Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum)

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Abbreviations: \(b\), regression coefficient; \(BW\), body weight; \(CV_G\), coefficient of genetic variation; \(CV_R\), coefficient of residual variation; \(DFI\), daily feed intake; \(DG\), daily gain; \(DHA\), docosahexaenoic acid; \(EPA\), eicosapentaenoic acid; \(FCR\), feed conversion ratio; \(h^2\), heritability; \(HP\), high protein; \(LifeFCR_{\text{Indicator}}\), indicator of lifetime feed conversion ratio; \(LifeFI_{\text{Indicator}}\), indicator of lifetime feed intake; \(LifeRFI_{\text{Indicator}}\), indicator of lifetime residual feed intake; \(LifeProtRetention_{\text{Indicator}}\), indicator of lifetime protein retention; \(LifeLipRetention_{\text{Indicator}}\), indicator of lifetime lipid retention; \(LifeERetention_{\text{Indicator}}\), indicator of lifetime energy retention; \(NP\), normal protein; \(RFI\), residual feed intake; \(r_G\), genetic correlation; \(r_P\), phenotypic correlation; \(V_G\), genetic variance; \(V_R\), residual variance; \(\Delta G\), rate of genetic gain.
Abstract

In farmed fish, selective breeding for feed conversion ratio (FCR) may be possible via indirectly selecting for easily-measured indicator traits correlated with FCR. We tested the hypothesis that rainbow trout with low lipid% have genetically better FCR, and that lipid% may be genetically related to retention efficiency of macronutrients, making lipid% a useful indicator trait. A quantitative genetic analysis was used to quantify the benefit of replacing feed intake in a selection index with one of three lipid traits: body lipid%, muscle lipid%, or percentage of viscera weight of total body weight (reflecting visceral lipid). The index theory calculations showed that simultaneous selection for weight gain and against feed intake (direct selection to improve FCR) increased the expected genetic response in FCR by 1.50-fold compared to the sole selection for growth. Replacing feed intake in the selection index with body lipid%, muscle lipid%, or viscera% increased genetic response in FCR by 1.29, 1.49, and 1.02-fold, respectively, compared to the sole selection for growth. Consequently, indirect selection for weight gain and against muscle lipid% was almost as effective as direct selection for FCR. The fish with genetically low body and muscle lipid% were more efficient in turning ingested protein into protein weight gain. Both physiological and genetic mechanisms promote that low-lipid% fish are more efficient. The results highlight that in breeding programmes of rainbow trout, control of lipid deposition improves not just FCR but also protein retention efficiency. This improves resource efficiency of aquaculture and reduces nutrient load to the environment.
Introduction

Feed is one of the largest costs of aquaculture production, making the improvement of feed conversion ratio (FCR), the ratio of feed intake to weight gain, of great importance. Selective breeding programmes aim for the genetic improvement of farmed animals. To directly select for FCR, feed intake needs to be recorded, preferably from individual fish. However, fish are typically held in schools and fed together making the recording of feed intake on individual fish a major challenge. A potential alternative is to improve FCR by indirect selection for traits that are genetically correlated with FCR. To be successful, such indicator traits need to have a firm biological and physiological relationship with FCR.

Individually recorded feed intake or FCR is currently not selected in any fish breeding programme, and indirect ways of improving FCR may be an effective alternative. Lipid deposition is one potential indicator trait of FCR because in livestock, lean animals are typically more efficient in converting feed to tissue growth compared to fat animals. In farmed fish, there is some evidence that the control of lipid deposition can be used to genetically improve FCR. An additional benefit of controlling lipid is that lipid deposition in different body parts influences fillet quality and slaughter yield. In fish, lipid can be recorded non-destructively, making trait recording appealing.

Studies on the genetic improvement of FCR in large rainbow trout *Oncorhynchus mykiss* (Walbaum), marketed at body weight of 1.5-3 kg, will especially benefit from the assessment of FCR when fish are reaching market size. This is the time when most of the feed is consumed, and hence the time when most of the feeding costs are realized. Moreover, rainbow trout become less efficient as fish grow. Simultaneously this is the time when lipid deposition is at high level, again reflecting the potential link between lipid deposition and FCR.

We quantified the benefit of using lipid deposition as a genetic indicator trait to indirectly select for improved FCR in farmed rainbow trout. Feed intake of individual fish was recorded using the x-ray method in which feed pellets are enriched with glass ballotini beads, the x-ray of a fish revealing the amount of feed consumed. Specifically, the objectives were: 1) To estimate the genetic correlations of FCR with whole body lipid%, muscle lipid%, and percentage of viscera weight of total body weight (reflecting visceral lipid); 2) To quantify the expected genetic response in FCR when lipid recording (indirect selection) is used as the substitute of feed intake recording (direct selection) in a breeding programme. We tested the benefit of replacing feed intake by three alternative lipid traits: body lipid%, muscle lipid%, and viscera%. Finally, 3) we tested whether lipid deposition is genetically
related to the indicators of retention efficiencies of energy, protein and lipid. The retention efficiencies explicitly quantify the utilization of macronutrients and energy. A fish can produce protein growth only from protein (amino acids) in feed, and high quality proteins are among the most expensive raw materials in an aquafeed formulation, and often of a limited supply\(^\text{17}\). Hence, effective conversion of protein in feed into tissue growth is preferred. Lipid in feed is intended to be used especially as an energy source, and excessive levels of lipid deposition in tissues and viscera are not preferred.

**Material and methods**

**Experimental fish population**

The experimental fish originated from the Finnish national breeding programme and were housed at the fresh water nucleus station, Tervo Fish Farm, in central Finland. All procedures involving animals were approved by the animal care committee of the Natural Resources Institute Finland. To enhance animal welfare and ameliorate suffering during all fish handling, the fish were always first anaesthetized using tricaine methanesulphonate (MS-222).

The fish were from 210 families, produced from 89 sires and 109 dams. Each sire was mated to an average of 2.3 dams (range: 1-5) and each dam to 1.9 sires (range: 1-3). Matings were completed over three days in April 2001. For the first 8 months after hatching, the families were held separately in 150 L family tanks, each family in their own tank. The broodstock fish had been selected for high body weight, late maturity age, silvery skin, spotless skin and body shape for three generations\(^\text{18}\).

In February 2002, each family was randomly split into two groups to be reared on different experimental diets. The diets were a standard low protein and high lipid diet with protein levels of 44.9%, 44.6% and 39.5%, and with lipid levels of 30.5%, 30.3% and 33.4% for the pellet sizes of 3 mm, 6 mm and 7 mm, respectively (NP diet) . The other diet was an experimental high protein and low lipid diet with protein levels of 56.4%, 56.3% and 49.4%, and with lipid levels of 20.7%, 20.6% and 23.8% for the pellet sizes of 3 mm, 6 mm and 7 mm, respectively (HP diet) . The impact of diets on fish performance has been detailed previously\(^\text{19,20}\). The diets were originally used to test hypothesis that high protein diet would reveal the individuals that are the most efficient in utilizing proteins.

The fish were individually tagged to link the individuals to the pedigree and to allow for repeated measurements of individuals (Trovan Ltd., Köln, Germany). At tagging, fish weight at the two dietary groups was very similar (mean±SD; NP=62.4±19.9 g, \(n=1355\) fish, and HP=62.3±19.4 g, \(n=1335\)). During their growth until 29 months of age, some of the fish were destructively recorded for body
composition for a purpose other than the current study\(^{(20)}\). Hence, at the end of the experiment, there were 1262 fish remaining.

Each diet treatment was replicated by four 20m\(^3\) indoor tanks with fish density of 20 kg/m\(^3\). The families were equally distributed among the tanks. Feeding was automated using computer-controlled pneumatic feeders (Arvo-Tec Inc., Finland), and fish were fed to satiation 4 h a day. Water temperature during the experiment was natural and exposed to seasonal fluctuations.

**Feed utilization traits recorded**

Body weight, daily feed intake and daily weight gain were recorded three times during growth, in May 2002 (age 11 months, body weight 142.5g), October 2002 (age 16 months, body weight 747g), and September 2003 (age 27 months, body weight 2113g).

At each time, a 3-week x-ray session with 3 repeated measurements of body weight and daily feed intake was performed. Before x-raying, all fish from a given tank were fed to satiation 4h a day the same way as any other day but the diet was labelled with radio-opaque ballotini glass beads (Jencons Scientific Ltd., Leighton Buzzard, UK). The labelled pellets used at months 11, 16, and 27 consisted of 1, 0.5, and 0.3% beads, respectively, with a diameter of 400 to 600\(\mu\)m.

To record individual feed consumption with the ballotini enriched feed, fish were x-rayed using a portable x-ray unit (Todd Research 80/20, Essex, UK)\(^{(1)}\). Each of the 8 tanks was measured once weekly (one NP and one HP tank per day). To avoid the potential effects of systematic feeding rhythms, the recording order of NP and HP tanks was reversed on successive days. To initiate a recording session, all fish (x-ray and non-x-ray) were weighed during the first week of each session, and daily feed intake was measured from predetermined randomly selected individuals from each family (average of 6-2 fish per family; range 5-7). In the second and the third weeks, the procedure was repeated but only the fish x-rayed in the first week were reweighed and x-rayed again.

**Body composition traits recorded**

Three lipid traits were recorded at month 29, November 2003, at an average body weight of 2607g. All fish \((n=1262)\) from all 210 families were sampled for whole body lipid\%, muscle lipid\% and viscera percentage (100 visceral weight / body weight). Body weight recorded from all fish at month 29 was also used in the analysis and abbreviated as \(BW_{M29}\). Muscle and chop lipid\% and protein\% of each fish was determined using spectroscopy based on infrared transmission\(^{(21)}\), calibrated against analyses.
according to\(^{(22,23)}\). Muscle was sampled above the lateral line as a 10 g portion of pure epaxial white muscle. Chop was a 3-cm thick cutlet cut directly from behind the dorsal fin from each fish. Whole body lipid% was predicted using predictive equation having chop lipid%, head%, viscera%, and body weight as predictors. The \(R^2\) of the predictive equation was 0.62 and the residual standard error 1.156\(^{(20)}\). Body protein% was predicted in the same way, using chop lipid% and chop protein% as the predictors \(R^2 = 0.58;\) residual standard error = 0.505\(^{(20)}\). To minimize the possibility that the relation of feed utilization with body composition was due to correlative effects with body weight, the statistical models of body lipid%, muscle lipid% and viscera% had body weight at the time of trait recording as a fixed covariate.

The state of maturity (mature, immature) and gender (male, female) were visually recorded at all trait recording times. Males matured at 2, 3, or 3+ years, females at 3 or 3+ years, and there were also fish with unknown gender and maturity state.

Definition of feed utilization traits analysed

Feed utilization traits were calculated for two different time periods that are of great importance for producers of large rainbow trout. First, at month 27 (2+ years), four traits were calculated based on the 3-week x-ray trial: Average daily weight gain (DG) and average daily feed intake (DFI) based on the records measured across the 3 week period, and FCR = DFI / DG. In all statistical models, body weight at the beginning of the 3-week trial was used as a fixed covariate, to correct for the impact of body weight on DG, DFI and FCR. Residual feed intake (RFI), defined as the difference between the observed feed intake and the feed intake predicted from the maintenance costs (metabolic body weight) and growth, was used as a complementary measure of efficiency\(^{(24)}\). RFI is phenotypically independent of body size, and is typically considered superior over FCR when animals with different sizes are compared for feed utilization. For this reason, RFI has been included in the selection indices of many terrestrial livestock species\(^{(25)}\). RFI was calculated as the residuals from a regression in which metabolic body weight and DG were used as predictors of DFI\(^{(24)}\). Metabolic body weight at the beginning of the 3-week trial was calculated as BW\(^{0.824}\). A low RFI value indicates an efficient fish that feeds less than expected based on its observed growth and maintenance requirements.

Second, five indicators of feed utilization were calculated across the whole lifetime. An indicator of lifetime FCR was calculated as: LifeFCR\(_{\text{Indicator}} = \) Cumulative feed intake / Final body weight at month 29, where cumulative feed intake (LifeFI\(_{\text{Indicator}}\)) is the sum of all 9 daily feed intake records.
measured at months 11, 16, and 27. An indicator of lifetime residual feed intake (LifeRFI Indicator) was calculated, separately for each diet, as the residuals from a regression in which cumulative feed intake was regressed against metabolic body weight at month 16 (measure of average maintenance costs during the feed intake recording) and body weight at month 29 (measure of weight gain). For LifeRFI, the partial regression coefficients for BWM29 were 0.0064 and 0.0056 ($P < 0.0001$) and for metabolic body weight 0.0035 ($P = 0.32$) and -0.0052 ($P = 0.05$) with the $R^2$'s of 33.3% and 14.1% for the regression models on NP and HP diets, respectively. At the three separate ages, the partial regression coefficients for DG ranged between 0.2035-0.3391 ($P < 0.0001$) and for metabolic body weight 0.0017-0.0234 (all but one significant) with the average $R^2$ of 32.0% for the regression models (range in $R^2$ = 7.2% - 57.8%). Indicators of lifetime retention efficiencies were calculated for three components, protein (LifeProtRetention Indicator), lipid (LifeLipRetention Indicator) and energy (LifeERetention Indicator) as: Final component weight in a fish (in g) / Cumulative component intake (in g). For instance, LifeProtRetention Indicator = Final protein weight at month 29 / Cumulative protein intake. In this formula, the numerator trait is recorded from the egg stage onwards, whereas the denominator trait is recorded from average body weight of 142.5g onwards during 9 days. Hence, all these traits are called indicators and their mean value per se has no explicit interpretation. Energy content of a fish was calculated from its protein and lipid weights, assuming energy concentration of 23.6 kJ/g for protein and 39.5 kJ/g for lipid\textsuperscript{(25,26)}. Feed intake was transformed to intake of the components using the known crude proximate composition of the diets\textsuperscript{(19)}.

**Statistical analysis**

Phenotypic and genetic variances and correlations were estimated using the DMUAI software. The software analyses multivariate mixed models using the restricted maximum likelihood method, and accounts for all relationships between all animals in the pedigree using a relationship matrix\textsuperscript{(27)}. The pedigree had 362 ancestors in four generations for the offspring generation used in the experiment. The statistical model for DG, DFI, FCR, body lipid%, muscle lipid% and viscera% to estimate (co)variance components was:

$$y_{ijkl} = anim_i + ExpTank_j + DietSexMat_k + b_{BW_{Diet_l}} + \varepsilon_{ijkl}, \quad (model\ 1)$$
where \( \text{anim} \) is the random genetic effect of an animal \((i = 1...\text{number of observations})\), \( \text{ExpTank} \) is the fixed test tank effect \((j = 1-8 \text{ tanks})\), and \( \text{DietSexMat} \) is the fixed interaction of gender, maturity stage and diet \((k = 1-12 \text{ levels})\), \( b_{\text{BW}} \) is the fixed regression coefficient of body weight on \( y \), fitted separately for the two diets, \( \text{Diet}_l \) \((l = 1-2 \text{ diets})\). These body weight corrected traits are indicated by \([\text{BW}]\) symbol in the trait abbreviations.

For residual feed intake and all lifetime traits, no additional correction for body weight was needed, and hence the statistical model was:

\[
y_{ijk} = \text{anim}_i + \text{ExpTank}_j + \text{DietSexMat}_k + \varepsilon_{ijk}, \quad \text{(model 2)}.
\]

For all traits, models with the random full-sib family effect (without a link to a pedigree) were also run, to quantify the environmental effect common to full sibs. The full-sib family variance \( (V_{FS}) \) includes common environment effects due to separate rearing of the full-sib families until tagging, but also potential non-additive genetic as well as parts of maternal additive genetic effects. Most of the traits had negligible \( V_{FS} \) (see Results), and when including the family effect into the multitrait models, the genetic and full-sib family covariances were severely confounded in our data. Hence, for all traits, the correlations were estimated using models excluding the full-family effect.

Heritability was calculated as the genetic variance explained by the animal effect divided by phenotypic variance \( (V_P) \), where \( V_P \) is the sum of genetic \( (V_G) \), full-sib family \( (V_{FS}) \), and residual variance \( (V_R) \). Full-sib family variance ratio was calculated as \( c^2 = V_{FS} / V_P \). To assess whether a low heritability of a trait results from low genetic variation or from high residual variation, coefficients of genetic \( (CV_G = 100 \sqrt{V_G} / \text{trait mean}) \) and residual variation \( (CV_R = 100 \sqrt{V_R} / \text{trait mean}) \) were calculated for traits recorded in the units of grams. \( CVs \) are not sensible for percentages or ratios\(^{28} \).

Heritability was considered significantly different from zero if the \( h^2 \) estimate - 0.98 SE did not include zero (one-tailed hypothesis) . Genetic correlation was considered smaller or greater than zero if \( r_G \) estimate +/- 1.96 SE did not include zero (two-tailed hypothesis) .

**Comparison of alternative selection scenarios**

A deterministic simulation was performed with SelAction computer software\(^{29} \) to quantify the expected genetic response in FCR \( (\Delta G_{FCR}) \) when using alternative selection indices. The expected genetic response in FCR\([BW]\) was calculated, firstly, when simultaneously selecting for \( \text{DG}_{[BW]} \) and
against DFI\textsubscript{[BW]} (direct selection for FCR), and then this scenario was compared to the genetic responses obtained with the index in which feed intake was replaced either by body lipid\%\textsubscript{[BW]}, muscle lipid\%\textsubscript{[BW]} or viscera\%\textsubscript{[BW]} (indirect selection). Selection was based on breeding values estimated using individuals' own and its sibs' trait records\textsuperscript{(29)}. For each scenario, the relative index weighting of DFI\textsubscript{[BW]} or a lipid trait was increased from zero (selection for DG\textsubscript{[BW]} only) to unity (no selection for DG\textsubscript{[BW]}). FCR\textsubscript{[BW]} was not used in the simulation directly, rather the genetic response in FCR\textsubscript{[BW]} was calculated from the responses of DFI\textsubscript{[BW]} and DG\textsubscript{[BW]}.

The phenotypic and genetic parameters estimated using the model 1, without the full-sib family effect, were used as input. The simulated population structure was the same for all selection scenarios, to make sure the proportion of selected individuals remained the same across all scenarios. The population size was held small, to obtain realistic genetic responses in growth (around 4-10\% per generation\textsuperscript{(18)}). The population was a full-sib design with 100 selected sires and 100 selected dams, full-sib family size of 4 animals, and the proportion of selected animals was 0.50.

Results

Feed utilization at age of 2+ years of age

Genetic variation for feed utilization and body composition

For DG\textsubscript{[BW]}, DFI\textsubscript{[BW]}, FCR\textsubscript{[BW]} and the composition traits, full-sib family variance ratio ranged between 0.00-0.034, so for these traits it was safe to focus on the estimates from the model excluding the full-sib family effect (Table 1). DG\textsubscript{[BW]}, DFI\textsubscript{[BW]}, FCR\textsubscript{[BW]} and the composition traits recorded at 2+ years of age displayed significant heritabilities (Table 1). Heritabilities of feed intake and FCR ranged between 0.10-0.11. Heritabilities of lipid traits ($h^2 = 0.43-0.57$) were 4.3-5.7 times higher compared to the heritability of feed intake. Growth and feed intake both showed high coefficients of genetic variation, ranging between 17.2-17.4. Coefficient of residual variation was higher for feed intake than for growth, explaining the low heritability observed for feed intake. Residual feed intake displayed limited heritability, and when full-family effect was included in the model, the $h^2$ estimate was reduced to 0.04 with large SE (Table 1).

Relationship of feed utilization and growth
Daily weight gain, corrected for body weight, was phenotypically and genetically favourably correlated with FCR\textsubscript{[BW]} (Table 2). The faster growing fish were more efficient. The correlations between DG\textsubscript{[BW]} and RFI were close to zero, which results from the method to calculate RFI. The correlations of DG\textsubscript{[BW]} with DFI\textsubscript{[BW]} were moderately positive. High RFI was related to high DFI\textsubscript{[BW]}, i.e. the fish with overly high feed intake were inefficient. Similar but a weaker pattern was observed between FCR\textsubscript{[BW]} and DFI\textsubscript{[BW]}. Residual feed intake and FCR\textsubscript{[BW]} were highly positively correlated, implying they describe partly the same phenomenon (Table 2).

**Relationships of feed utilization and lipid traits**

The low body lipid\%\textsubscript{[BW]} and muscle lipid\%\textsubscript{[BW]} were both genetically related to low FCR\textsubscript{[BW]} and RFI, confirming the hypothesis that low-lipid% fish were genetically more efficient (Table 3). This results because DFI\textsubscript{[BW]} was positively, yet non-significantly, genetically related with body lipid\%\textsubscript{[BW]} and muscle lipid\%\textsubscript{[BW]}, whereas DG\textsubscript{[BW]} was weakly or even negatively genetically related to these lipid traits.

The genetic correlations of viscera\%\textsubscript{[BW]} with growth and feed utilization were of the opposite sign compared to those of body lipid\%\textsubscript{[BW]} and muscle lipid\%\textsubscript{[BW]}, and none reached significance (Table 3).

**Expected genetic responses**

The selection index calculations showed that selection solely for DG\textsubscript{[BW]} is expected to lead to +7.2% genetic increase in DG\textsubscript{[BW]}, +2.53% increase in DFI\textsubscript{[BW]}, and consequently to -4.36% change in FCR\textsubscript{[BW]}, i.e. improvement in FCR (Table 4).

Figure 1 was used to indentify the index weightings that maximize the expected genetic response in FCR in alternative selection index scenarios. When having DG\textsubscript{[BW]} and one of the alternative traits in the index, the index weighting that produced the greatest genetic response in FCR was -0.52 for DFI\textsubscript{[BW]}, -0.68 for BodyLipid\%\textsubscript{[BW]}, -0.70 for MuscleLipid\%\textsubscript{[BW]}, and -0.10 for Viscera\%\textsubscript{[BW]} (Table 4). Simultaneous selection for DG\textsubscript{[BW]} and against DFI\textsubscript{[BW]} (direct selection to improve FCR) increased genetic response in FCR\textsubscript{[BW]} by 1.50 fold to -6.54% compared to the sole selection for DG\textsubscript{[BW]} (Table 4). Yet, this occurred at the expense of genetic response in DG\textsubscript{[BW]} reducing from 7.2% to 4.83%.

Replacing DFI\textsubscript{[BW]} in the selection index by body lipid\%\textsubscript{[BW]}, muscle lipid\%\textsubscript{[BW]} or viscera\%\textsubscript{[BW]}, increased genetic response in FCR\textsubscript{[BW]} by 1.29, 1.49, and 1.02 fold, respectively, compared to the sole
selection for DG_{BW} (Table 4). Hence, using muscle lipid %_{BW} to indirectly select for FCR was
effective and simultaneously DG_{BW} improved by 5.93%. These results are in line with the positive
genetic correlations of muscle lipid%_{BW} with FCR_{BW} (and RFI) (Table 3).

**Lifetime feed utilization**

*Genetic variation for the indicators of lifetime feed utilization*

For the lifetime traits, $c^2$ estimates ranged between $0.037-0.065$, and in 3 out of 7 traits, the SE was
smaller than the $c^2$ estimate (Table 5). For these traits, the real heritability is likely to be between the
estimates obtained using the two models, one with and one without the full-sib family effect. Similar to
+2 years of age, the indicators of lifetime feed intake, FCR, residual feed intake and retention
efficiencies (Table 5) displayed lower heritability than growth and lipid traits (Table 1). Similar to the
traits in +2 age, the coefficient of genetic variation was of similar magnitude for BW_{M29} ($CV_G = 11.6\%$;
$CV_R = 15.5\%$) and LifeFI_{Indicator} ($CV_G = 12.7\%$; $CV_R = 40.3\%$), but coefficient of residual variation was
higher for LifeFI_{Indicator}, explaining the low heritabilities of LifeFI_{Indicator} (Table 5).

**Relationship of lifetime feed utilization and lipid traits**

Body weight at month 29 was phenotypically and genetically favourably correlated with
LifeFCR_{Indicator} (Table 6). The correlations of BW_{M29} with lifetime energy, lipid and protein retention
efficiency indicators were also favourably positive but with large standard errors.

The correlations of body lipid%_{BW}, muscle lipid%_{BW} and viscera%_{BW} with LifeFCR_{Indicator} and
LifeRI_{Indicator} had the same pattern as at +2 age, muscle lipid%_{BW} and body lipid%_{BW} having the
strongest correlations and viscera%_{BW} the weakest (Table 6). Decreasing muscle lipid%_{BW} was
genetically related to increased efficiency to use feed (both lifeFCR_{Indicator} and lifeRI_{Indicator}).

Decreasing muscle lipid%_{BW} was genetically related to improving lifetime protein retention
efficiency, and the phenotypic correlation of body lipid%_{BW} with LifeProtRetention_{Indicator} showed the
same trend (Table 6). The relationship between body lipid%_{BW} and muscle lipid%_{BW} with lifetime
lipid and energy retention indicators was weaker than with lifetime protein retention efficiency.
Discussion

Improving FCR via control of lipid deposition

Body composition was genetically related to the efficiency in which fish used feed. At +2 age, the lower body lipid% and muscle lipid% were genetically related to improved FCR and residual feed intake, confirming the hypothesis that fish with low lipid% are genetically more efficient. For the feed utilization indicators recorded across the whole lifetime until age of 29 months, the pattern was similar. The results highlight the benefit of controlling especially muscle lipid on the genetic improvement of FCR in rainbow trout. The index theory calculations showed that direct selection to improve FCR, via simultaneous selection for weight gain and against feed intake, is expected to decrease FCR by 1.50-fold ($\Delta G_{FCR} = -6.54\%$) compared to the sole selection for weight gain. There is hence room to improve FCR via methods other than growth selection. When feed intake is replaced in the selection index with muscle lipid%, such indirect selection results in maximum genetic response of $-6.50\%$ in FCR. These results are similar to the ones observed for the use of body lipid% to indirectly improve FCR in European whitefish *Coregonus lavaretus* L. Also in terrestrial livestock leaner animals are typically more efficient, and fat traits have positive genetic correlations with FCR.

In our selection index calculations, selection responses are determined by (co)variances of the traits. The efficiency of muscle lipid% as an indirect indicator to improve FCR results, firstly, because of the strong genetic correlation of muscle lipid% with feed intake, and a weaker correlation with weight gain. Selection against muscle lipid% will hence suppress feed intake more than growth, leading to improved FCR. High level of feed intake is likely related to high level of lipid deposition. Secondly, muscle lipid% has higher heritability than feed intake. Lipid traits in general are highly heritable in fish. Selection on a highly heritable trait is expected to result in higher genetic responses than selection for a low heritability trait. Hence, indirect selection for a highly heritable trait, like lipid traits, can be even more effective than direct selection. Feed intake and also FCR and retention efficiencies displayed low heritabilities compared to weight gain and BW. Daily feed intake is an unusually variable trait in fish. Additionally, recording of the long-term feed intake is a major challenge in fish. Using the x-ray method, only snapshots of fish behaviour can be recorded. In our data this is indicated by the very high residual variation for feed utilisation traits ($CV_R > 40\%$). The high residual variance reduces the heritability estimate, even though the genetic variation, measured as $CV_G$, in feed intake is of similar magnitude compared to growth.
In the current study, all lipid traits were recorded destructively, but fillet and muscle lipid can be recorded non-destructively in fish\(^{10,12,13}\). It is well established that the non-destructive methods can be effectively used to obtain realised genetic response in lipid traits in rainbow trout\(^{7,9}\), but the non-destructive methods are predictive tools that have measurement error and are not 100% accurate\(^{10,12,13}\). Hence, the use of non-destructive methods to record lipid will reduce the efficiency of indirect selection to improve FCR. Moreover, in line with a general finding\(^{31}\), in our study the genetic correlations were higher than the phenotypic correlations. This may be a real phenomenon, but additionally, genetic correlations may become biased when data set is small.

Naturally, lipid deposition should not be reduced to an extreme because lipid is essential for fish reproduction, lipid is an important source of healthy fatty acids for humans\(^{32}\), and lipid\% of tissues may have an intermediate optimum for product quality\(^{33}\). Similar to pigs\(^{34}\), to define the optimum lipid level would require the combined analysis of economics, biology and novel information on the genetics of the fatty acid profiles. Selection strategies should be further coupled with feeding practices to obtain the desired lipid and fatty acid levels in farmed fish.

It is reliable to use lipid deposition as a genetic indicator trait of FCR in a breeding programme because it has a physiological relationship with FCR. Assume two different fish, one with 17\% and the other with 25\% body lipid\%. For the time being, we can assume that body protein\% is the same 16\% for both fish, because in general, protein\% of tissues is both phenotypically and genetically very invariable in fish\(^{20,35,36}\). Lipid\% and water\% are inversely correlated in rainbow trout above 50 g\(^{14,35}\), and hence only lipid\% and water\% (with no energy value) differ between the two fish. Next, assume the two fish grow 1 g of weight and their body composition remains unchanged. The energy content needed for 1 g of growth for the low and high lipid\% fish are 10.5 and 13.7 kJ (assuming the energy concentration of 23.6 kJ/g for protein and 39.5 kJ/g for lipid). The cost of depositing different body components does not need to be taken into account because only lipid differs between the fish.

Assuming energy concentration of 20 kJ/g for feed and 50\% energy retention efficiency for both fish, the low and high lipid\% fish need 1.05 g and 1.37 g of feed to gain 1 g of weight. These are simply the FCR values of 1.05 for the low lipid\% fish and 1.37 for the high lipid\% fish because we assumed 1 g of weight gain, proving that decreasing body lipid\%, adjusted for fixed growth, is related to improved efficiency on wet weight basis. On the energy retention basis, the two fish were in fact equally efficient.
Above we assumed that body protein% remained invariable among individuals. It is noteworthy to consider the impact of protein deposition on the efficiency of low lipid% fish. In rainbow trout, genetic variation in body and muscle protein% seem to increase significantly, yet remain low, when fish obtain body weight of 2 kg\(^2\). The size which is of greatest commercial interest for producers of large rainbow trout. The increased genetic variation in protein% may be due to the extensive lipid deposition and the large increase in differences for lipid% between families at this age, forcing protein%, as a side effect, to vary\(^2\). Moreover, in our data, both body lipid% \((r_p = -0.57; r_G = -0.95 \pm 0.05)\) and muscle lipid% \((r_p = -0.33; r_G = -0.82 \pm 0.12)\) are phenotypically and genetically negatively correlated with the respective protein% trait. Hence, a low lipid% fish was in fact a high protein% fish.

One factor making lean animals more efficient is that deposition of protein induces more weight gain compared to deposition of lipid\(^{25,37}\). In fish, deposition of 1 g of lipid is associated with deposition of around 0.1 g of water. Deposition of 1 g of protein, in turn, is associated with deposition of over 3 g of water. Consequently, the deposition of 1 g of lipid is expected to lead to wet weight increase of 1.1 g (partial regression coefficient \(b_{lipid} = 1.1\)), whereas the deposition of 1 g of protein is expected to lead to 4-5 g wet weight gain \((b_{prot} = 4-5){}^{25,37, but see 38}\). The partial regression coefficients can be calculated from our data by regressing simultaneously both lipid and protein body weight (on x-axis) against final wet weight (y-axis). In line with the literature, our data have \(b_{lipid} = 1.45\) and \(b_{prot} = 4.24\) for NP diet \((n = 416\) fish), and \(b_{lipid} = 1.55\) and \(b_{prot} = 4.12\) for HP diet \((n = 482\) fish). Consequently, protein weight gain generally results in significantly more wet weight gain compared to lipid gain. This phenomenon facilitates that lean fish, with high protein weight gain, are more efficient, when efficiency is measured on wet weight basis.

However, depositing 1 g of protein \((59.9\) kJ / g of protein) is energetically more expensive than depositing 1 g of lipid \((55.3\) kJ/g and 43.5 kJ/g from non-lipid and lipid origins). These approximate values were calculated assuming energy concentration of protein and lipid of 23.6 kJ/g and 39.5 kJ/g, and net energy costs of 2.54, 1.4 and 1.1 kJ per kJ for protein and lipid retention from non-lipid or lipid origins, respectively\(^{39}\). The values that Emmans\(^{39}\) provides are calculated for terrestrial animals, but costs of protein deposition appear to be similar across terrestrial and aquatic animals, whereas costs of lipid deposition vary more\(^{39}\). The higher cost of protein deposition does not overrule the efficiency of protein deposition because the higher energy cost is small compared to the 4-5 fold effect on the increased wet weight gain.
Maximising genetic improvement in FCR reduces considerably the genetic response in weight gain, which may not be desirable (Fig. 1). Hence, the target of selection should be to obtain economically optimized balance between genetic changes in weight gain, feed intake and FCR, to make economically more efficient fish. This can be obtained by calculating economic values of the traits, e.g., by using bio-economic models\textsuperscript{(33,40)}.

Muscle lipid% but not viscera% was related to feed utilization. Visceral lipid is a major portion of viscera weight, and viscera% can be regarded as a lipid trait\textsuperscript{(11)}. Lipid deposits at different body locations are genetically different traits, and hence they are expected to have different correlations with other traits\textsuperscript{(20,41-43)}. Viscera% is easy to record in a breeding programme when sibs of breeding candidates are slaughtered, and selection against viscera% can be used to genetically improve fillet% and reduce slaughter waste, as is practiced in the Finnish breeding programme for rainbow trout\textsuperscript{(11)}. Unfortunately our data indicate no additional impact on improved feed utilization.

Getting around wet weight based traits: The retention efficiencies

The wet weight based traits like FCR, weight gain and body weight are traits important to fish farmers. Farmers that sell their fish to processors or directly to retailers are paid based on wet weight growth of fish, typically gutted weight. However, pelleted feed has low water concentration (2-10%) and fish ingest large amounts of water to obtain high body water concentration (70-80%). To directly assess the efficiency in which macronutrients and energy of the feed are used, the analysis of indicators of protein, lipid and energy retention efficiency was performed.

The results show that restricting excessive lipid deposition in a rainbow trout breeding programme improves protein retention efficiency. This is favorable for aquaculture because even a small improvement in protein retention efficiency has a large economic impact on the industry. High quality protein raw materials are among the most expensive components in an aquafeed formulation, and often of a limited supply\textsuperscript{(17)}. Moreover, protein is the source of nitrogen, and the more nitrogen from feed is deposited into a fish, the smaller the nutrient load to the environment will be per produced kg of fish.

In contrast to protein retention efficiency, the effective genetic improvement of lipid retention may be of less importance. In feed formulation, lipid is especially meant to be used as a major energy source for a fish, sparing protein to be used for tissue growth\textsuperscript{(44)}. Hence, improving lipid retention efficiency too much would make fish to allocate more of the ingested lipid to deposited lipid, which
may be unoptimal. Yet, the improvement of retention of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) n-3 fatty acids would be of importance as these are the main healthy components for humans. Moreover, fish need lipid deposits for basic life functions, and a suitable level of lipid is required in farmed fish for fulfilling standards of eating quality. Accordingly, the ultimate goal for both animal breeding and feed development would be a fish that optimally partitions different macronutrients between tissue growth and energy requirements.

The observation that the fish with genetically low body lipid% and muscle lipid% were more efficient in turning ingested protein into protein weight gain can be partly explained by the negative relationship between lipid% and protein%. The 'low lipid%-high protein%’ fish have high protein retention efficiency. Indeed, in our data, body protein%\(_{[BW]}\) is phenotypically and genetically related to improved indicator of lifetime protein retention efficiency \((r_p = 0.15; r_G = 0.81 \pm 0.32)\). Our findings are similar to the genetic responses observed when selecting for low and high muscle lipid%, corrected for body weight, lines in rainbow trout. The line with low muscle lipid% has improved feed efficiency and protein retention efficiency\(^{(7,9,45,46)}\).

Detailed studies on protein synthesis have revealed some of the mechanisms behind the highly efficient fish. The protein synthesis is costly, about 11-42% of energy expenditure\(^{(47)}\), and hence, fish which grow more efficiently achieve this through adopting the low-protein turnover strategy\(^{(48)}\). A reduction in protein turnover, brought about by lower degradation of synthesised proteins, leads to increased protein and wet weight growth efficiency. In this way, some individuals achieve faster and more efficient protein accretion when consuming the same amount of food as individuals with slower and less efficient growth\(^{(49)}\).

It is worth noting that our and the previous observations\(^{(7,9,45,46)}\) on among-individual variation differ from the results of diet comparisons. In contrast to our results, it is commonly found in diet comparisons that high lipid diet enhancing lipid deposition improves protein retention efficiency. This protein sparing effect occurs because the excess lipid in the diet fulfils the energy requirements of a fish, allowing the fish to allocate ingested protein for growth, and less to maintenance\(^{(44)}\). Naturally, effects of diets on a pair of fish traits do not need to be of the same direction as the phenotypic, and especially the genetic correlations between the same traits. For instance, the use of plant-based ingredients in feed can increase feed intake and decrease body lipid% compared to a fully fish-based diet, but simultaneously, within each diet, a fish with high feed intake can have high lipid%\(^{(8)}\).
Implications

In many fish species, lipid deposition is controlled in fish breeding programmes because of its impact on reduced slaughter waste, increased fillet% and quality\(^{(11)}\). The present and other studies\(^{(7-9,45,46)}\) contribute to the growing evidence that the control of excess lipid deposition by selective breeding programmes would bring an additional benefit of improving not just feed conversion ratio but also protein retention efficiency in fish.

Acknowledgments

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Fig 1. Expected genetic response in A) feed conversion ratio (FCR\_{BW}) and B) daily weight gain (DG\_{BW}) when selecting simultaneously for DG\_{BW} and against one of the alternative traits: DFI\_{BW} or one of the lipid traits.
Table 1. Sample size \((n)\), trait mean, phenotypic variance \((V_P)\), heritability and its standard error \((h^2 \pm SE)\), coefficients of genetic \((CV_G)\) and residual variation \((CV_R)\), and full-sib effect ratio \((c^2 \pm SE)\) for lipid traits and feed utilization traits recorded at +2 years of age estimated with an animal model either including or excluding the random full-sibs effect

<table>
<thead>
<tr>
<th>Trait*</th>
<th>Full-sib effect excluded</th>
<th>Full-sib effect included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Mean</td>
</tr>
<tr>
<td>DG([BW])</td>
<td>891</td>
<td>16.19</td>
</tr>
<tr>
<td>DFI([BW])</td>
<td>815</td>
<td>16.11</td>
</tr>
<tr>
<td>FCR([BW])</td>
<td>756</td>
<td>1.113</td>
</tr>
<tr>
<td>RFI</td>
<td>756</td>
<td>0.000</td>
</tr>
<tr>
<td>BodyLipid%([BW])</td>
<td>989</td>
<td>21.27</td>
</tr>
<tr>
<td>MuscleLipid%([BW])</td>
<td>998</td>
<td>7.700</td>
</tr>
<tr>
<td>Viscera%([BW])</td>
<td>1001</td>
<td>11.80</td>
</tr>
</tbody>
</table>

* Abbreviations: DG - daily weight gain; DFI - daily feed intake; FCR - feed conversion ratio; RFI - residual feed intake; BodyLipid\% - body lipid percentage; MuscleLipid\% - muscle lipid percentage; Viscera\% - viscera percentage of body weight; [BW] - A trait corrected for a constant body weight.

† Variance from the model 1 or 2 using which all the fixed effects have been removed.
Table 2. Phenotypic (above diagonal) and genetic correlations (below diagonal; ± their standard error) for growth and feed utilization traits recorded at +2 years of age*

<table>
<thead>
<tr>
<th></th>
<th>(\text{DG}_{\text{BW}})</th>
<th>(\text{DFI}_{\text{BW}})</th>
<th>(\text{FCR}_{\text{BW}})</th>
<th>RFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{DG}_{\text{BW}})</td>
<td>0.29</td>
<td>0.34</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>(\text{DFI}_{\text{BW}})</td>
<td>0.36 (0.25)</td>
<td>0.65</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>(\text{FCR}_{\text{BW}})</td>
<td>-0.63 (0.30)</td>
<td>0.36 (0.36)</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>RFI</td>
<td>-0.05 (0.29)</td>
<td>0.93 (0.042)</td>
<td>0.91 (0.10)</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 1.

Table 3. Phenotypic \((r_p)\) and genetic correlations \((r_g \pm \text{their standard error})\) between lipid, growth and feed utilization traits recorded at +2 years of age*

<table>
<thead>
<tr>
<th></th>
<th>(\text{BodyLipid%}_{\text{BW}})</th>
<th>(\text{MuscleLipid%}_{\text{BW}})</th>
<th>(\text{Viscera%}_{\text{BW}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r_p)</td>
<td>(r_g)</td>
<td>SEM</td>
</tr>
<tr>
<td>(\text{DG}_{\text{BW}})</td>
<td>0.14</td>
<td>-0.07</td>
<td>0.18</td>
</tr>
<tr>
<td>(\text{DFI}_{\text{BW}})</td>
<td>0.09</td>
<td>0.37</td>
<td>0.26</td>
</tr>
<tr>
<td>(\text{FCR}_{\text{BW}})</td>
<td>0.01</td>
<td>0.58</td>
<td>0.28</td>
</tr>
<tr>
<td>RFI</td>
<td>0.07</td>
<td>0.48</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 1.
Table 4. Expected maximum genetic response (ΔG) in growth, feed utilization and lipid traits in response to alternative selection index scenarios*

<table>
<thead>
<tr>
<th>Traits in a selection index*</th>
<th>ΔG (% of original trait mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG(BW)</td>
<td>7.20</td>
</tr>
<tr>
<td>DG(BW) - DFI(BW) (-0.52)</td>
<td>4.83</td>
</tr>
<tr>
<td>DG(BW) - BodyLipid%[BW] (-0.68)</td>
<td>6.09</td>
</tr>
<tr>
<td>DG(BW) - MuscleLipid%[BW] (-0.70)</td>
<td>5.93</td>
</tr>
<tr>
<td>DG(BW) - Viscera%[BW] (-0.10)</td>
<td>7.31</td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 1.

† Relative index weighting given in parenthesis.

Table 5. Sample size (n), trait mean, phenotypic variance (Vp), heritability and its standard error (h² ± SE), and full-sib effect ratio (c² ± SE) for lifetime traits estimated with an animal model either including or excluding the random full-sibs effect

<table>
<thead>
<tr>
<th>Trait*</th>
<th>Full-sib effect excluded</th>
<th>Full-sib effect included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>BW_M29</td>
<td>1262</td>
<td>2591</td>
</tr>
<tr>
<td>LifeFIIndicator</td>
<td>736</td>
<td>21.79</td>
</tr>
<tr>
<td>LifeFCRIndicator</td>
<td>692</td>
<td>0.845</td>
</tr>
<tr>
<td>LifeRFIIndicator</td>
<td>692</td>
<td>0.000</td>
</tr>
<tr>
<td>LifeERetentionIndicator</td>
<td>545</td>
<td>73.69</td>
</tr>
<tr>
<td>LifeLipidRetentionIndicator</td>
<td>545</td>
<td>124.2</td>
</tr>
<tr>
<td>LifeProtRetentionIndicator</td>
<td>545</td>
<td>48.76</td>
</tr>
</tbody>
</table>

* Abbreviations: BW_M29 - Body weight at month 29; LifeFIIndicator - Lifetime feed intake; LifeFCRIndicator - Lifetime feed conversion ratio; LifeRFIIndicator - Lifetime residual feed intake; LifeERetentionIndicator - Lifetime energy retention; LifeLipidRetentionIndicator - Lifetime lipid retention; LifeProtRetentionIndicator - Lifetime protein.

† Variance from the model 1 or 2 using which all the fixed effects have been removed.
Table 6. Phenotypic ($r_p$) and genetic correlations ($r_g \pm \text{SEM}$) for lifetime feed utilization and lipid traits*

<table>
<thead>
<tr>
<th></th>
<th>BW_{M29}</th>
<th>BodyLipid%_{(BW)}</th>
<th>MuscleLipid%_{(BW)}</th>
<th>Viscera%_{(BW)}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_p$</td>
<td>$r_g$ SEM</td>
<td>$r_p$</td>
<td>$r_g$ SEM</td>
</tr>
<tr>
<td>LifeFCR_Indicator</td>
<td>-0.15</td>
<td>-0.47 0.24</td>
<td>0.13</td>
<td>0.60 0.29</td>
</tr>
<tr>
<td>LifeRFI_Indicator</td>
<td>0.05</td>
<td>-0.04 0.27</td>
<td>0.09</td>
<td>0.29 0.28</td>
</tr>
<tr>
<td>BW_{M29}</td>
<td>na†</td>
<td>na†               na†</td>
<td>0.08</td>
<td>-0.19 0.17</td>
</tr>
<tr>
<td>LifeFI_Indicator</td>
<td>0.30</td>
<td>0.31 0.25</td>
<td>0.15</td>
<td>0.59 0.22</td>
</tr>
<tr>
<td>LifeERetention_Indicator</td>
<td>0.02</td>
<td>0.24 0.28</td>
<td>-0.04</td>
<td>-0.08 0.29</td>
</tr>
<tr>
<td>LifeLipidRetention_Indicator</td>
<td>0.04</td>
<td>0.24 0.27</td>
<td>0.01</td>
<td>0.03 0.27</td>
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<tr>
<td>LifeProtRetention_Indicator</td>
<td>-0.04</td>
<td>0.20 0.29</td>
<td>-0.18</td>
<td>-0.38 0.30</td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 1 and Table 5.
† Not estimable.
Fig 1. Expected genetic response in A) feed conversion ratio (FCR[BW]) and B) daily weight gain (DG[BW]) when selecting simultaneously for DG[BW] and against one of the alternative traits: DFI[BW] or one of the lipid traits.
Fig 1. Expected genetic response in A) feed conversion ratio (FCR[BW]) and B) daily weight gain (DG[BW]) when selecting simultaneously for DG[BW] and against one of the alternative traits: DFI[BW] or one of the lipid traits.