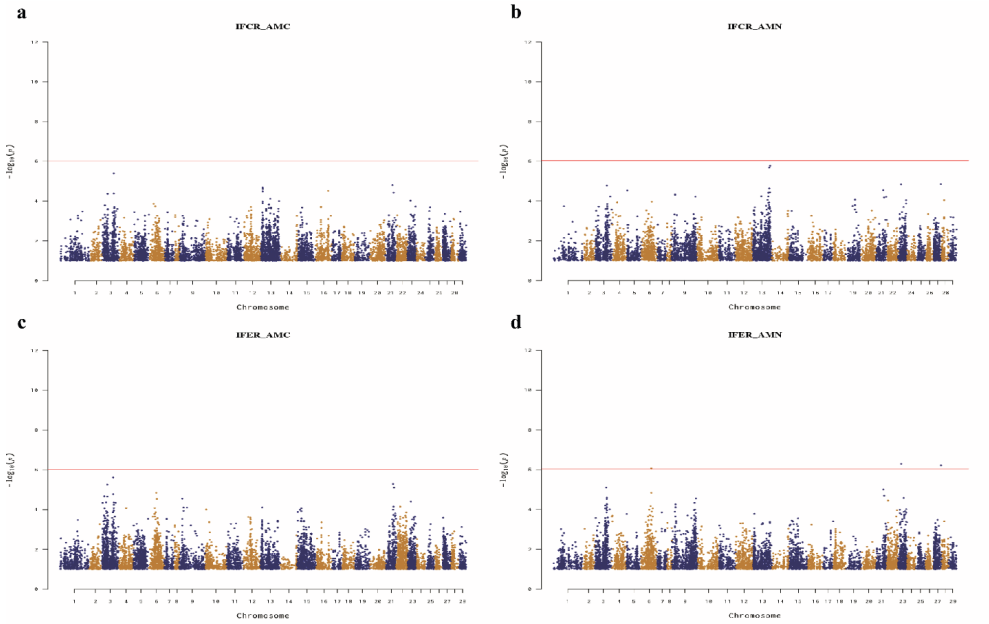


Figure 3.

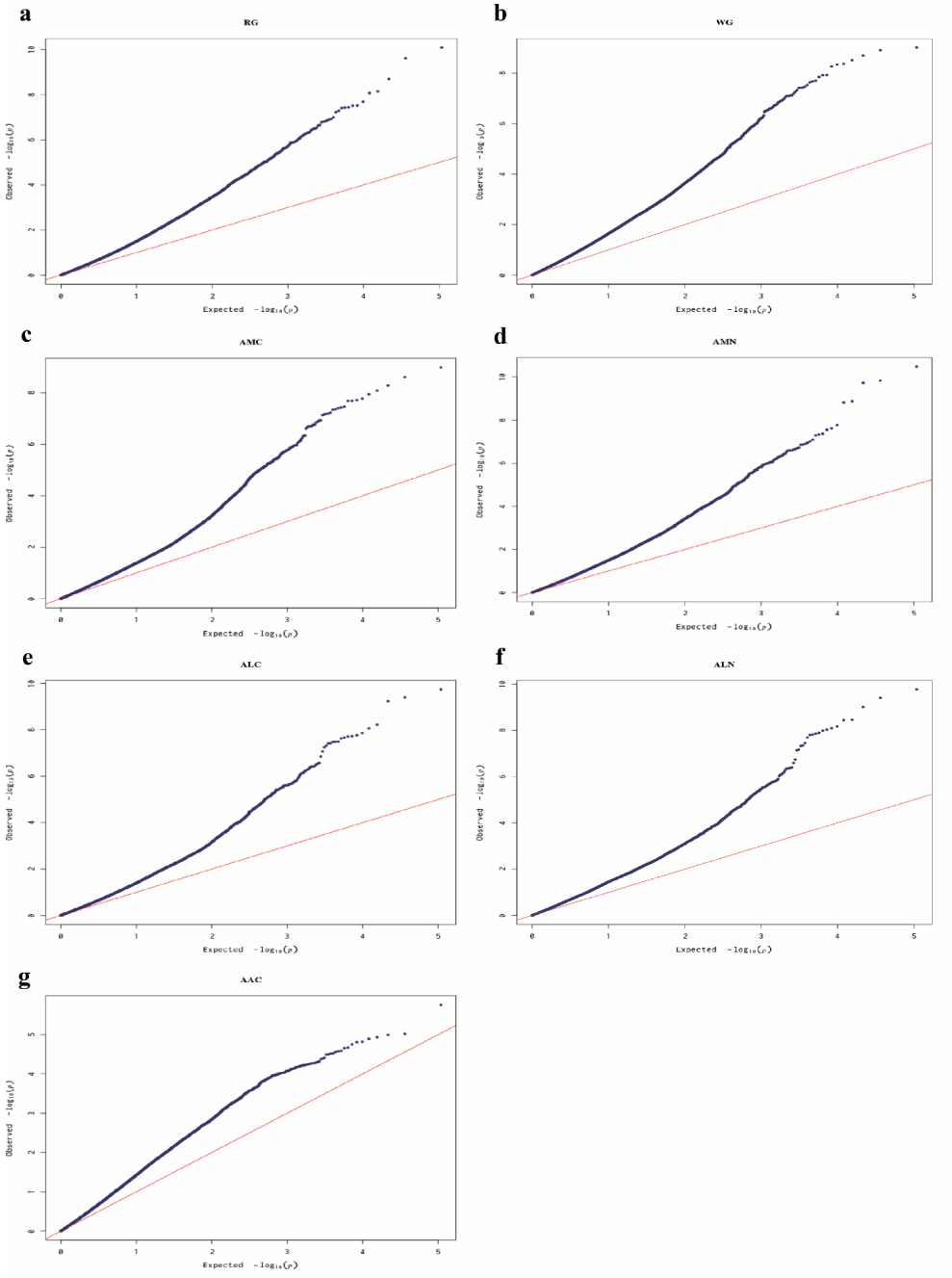
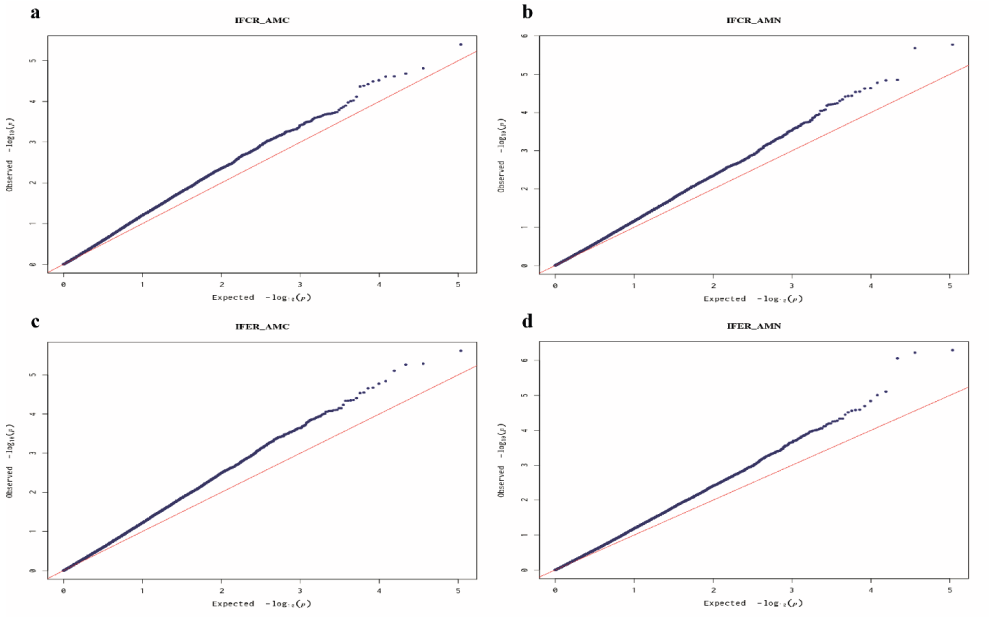

a
RG
b
WG
c
AMC
d
AMN
e
ALC
f
ALN
g
AAC
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$

Figure 4.



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