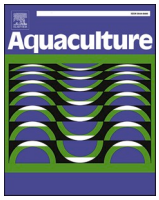
Aquaculture 562 (2023) 738806

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



$\delta^{13}C$ in muscle, liver, and adipose fin and their relationship to weight change during both growth and starvation in rainbow trout *(Oncorhynchus mykiss),* after feeding a diet low in ^{13}C

Hanne Dvergedal [a,*], Jørgen Ødegård [a,b], Trina F. Galloway [b], Sandeep Sharma [c], Monica Juarez [c], Margareth Øverland [a], Gunnar Klemetsdal [a]

[a] Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Post Box 5003 NMBU, N-1432 Aas, Norway
[b] AquaGen AS, Post Box 1240, Torgard, 7462 Trondheim, Norway
[c] Biomar AS, Post Box 1282, Havnegata 9, 7010 Trondheim, Norway

ARTICLE INFO

Keywords:
Aquaculture
Carbon turnover
Growth
Starvation
Metabolism

ABSTRACT

A 35-day experiment was conducted with a total of 105 rainbow trout (*Oncorhynchus mykiss*) with an average initial weight of 192.9 g, sampled over four time points (day 7, 14, 21, and 28) to study the relationship between respectively, $\delta^{13}C$ in muscle, liver, and adipose fin and relative weight gain, when fed a diet based on plant C3 ingredients, with a lower level of $\delta^{13}C$ compared to the baseline diet. From day 28 to 35, seven fish per tank were starved to investigate the effect of starvation on $\delta^{13}C$ in muscle, liver, and the adipose fin. Relative weight gain (as a proxy for feed efficiency) significantly ($P < 0.0001$) affected $\delta^{13}C$ in muscle and liver, respectively, implying that a diet with a lower natural abundance (compared to the baseline diet) can be used to phenotype feed efficiency. Moreover, the change in $\delta^{13}C$ due to weight loss was considerable in liver (2.6‰), but only 1.7‰ in muscle under starvation.

1. Introduction

Selection for improved feed efficiency (growth per unit of feed consumed, FER) or feed conversion ratio (feed consumed per unit of growth, FCR) in commercial breeding programs requires both records of individual growth rate and feed intake. Both on the phenotypic (de Verdal et al., 2017; Kolstad et al., 2004; Thodesen et al., 1999, 2001) and genetic (Dvergedal et al., 2019b; Henryon et al., 2002; Kinghorn, 1983) levels high favorable correlations have been found between growth and feed efficiency. In fish farming, recording the growth rate is relatively simple, but the fish are typically kept in large units (e.g., sea cages or ponds) and group-fed by dispersing feed into the water. No method, however, exists for recording individual feed intake for a large number of individuals under field conditions, which complicates the implementation of direct selection for improved feed efficiency in fish breeding schemes. Thus, in practice selection for improved feed efficiency has been carried out by indirect selection for increased growth rate, since individual body weight is easy to record and all fish are harvested at the same age (e.g., Kristjánsson et al., 2020). Fast-growing fish will reach appropriate harvest weight at a younger age, resulting in saved feed for maintenance and thus improved FCR (Gjedrem and 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 new traits could have the potential to improve selection for feed efficiency.

Investigating potential phenotypes which could explain more of the variation in feed efficiency is an alternative approach. Assessing feed efficiency through use of stable isotope profiling of carbon (^{13}C) metabolism in important tissues such as muscle, liver, and adipose tissue was investigated by Dvergedal et al. (2019b). Results indicated the existence of individual variation in metabolic efficiency, with efficient fish allocating more of the nutrients from feed to growth and less to the maintenance of existing body tissues (Dvergedal et al., 2019a, 2019b). Using leave-one-out cross-validation, as much as 79% of the between-tank variation in FCR was explained by growth, isotope profile, and sampling day (Dvergedal et al., 2019b). In comparison, only 62% of the variation was explained by growth and sampling day. In the latter study, the ratio of tissue turnover, estimated as the change in isotope fractions

* Corresponding author.
E-mail address: hanne.dvergedal@nmbu.no (H. Dvergedal).

https://doi.org/10.1016/j.aquaculture.2022.738806
Received 15 June 2022; Received in revised form 31 August 2022; Accepted 5 September 2022
Available online 9 September 2022

to body growth, was used as an individual indicator of feed conversion efficiency and the reciprocal ratio for feed efficiency. For these indicator traits, the heritabilities ranged from 0.06 to 0.11, and estimated genetic correlations to the FCR, on a group level, approached unity. In summary, the study showed that individual indicator traits have the potential to assess individual feed efficiency in salmonids. However, the experiment was performed with juvenile Atlantic salmon, and factors affecting feed efficiency early and later in life are not necessarily identical. These factors may also be species-dependent.

One prerequisite to applying this approach in a breeding program is cost-effective production of feed with a contrasting isotope profile (based on ingredients with a higher or lower natural abundance of ^{13}C) relative to the level of the isotope in the diet previously fed (baseline). One way to achieve this is to replace C3 plant ingredients in the diet (e.g., wheat gluten) with C4 plant ingredients (e.g., maize gluten). Due to different photosynthetic pathways (e.g., Gannes et al., 1998), C4 plants make an intermediate four-carbon compound that splits into a three-carbon compound for the Calvin cycle during photosynthesis (Gannes et al., 1998). The difference in ^{13}C signatures of biological (C3 vs. C4 plants) material occurs because of differing discrimination against ^{13}C in different photosynthetic pathways (Gannes et al., 1998; Staddon, 2004). Both C3 and C4 plants are low in ^{13}C compared with atmospheric carbon, but this is less for C4 plants (C4; $\delta^{13}C$ -14.35, C3; $\delta^{13}C$ -28.79) (Bahar et al., 2005). These differences can be exploited as a cost-efficient way to produce experimental feeds with contrasting isotope profiles.

In this study, we aimed to verify whether the relationship found in juvenile Atlantic salmon (*Salmo salar*) by Dvergedal et al. (2019a, 2019b) (that more of the nutrients were prioritized for growth and less for maintenance) could be obtained in rainbow trout (*Oncorhynchus mykiss*) when feeding a diet low in $\delta^{13}C$. If the fish uses less energy for maintenance, it has the potential to allocate these resources to growth and become more feed efficient. Thus, a relationship is expected between growth and the change in the isotope profile, and we, therefore, used relative weight gain as a proxy for feed efficiency. We examined this relationship by regressing the change in isotope profile on relative weight gain. Also, we aimed to investigate the effect of starvation on the $\delta^{13}C$ in muscle, liver, and adipose fin. This was motivated by the common practice of starvation of salmonids prior to slaughter for 3–20 days to ensure complete evacuation of the gut to improve hygiene and quality (Imsland et al., 2020; Einen et al., 1998; Einen and Thomassen, 1998), and to increase stress tolerance in handling through a reduced metabolic rate (Waagbø et al., 2017). This change in metabolic rate might affect the stable isotope profile in tissues since Dvergedal et al. (2020) have shown that the ^{15}N levels in both muscle, liver, and mid-intestine decreased significantly with time in starved juvenile Atlantic salmon.

2. Materials and methods

2.1. Ethical statement

A 35-day experiment was carried out at the Center for Sustainable Aquaculture at the Norwegian University of Life Sciences (NMBU), Aas, Norway, following the laws and regulations for experiments on live animals in the EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761).

2.2. Fish and housing

The experiment was approved by the Norwegian Food Safety Authority (FOTS ID 20601). A total of 105 rainbow trout of both sexes from the breeding company AquaGen AS with an average initial body weight of 192.9 g were randomly distributed into three tanks (35 fish per tank). Prior to the start of the 35-day experimental period, all fish were pit-tagged with a 2 × 12 mm unique glass tag (RFID Solutions, Hafrsfjord, Norway), and the initial length and weight were recorded. The circular tanks (height = 70 cm and diameter = 78 cm), each with a 300 l capacity, were supplied with recirculated freshwater from the recirculating aquaculture system (RAS), at a flow rate of 7–8 l min^{-1}, and the fish were kept under a 24 h light regime, with an average water temperature of 15.0 °C. The tanks were controlled by OxyGuard water quality monitoring and control systems for aquaculture (OxyGuard International AS, Denmark), and the water quality was within legal legislation (<0.05, 0.03, and 8.15 mg l^{-1} for ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3-), respectively). Dissolved oxygen was measured daily and kept above 8 mg l^{-1} in the outlet water. One day after the fish were distributed into tanks, three fish were euthanized because they had problems with the swim bladder, while the rest of the fish stayed healthy throughout the experiment.

2.3. Dietary treatment and feeding

A priori to the experiment the fish were fed a commercial diet from Skretting AS (Nutra Olympic 3 mm with a $\delta^{13}C$ of −24.18). In the experiment, a diet with a low level of ^{13}C was fed for 28-days (Table 1, $\delta^{13}C$ of −27.7). From day 28 to day 35 the fish were starved to investigate the effect of starvation on the $\delta^{13}C$ in muscle, liver, and adipose fin. All fish were healthy, and no mortality occurred throughout the experiment. The formulation and analyzed chemical composition of the diet are presented in Table 1. The experimental diet was formulated to create a considerable difference in $\delta^{13}C$ (between the baseline and experimental diets) and to meet the nutritional requirements of rainbow trout and was produced at the BioMar AS pilot plant (Tech Center, Brande, Denmark). The fish were fed twice a day (07:00 and 15:00) for a period of 1 h, by automatic belt feeders. The feeding level equaled 1–3% of the estimated body weight and was adjusted for uneaten feed. Registrations of uneaten feed and calculations of feed intake were performed according to Helland et al. (1996). The daily feed intake per tank was calculated by first collecting the waste feed on a wedge wire screen (Shomorin et al., 2019) and correcting the total waste feed for leaching losses. As explained by Shomorin et al. (2019), the wedge wire screen 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

Table 1
Formulation and analyzed content of the experimental diet for ~200 g rainbow trout.

Formulation and content	
Formulation, g kg^{-1}	
Fish meal	293.9
Sunflower expeller	117.7
Wheat gluten	157.1
Pea protein	132.8
Wheat	158.8
Rapeseed oil	105.1
Premix and others	34.5
Y_2O_3	0.5
Analyzed content, g kg^{-1}	
Dry matter	927.4 ± 0.07
Crude protein	439.9 ± 0.18
Lipid	195.5 ± 5.58
Starch	133.2 ± 0.24
Ash	54.3 ± 0.06
Gr oss energy, MJ kg^{-1}	23.0 ± 0.02
Analyzed content, ‰	
$\delta^{13}C$	−27.7 ± 0.04

The analysis was a mean of duplicates with standard deviations; Fish Meal SA Krill, Aker Biomarine, Norway; Sunflower expeller, Bunge, Hungary; Wheat gluten, Roquette, EU; Pea protein, Norsildmel, China; Wheat, Hedegaard, Denmark; Rapeseed oil, Emmelev, Denmark; Owned by BioMar AS, used under license for this study, and not publicly available; Yttrium oxide (Y_2O_3), Metal Rare Earth Limited, Shenzhen, Guangdong, China.

dry matter intake, after drying the uneaten feed at 105 °C overnight. The tank-based feed conversion ratios (FCR) were 0.77, 0.78 and 0.79 for tanks 1, 2 and 3, respectively.

2.4. Tissue sampling

Seven fish from each tank were sampled on days 7, 14, 21, 28, and 35 after the dietary switch. Fish were anesthetized with Finquel vet. (Tricaine Methanesulfonate; Scanvacc) and killed with a sharp blow to the head prior to dissection. Tissue samples from muscle, liver and adipose fin were collected, snap-frozen in liquid nitrogen, and stored at −20 °C until stable isotope analysis. Tissue sampling was standardized; muscle was sampled in front of the dorsal fin on the left side in the filet (1 × 1 cm cube), the liver was divided into four small pieces, and the whole adipose fin was collected, but only the tip of the adipose fin was utilized for stable isotope analysis.

2.5. Chemical analysis

The diet was dried and ground prior to analysis, which were performed in duplicates (Table 1). The diet was analyzed 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 et al. (1994). 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 the PARR 1281 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831.

2.6. Stable isotope analysis

Prior to analysis, the diet was homogenized and weighed out in duplicates, and tissue samples (muscle, liver, and adipose fin) were freeze-dried and homogenized (except adipose fin), and for both diet and tissues approximately 1 mg per sample was weighed into small tin capsules (8 × 5 mm, Elemental Microanalysis, Devon, UK). Samples were analyzed for C-isotope composition using a Nu Horizon isotope-ratio mass spectrometer (IRMS) (Nu Instruments, Wrexham, UK) coupled to a Eurovector element analyzer (EA) 3028 (Eurovector S.p. A, Redavalle, Italy) at the Institute for Energy Technology (Kjeller, Norway), and δ^{13}C was calculated as follows (Fry, 2006):

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C} Sample}{\frac{^{13}C}{^{12}C} Standard} - 1 \right) 1000,$$

where the two ratios are the proportions of ^{13}C divided by the proportion of ^{12}C, in the sample and the reference standard, per ‰ ((Vienna Pee Dee Belemnite for carbon, VPDB); $\delta^{13}C_{Standard}$= 0.0112372 (Craig, 1957)).

The calibration of ^{13}C was performed against international certified reference materials and internal standards, and the results of δ^{13}C analyses were plotted on a two-point calibration line calculated from the analysis of the USGS-24 standard (−16.05‰) from the United States Geological Survey and an in-house (Institute for Energy Technology, IFE) graphite standard (−31.56‰) from Spectrapure. The internal IFE trout standard was prepared by Soxhlet extraction with CH_2Cl_2: 7% CH_3OH for approximately two hours, cleansed with 2 N HCl, and rinsed with distilled water to a neutral pH. The δ^{13}C composition of IFE trout was calibrated against the USGS-24 standard. The average δ^{13}C from six analyses of the IFE trout was −20.05‰, with a standard deviation of 0.11.

2.7. Phenotypes analyzed

The initial (W_0) and final (W_{28}) weights (weights before and at day 28) were recorded for each fish. From these variables, individual weight gain (*WG*) and relative weight gain (*RG*) were calculated as follows:

$$WG = W_{28} - W_0,$$

$$RG = \frac{W_{28} - W_0}{W_{28}} \times 100.$$

For fish that were starved from day 28 to 35 the individual relative weight loss (*RWL*) was calculated as follows:

$$RWL = \left(\left(\frac{(W_{35} - W_{28})}{W_{28}} \right) \times 100 \right),$$

where W_{28} and W_{35} are the individual weights before (day 28) and after (day 35) the starvation period.

From the tissue samples, the following individual variables were available: δ^{13}C in muscle (MC), δ^{13}C in the liver (LC), and δ^{13}C in adipose fin (FC).

Three fish were removed from the dataset due to negative growth rates, indicating abnormal development or phenotyping error, respectively.

2.8. Relationship between isotope-derived variables and relative weight gain

The isotope-derived variables MC, LC, and FC (Y_{ijk}) were analyzed with the following model:

$$Y_{ijk} = \mu + t_i + d_j + b_1 RG + b_2 RG(d_j) + \varepsilon_{ijk},$$

where μ is the overall mean, t_i is the fixed effect of tank i ($i = 1, 2, 3$), d_j is the fixed effect of sampling-day j (j = 7, 14, 21, and 28), b_1 is the regression coefficient of RG, b_2 is the regression coefficients of RG nested within sampling-day and ε_{ijk} is a random residual for the k^{th} fish (in tank i, at sampling day j). The analyses were conducted using PROC GLM in SAS®, V.9.4 (SAS Inst. Inc., Cary, NC).

2.9. Effect of starvation on $\delta^{13}C$ in muscle, liver, and adipose fin

To estimate what effect the relative weight loss (X) had on the variables MC, LC, and FC (Y_{ijk}) the following model was run:

$$Y_{ijk} = \mu + t_i + d_j + bX + \varepsilon_{ijk},$$

where μ is the overall mean, t_i is the fixed effect of tank i ($i = 1, 2, 3$), d_j is the fixed effect of sampling-day j (j = 28 and 35; day 0 and 7 from the start of starvation, respectively), b is the regression coefficient of relative weight loss (RWL = X), and ε_{ijk} is a random residual for the k^{th} fish (in tank i, at sampling day j). These analyses were also carried out with PROC GLM in SAS®. Least-squares means were calculated, and differences were tested (*t*-test).

3. Results

Table 2 shows that RG explained a major fraction of the variance in MC and LC in addition to tank for liver, respectively $R^2 = 0.66$ and 0.68 for the full model. The relationships between MC, LC, FC, and RG over time are shown in Fig. 1. Both Table 2 and Fig. 1a and b show a strong negative and significant relationship between MC, LC, and relative weight gain, which is expected since protein growth is necessarily based on the deposition of newly consumed feed with a low level of ^{13}C. No such relationship was found for FC (Table 2; Fig. 1c). However, no significant interaction between RG and sampling day was found (Table 2). Due to the fast metabolic rate the most negative $\delta^{13}C$ was

Table 2
Individual $\delta^{13}C$ in muscle (MC), liver (LC), or adipose fin (FC) and estimated effects of sampling day, tank, relative weight gain (RG), and the interaction between relative weight gain and sampling day in three univariate analyses of variance.

Trait	Fixed effect	$\hat{b}\pm$ s.e.	*F*-value	*P*-value	R^2
MC	Intercept	−23.81 ± 1.40			0.66
	Day 7	1.63 ± 1.60			
	Day 14	2.13 ± 1.67	1.12	0.35	
	Day 21	0.41 ± 1.56			
	Day 28	0			
	Tank 1	−0.02 ± 0.16			
	Tank 2	−0.31 ± 0.17	2.08	0.13	
	Tank 3	0			
	RG	−0.04 ± 0.03	17.16	<0.0001	
	RG*DAY 7	−0.03 ± 0.05			
	RG*DAY 14	−0.06 ± 0.04	1.10	0.36	
	RG*DAY 21	−0.008 ± 0.04			
	RG*DAY 28	0			
LC	Intercept	−25.61 ± 0.57			0.68
	Day 7	0.84 ± 0.65			
	Day 14	0.38 ± 0.68	1.56	0.21	
	Day 21	1.15 ± 0.64			
	Day 28	0			
	Tank 1	0.22 ± 0.07			
	Tank 2	0.06 ± 0.07	5.99	0.004	
	Tank 3	0			
	RG	−0.01 ± 0.01	23.49	<0.0001	
	RG*DAY 7	−0.02 ± 0.02			
	RG*DAY 14	−0.01 ± 0.02	1.40	0.25	
	RG*DAY 21	−0.03 ± 0.01			
	RG*DAY 28	0			
FC	Intercept	−20.18 ± 1.22			0.59
	Day 7	−2.34 ± 1.40			
	Day 14	−1.84 ± 1.46	1.00	0.40	
	Day 21	−2.16 ± 1.36			
	Day 28	0			
	Tank 1	0.07 ± 0.14			
	Tank 2	−0.10 ± 0.15	0.63	0.54	
	Tank 3	0			
	RG	−0.07 ± 0.03	2.09	0.15	
	RG*DAY 7	0.10 ± 0.04			
	RG*DAY 14	0.06 ± 0.04			
	RG*DAY 21	0.06 ± 0.03			
	RG*DAY 28	0	2.08	0.11	

$\hat{b}$: Estimated regression coefficient; R^2: The coefficient of determination of model.

observed in the liver on day 28, followed by muscle and adipose fin (averages: −26.2, −25.6, and − 23.6, respectively). However, none of the tissues had reached an equilibrium with the diet (Table 1; −27.7).

During the 7-days of starvation (days 28 to 35), the RWL ranged from −5.7 to −11.5% (data not shown), meaning that all fish lost weight. Moreover, Table 3 shows that the RWL, in addition to sampling day, significantly ($P < 0.05$) affected two out of three isotope-derived variables (MC and LC). The estimated regression coefficients of RWL on the isotope-derived variables were all positive (Table 3), meaning that the more bodyweight the fish lost, the lower $\delta^{13}C$ content. However, Table 4 shows that MC and LC least-squares means were significantly ($P < 0.05$) enlarged after 7-days of starvation (higher than at day 28), 1.7 and 2.6‰, respectively.

4. Discussion

The results showed a strong relationship to exist between the isotope-derived variables in muscle and liver (MC and LC, respectively) and RG (Table 2), in accordance with Dvergedal et al. (2019a). However, in the adipose fin (FC) the relationship to RG was not significant ($P = 0.15$), as it neither was in Dvergedal et al. (2022). The experimental design allowed to test both the heterogeneity of slopes and the different intercepts for sampling day, but neither were significant (Table 2). However, the main effect of relative weight gain across sampling days showed a consistent strong negative relationship with MC and LC, substantiating an effect of isotopes on growth/feed efficiency. From this, one could reason it sufficient to only observe growth, but initially, we have motivated that the isotope-derived variables add information to improve selection for feed efficiency. In consequence, we would advise phenotyping both variables when aiming to improve selection for feed efficiency. The main reason to sample the adipose fin was to elucidate whether a fin-clip can be used to identify feed-efficient fish, to avoid the need of slaughtering the individuals. If such an association would have been discovered the phenotyping of individual feed efficiency could have been done on the breeding candidates themselves. However, one should keep in mind that we were not able to homogenize the tissue to account for the intra-fin variability (Hayden et al., 2015). Moreover, the adipose fin might not be the most suitable for this purpose and sampling a fin-clip from another fin (e.g., caudal; Cano-Rocabayera et al., 2015) or fish scales (Rodde et al., 2020) has both been shown related to $\delta^{13}C$ in muscle and might be more relevant and should be investigated for this purpose.

Switching from the baseline diet to a feed based on ingredients low in $\delta^{13}C$ (more C3 plants) reduced the $\delta^{13}C$ profile of the fish accordingly (Fig. 1). As explained by Dvergedal et al. (2019a), the change in the carbon isotope profile is due to protein and fat metabolism, i.e., growth dilution in addition to the replacement of existing body tissues (losses). This implies that fish obtaining a certain level of relative growth over a shorter time period will reduce the average replacement of existing body tissue (i.e., reduced maintenance costs from a shorter growth period), and thus improve average FCR. Thus, metabolic feed efficient fish can logically be characterized by having a large fraction of their metabolism allocated to growth, which can be assessed as the ratio between $\delta^{13}C$ in the muscle and relative weight gain, the measure used by Dvergedal et al. (2019b). Dvergedal et al. (2019b) explored the genetic component of individual phenotypes for this measure in juvenile Atlantic salmon and found it heritable and with a high genetic correlation to FCR (close to unity).

When feeding an experimental diet low in ^{13}C using natural ingredients (i.e., moderately low diets), one should be aware of a potential confounding with isotope fractionation between fish and diet. Isotope fractionation means that the heavy isotope (^{13}C, the light being ^{12}C) tends to bioaccumulate in tissues of consuming animals due to preferential metabolic retention of ^{13}C (Gamboa-Delgado, 2021), leading to an isotopic difference between animals and their respective diet. Higher nutrient retention with less excretion of waste products is expected to give the lowest diet-animal fractionation (Martin-Perez et al., 2013; Trueman et al., 2005), and individual fractionation will thus likely be negatively correlated to FCR, it means ^{13}C will be highest for the inefficient fish (^{13}C content in a fish in equilibrium with its' feed, i.e., before being fed with the low ^{13}C diet). However, after the dietary switch, fast replacement of body nutrients per unit growth (poor efficiency) would generate the fastest decline of ^{13}C content in body tissue per unit growth. Consequently, a dietary switch to a ^{13}C-low diet and the general diet-animal fractionation may have opposing effects on the relationship between FCR and ^{13}C content in body tissues. With enrichment (e.g., using C4 plants), however, the relationship between FCR and ^{13}C content will tend to be affected in the same direction, meaning that the latter approach should have preference. However, the clear association between growth rate (through increased efficiency) and ^{13}C content in body tissues (MC and LC) indicates that a diet with a lower natural abundance compared to the baseline diet can be used to phenotype feed efficiency.

In this study, a strong relationship existed between LC and RG (Table 2). In juvenile Atlantic salmon, the metabolic drive is towards growth of new muscle tissue, and there is a high demand for protein. As explained in Dvergedal et al. (2019b), protein is likely the main source of both nitrogen and carbon in muscle, and of nitrogen (but not

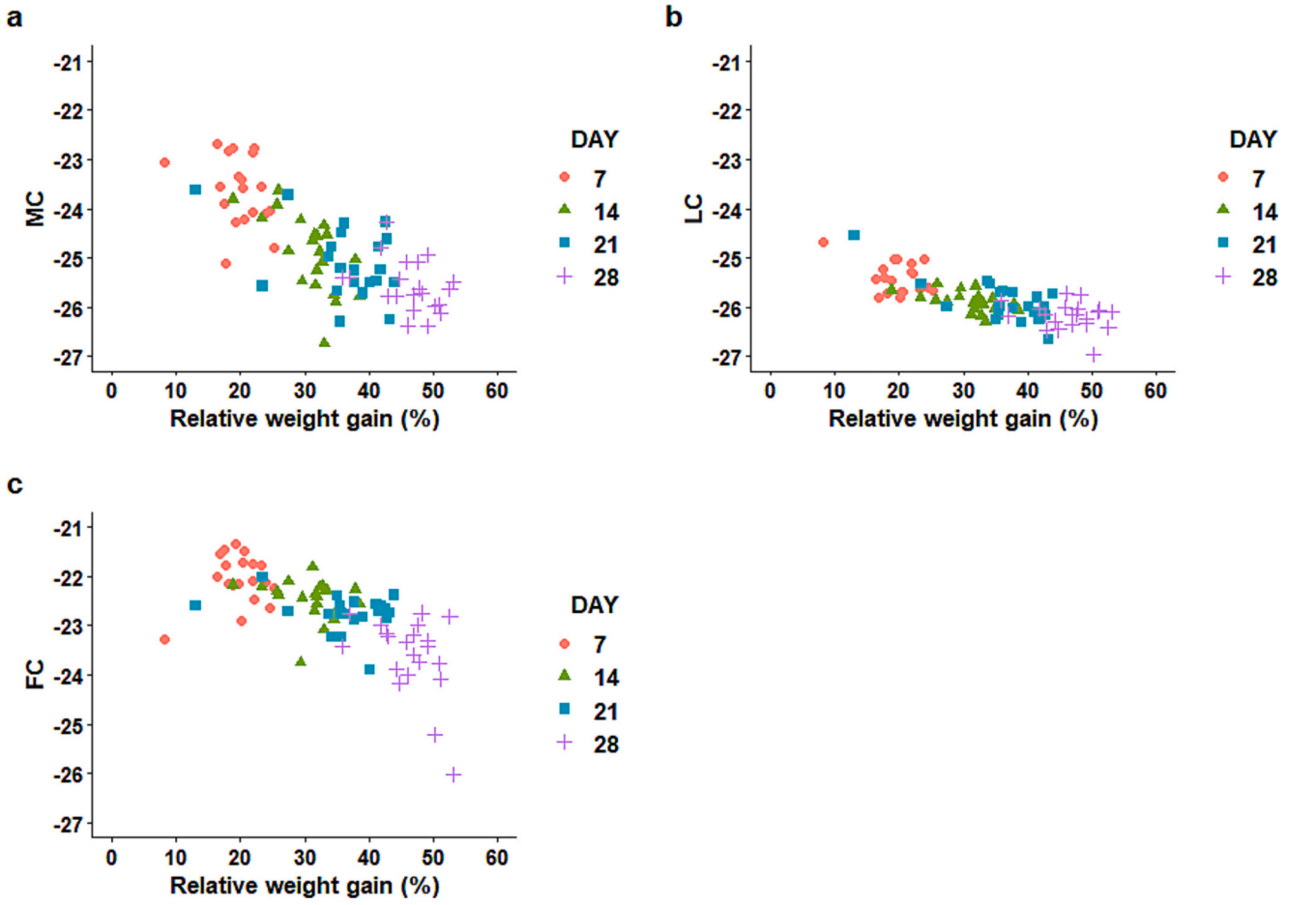


Fig. 1. Scatter plots of the relationships between $\delta^{13}C$ in a) muscle (MC), b) liver (LC), and c) adipose fin (FC) and relative weight gain over time ($n = 7$ at 7, 14, 21, and 28 days).

Table 3
Individual $\delta^{13}C$ in either muscle (MC), liver (LC), or adipose fin (FC) and estimated effects of sampling day, tank, and relative weight loss (RWL) under starvation in three univariate analyses of variance.

Trait	Fixed effect	$\widehat{b}\pm$ s.e.	*F*-value	*P*-value
	Intercept	−23.71 ± 0.89		
	Day 28	−1.69 ± 0.84		
	Day 35	0	4.04	0.05
	Tank 1	−0.36 ± 0.24		
	Tank 2	−0.14 ± 0.23		
	Tank 3	0	1.15	0.33
MC	RWL	0.20 ± 0.10	4.12	0.05
	Intercept	−23.64 ± 0.61		
	Day 28	−2.55 ± 0.57		
	Day 35	0	19.72	<0.0001
	Tank 1	−0.01 ± 0.16		
	Tank 2	0.03 ± 0.16		
	Tank 3	0	0.03	0.97
LC	RWL	0.22 ± 0.07	11.02	0.002
	Intercept	−22.01 ± 1.18		
	Day 28	−1.61 ± 1.11		
	Day 35	0	2.09	0.16
	Tank 1	−0.09 ± 0.32		
	Tank 2	0.15 ± 0.30		
	Tank 3	0	0.34	0.71
FC	RWL	0.12 ± 0.13	0.87	0.36

$\widehat{b}$: Estimated regression coefficient.

Table 4
Least-square means ± standard error of $\delta^{13}C$ in muscle (MC), liver (LC), and adipose fin (FC) by day of starvation.

	Day	
	28	35
MC	-26.32 ± 0.39^{a}	-24.62 ± 0.47^{b}
LC	-27.01 ± 0.26^{a}	-24.46 ± 0.32^{b}
FC	-24.06 ± 0.51^{a}	-22.45 ± 0.63^{a}

Significantly different ($P < 0.05$) when letters are different.

necessarily carbon) in the liver. With increased fish size and age, white muscle adiposity will increase in both rainbow trout and Atlantic salmon (Weil et al., 2013), and the strong relationship between RG and LC in this study might also be due to increased fat deposition in the muscle tissue (if fat has a lower isotope profile than protein). This agrees with the results of Dvergedal et al. (2019b); where LC was estimated with close phenotypic/genetic correlations to FCR (−0.73/−0.90) but with lower correlations to RG (0.19/0.12), suggesting that LC might explain additional variation in the feed efficiency complex related to deposition efficiency.

Since salmonids are routinely starved prior to slaughter, also to reduce the metabolic rate (Waagbø et al., 2017), a random sample of the fish were starved at the end of the experiment to investigate the effect of starvation on $\delta^{13}C$ in muscle, liver, and adipose fin. From the result obtained by Dvergedal et al. (2020), it was expected that a starvation period of only 7 days should not have a sizeable effect on the isotope profile in muscle, liver, and adipose fin. However, in this study, the

average RWL was relatively large (−8.5%, data not shown) and the least-square means of ^{13}C on day 35 relative to day 28 (after 7 days of starvation) became increased for both MC and LC (Table 4). However, note that the least-squares mean differences (Table 4) might have been somewhat scaled up because fish with the largest RWL can be assumed with the most negative $\delta^{13}C$ values before starvation, increasing the steepness of the estimated regression coefficient for RWL. This logic assumes that fish with high maintenance requirements when feeding is expected to have the largest maintenance requirements under starvation. As estimated, the largest increase was observed in the liver because it is an organ with high metabolic activity independent of the fish being fed or starved (Table 4): The $\delta^{13}C$ values increase in the tissues because ^{12}C compounds are being prioritized metabolically over ^{13}C (the lightest isotope is preferred; Gamboa-Delgado, 2021) to produce energy during starvation. This is the same process that drives isotopic fractionation, but starvation of the fish also avoids new nutrients to be supplied simultaneously. Therefore, the ratio between $^{13}C/^{12}C$ in the tissues is enhanced, and thus also $\delta^{13}C$ is increased (Table 4). The change in $\delta^{13}C$ in muscle (MC) and liver (LC) in this experiment occurred earlier than in the experiment reported by Dvergedal et al. (2020), this indicates that juvenile Atlantic salmon might, thus, be more resilient and able to withstand periods of starvation or feed deprivation compared to a larger rainbow trout (~200 g). The results suggest that the change in $\delta^{13}C$ during starvation needs to be considered when phenotyping the isotope-derived variables for individual feed efficiency in the field.

5. Conclusion

The finding of a strong association between growth rate (through increased efficiency) and $\delta^{13}C$ contents in both muscle and liver indicates that a diet with a lower natural abundance (compared to the baseline diet) can be used to phenotype feed efficiency. Also, our results suggest that the $\delta^{13}C$ in the muscle and liver under starvation is affected by the relative weight loss (relative to the weight when initiating starvation), most in liver.

Submission declaration

All authors read and approved the final manuscript, which has not been published or submitted for publication elsewhere.

Authors' contributions

H.D., J.Ø., T.F.G., S·S., M.J., M.Ø., and G.K. designed the experiment. S.S and M.J. formulated and produced the diet. H.D. conducted the experiment, the sampling, and prepared samples for stable isotope analysis. H.D. and G.K. conducted the statistical analysis. H.D. wrote the first draft of the manuscript.

Declaration of Competing Interest

The authors declare no interest.

Data availability

Data will be made available on request.

Acknowledgments

This study received support from AquaGen AS, BioMar AS, and Foods of Norway, a Centre for Research-based Innovation (the Research Council of Norway; grant no. 237841/O30). We thank Bjørn Reidar Hansen, Harald Støkken, and Ricardo Tavares Benicio, for help and assistance during the experiment.

References

Bahar, B., Monahan, F.J., Moloney, A.P., O'Kiely, P., Scrimgeour, C.M., Schmidt, O., 2005. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. Rapid Commun. Mass Spectrom. 19 (14), 1937–1942. https://doi.org/10.1002/rcm.2007.

Cano-Rocabayera, O., Maceda-Veiga, A., de Sostoa, A., 2015. Fish fins and scales as non-lethally sampled tissues for stable isotope analysis in five fish species of north – eastern Spain. Environ. Biol. Fish 98, 925–932. https://doi.org/10.1007/s10641-014-0328-6.

Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12, 133–149. https://doi.org/10.1016/0016-7037(57)90024-8.

de Verdal, H., Mekkawy, W., Lind, C.E., Vandeputte, M., Chatain, B., Benzie, J.A.H., 2017. Measuring individual feed efficiency and its correlations with performance traits in Nile tilapia, *Oreochromis niloticus*. Aquaculture 468, 489–495. https://doi.org/10.1016/j.aquaculture.2016.11.015.

Dvergedal, H., Ødegård, J., Mydland, L.T., Øverland, M., Hansen, J.Ø., Ånestad, R.M., Klemetsdal, G., 2019a. Stable isotope profiling for large-scale evaluation of feed efficiency in Atlantic salmon (*Salmo salar*). Aquac. Res. 50, 1153–1161. https://doi.org/10.1111/are.13990.

Dvergedal, H., Ødegård, J., Øverland, M., Mydland, L.T., Klemetsdal, G., 2019b. Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling. Genet. Sel. Evol. 51, 13. https://doi.org/10.1186/s12711-019-0455-9.

Dvergedal, H., Mydland, L.T., Klemetsdal, G., 2020. The change in ^{15}N stable isotope content in muscle, liver and mid-intestine in juvenile Atlantic salmon (*Salmo salar*) under starvation. Aquac. Res. 51, 5265–5268. https://doi.org/10.1111/are.14840.

Dvergedal, H., Ødegård, J., Galloway, T., Sharma, S., Juarez, M., Klemetsdal, G., 2022. Verifying the relationship between $\delta^{13}C$ isotope profile variables and individual feed conversion ratio in large rainbow trout (*Oncorhynchus mykiss*). Aquaculture 558, 738355. https://doi.org/10.1016/j.aquaculture.2022.738355.

Einen, O., Thomassen, M.S., 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar)* II. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. Aquaculture 169 (1–2), 37–53. https://doi.org/10.1016/S0044-8486(98)00332-9.

Einen, O., Waagen, B., Thomassen, M.S., 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar)* I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition. Aquaculture 166 (1–2), 85–104. https://doi.org/10.1016/S0044-8486(98)00279-8.

Fry, B., 2006. Isotope notation and measurement. In: Fry, B. (Ed.), Stable Isotope Ecology. Springer, New York, pp. 21–39.

Gamboa-Delgado, J., 2021. Isotopic techniques in aquaculture nutrition: state of the art and future perspectives. Rev. Aquac. 14, 456–476. https://doi.org/10.1111/raq.12609.

Gannes, L.Z., Martínez del Rio, C., Koch, P., 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 119 (3), 725–737. https://doi.org/10.1016/S1095-6433(98)01016-2.

Gjedrem, T., Baranski, M., 2010. Selective Breeding in Aquaculture: An Introduction. Springer Science & Business Media, Berlin.

Hayden, B., Soto, D.X., Jardine, T.D., Graham, B.S., Cunjak, R.A., Romakkaniemi, A., Linnansaari, T., 2015. Small tails tell tall Tales – intra-individual variation in the stable isotope values of fish fin. PLoS One 10 (12), e0145154. https://doi.org/10.1371/journal.pone.0145154.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139 (1), 157–163. https://doi.org/10.1016/0044-8486(95)01145-5.

Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209, 59–76. https://doi.org/10.1016/S0044-8486(01)00729-3.

Imsland, A.K.D., Roth, B., Christensen, L.B., Siikavuopio, S.I., Foss, A., 2020. Effects of short-term starvation periods on flesh quality in Arctic charr (*Salvelinus alpinus*) in different seasons. Aquac. Res. 51, 4022–4029. https://doi.org/10.1111/are.14745.

Kinghorn, B., 1983. Genetic variation in food conversion efficiency and growth in rainbow trout. Aquaculture 32, 141–155. https://doi.org/10.1016/0044-8486(83)90276-4.

Kolstad, K., Grisdale-Helland, B., Gjerde, B., 2004. Family differences in feed efficiency in Atlantic salmon (*Salmo salar*). Aquaculture 241, 169–177. https://doi.org/10.1016/j.aquaculture.2004.09.001.

Kristjánsson, Ó.H., Gjerde, B., Ødegård, J., Lillehammer, M., 2020. Quantitative genetics of growth rate and filet quality traits in Atlantic Salmon inferred from a longitudinal Bayesian model for the left-censored Gaussian trait growth rate. Front. Genet. 11, 573265 https://doi.org/10.3389/fgene.2020.573265.

Martin-Perez, M., Fernandez-Borras, J., Ibarz, A., Felip, O., Fontanillas, R., Gutierrez, J., Blasco, J., 2013. Naturally occurring stable isotopes reflect changes in protein turnover and growth in Gilthead Sea bream (*Sparus aurata*) juveniles under different dietary protein levels. J. Agric. Food Chem. 61 (37), 8924–8933. https://doi.org/10.1021/jf402617h.

McCleary, B.V., Solah, V., Gibson, T.S., 1994. Quantitative measurement of total starch in cereal flours and products. Cereal Sci. 20, 51–58. https://doi.org/10.1006/jcrs.1994.1044.

Rodde, C., de Verdal, H., Lefebvre, S., Menniti, C., Vandeputte, M., Clota, F., Nahon, S., 2020. Variations in isotope incorporation rates and trophic discrimination factors of carbon and nitrogen stable isotopes in scales from three European sea bass

(*Dicentrarchus labrax*) populations. J. Exp. Mar. Biol. Ecol. 533, 151468 https://doi.org/10.1016/j.jembe.2020.151468.

Shomorin, G.O., Storebakken, T., Kraugerud, O.F., Øverland, M., Hansen, B.R., Hansen, J.Ø., 2019. Evaluation of wedge wire screen as a new tool for faeces collection in digestibility assessment in fish: the impact of nutrient leaching on apparent digestibility of nitrogen, carbon and sulphur from fishmeal, soybean meal and rapeseed meal-based diets in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 504, 81–87. https://doi.org/10.1016/j.aquaculture.2019.01.051.

Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends Ecol. Evol. 19 (3), 148–154. https://doi.org/10.1016/j.tree.2003.12.003.

Thodesen, J., Grisdale-Helland, B., Helland, S.J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture 180, 237–246. https://doi.org/10.1016/S0044-8486(99)00204-5.

Thodesen, J., Gjerde, B., Grisdale-Helland, B., Storebakken, T., 2001. Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (*Salmo salar*). Aquaculture 194, 273–281. https://doi.org/10.1016/S0044-8486(00)00527-5.

Trueman, C.N., McGill, R.A.R., Guyard, P.H., 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). Rapid Commun. Mass Spectrom. 19 (22), 3239–3247. https://doi.org/10.1098/rspb.2014.0669.

Waagbø, R., Jørgensen, S.M., Timmerhaus, G., Breck, O., Olsvik, P.A., 2017. Short-term starvation at low temperature prior to harvest does not impact the health and acute stress response of adult Atlantic salmon. PeerJ 5, e3273. https://doi.org/10.7717/peerj.3273.

Weil, C., Lefèvre, F., Bugeon, J., 2013. Characteristics and metabolism of different adipose tissues in fish. Rev. Fish Biol. Fish. 23, 157–173. https://doi.org/10.1007/s11160-012-9288-0.