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 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 using individually recorded phenotypes and genotypes. 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 were 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. 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, and activity (Byerly, 1967; Gjedrem, 2005; Varley, 2009). In addition to growth, other traits (e.g., digestibility) potentially add 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.

2.3 Chemical analyses

The diet was dried and ground prior to analysis, and results of the chemical analyses are presented as an average of two samples (Table 1). The diet was analyzed for dry matter by drying to a constant weight at 104 ℃, for ash by combustion at 550 ℃, 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. (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 a PARR 1281 Adiabatic Bomb calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. Amino acids were analyzed according to Commission Regulation (EC) No 152/2009 on a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd,. Cambridge, UK), for all amino acids except tryptophan. Tryptophan analysis was performed according to Commission Regulation (EC) No 152/2009 using a Dionex Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) equipped with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Three replicates of the diet were homogenized, and to obtain enough feces per fish the four feces samples per fish were pooled, freeze-dried and homogenized, before analyses of nitrogen (N) and carbon (C) using a CHNS Elemental Analyzer (Vario El Cube elemental analyzer system GmbH, Hanau, Germany). The internal digestibility marker, Yttrium oxide (Y) (Metal Rare Earth Limited, Shenzhen, Guangdong, China), in diets and feces were determined by ICP-MS

(Agilent 8800 Triple Quadrupole mass spectrometer, Agilent Technologies Inc., Santa Clara, CA, USA). The samples were decomposed with concentrated ultrapure HNO_3 at 250°C using a Milestone microwave UltraClave III (Milestone Srl, Sorisole, Italy). The Y analyses were validated using certified reference material no. NIM-GBW07603 (National Analysis Centre for Iron & Steel, Beijing, China).

2.4 Calculation of apparent digestibility coefficients

Individual ADC was calculated as follows, using Y_2O_3 as the inert marker (Austreng et al., 2000) for both elements nitrogen and carbon:

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

where $a = \frac{\%\ element\ in\ feed}{\%\ Y_2O_3\ in\ feed}$, and $b = \frac{\%\ element\ in\ feces}{\%\ Y_2O_3\ in\ feces}$. The rationale behind the formula for ADC can be outlined as follows:

$$\frac{a-b}{a} = \frac{\frac{\%\ element\ in\ feed}{\%\ Y_2O_3\ in\ feed} - \frac{\%\ element\ in\ feces}{\%\ Y_2O_3\ in\ feces}}{\frac{\%\ element\ in\ feed}{\%\ Y_2O_3\ in\ feed}} = \frac{\%\ element\ in\ feed - \%\ element\ in\ feces \times \frac{\%\ Y_2O_3\ in\ feed}{\%\ Y_2O_3\ in\ feces}}{\%\ element\ in\ feed}$$

$$= \frac{\%\ element\ in\ feed - \%\ element\ in\ feces \times (1 - Digestibility)}{\%\ element\ in\ feed} \times \frac{g\ dry\ matter\ intake}{g\ dry\ matter\ intake}$$

$= \frac{g\ element\ consumed - g\ element\ in\ feces}{g\ element\ consumed}$. Hence, the ADC formula gives the expected fraction of digested nutrients.

2.5 Phenotypes analyses

After one week in the experiment, 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:

$$WG_i = FW_i - IW_i$$

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

From the feces samples, the apparent digestibility coefficient for nitrogen (ADC_N_i) and the apparent digestibility coefficient for carbon (ADC_C_i) were available at an individual level.

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.

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 the residual variance of the trait, for the pooled sample of four stripping's per fish. For each trait, the coefficient of phenotypic as well as a genetic variation (CV_P and CV_G, respectively) were calculated (e.g. Felix et al., 2012).

The significance of the genetic effect was tested using a likelihood-ratio (LR) test-statics, comparing a single-trait model with genetic effect (H_1) to a model without genetic effects (H_0) with the **G** matrix in H_1. LR was then calculated as:

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

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

All genetic analyses were performed using the ASReml4 software package (Gilmour et al., 2015).

3. Results

Descriptive statistics of the data are given in Table 2. Initial body weight for fish at the same age ranged 32.6-337.7 g. A large variation in IW, FW, WG, and RG was observed between families (Figure 1a-d). Even though the experimental period was relatively short (30 days) the growth was substantial, averaging 42% (relative to initial body weight).

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, give 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

236 estimated genetic correlation between ADC_N and ADC_C was positive and very high (fixed at
237 the boundary of 1.0 by the program), indicating that digestibility of nitrogen and carbon are
238 largely the same genetic trait.
239

240 ## 4. Discussion

241

242 The two growth traits, WG and RG, were as expected highly genetically correlated (Table 4).
243 The same result was obtained for the two digestibility traits, ADC_N and ADC_C (Table 4),
244 which was expected as protein was the main source of both nitrogen and carbon in the diet. The
245 apparent digestibility of nitrogen and carbon had a strong adverse genetic correlation to growth (-
246 0.77 ± 0.24 to -0.85 ± 0.16; Table 4). The negative genetic correlation might be explained by a
247 high positive correlation between growth rate and feed intake (0.98) in Atlantic salmon (Kolstad
248 et al., 2004). Low feed intake is associated with a reduced gastric evacuation time (Venou et al.,
249 2009), which leads to a longer time for digestion and absorption of nutrients, improving
250 digestibility (Aas et al., 2011; Adamidou et al., 2009). The effect of feed intake on gastric
251 evacuation time will likely depend on the size of the fish. The relative feed intake as a percentage
252 of body weight is likely more relevant than feed intake, as such. For this reason, both RG
253 (closely associated with the relative feed intake) and WG (closely associated with the absolute
254 feed intake) were included in the analyses.
255

256 Given a fixed average slaughter weight, genetically increased growth rate will reduce time to
257 slaughter, reduce the energetic cost for maintenance and thereby improve feed conversion ratio
258 (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 the 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) and also holds for larger fish in the saltwater phase, 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 was 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 the 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 $R^2 = 0.32$.

Figure 1.

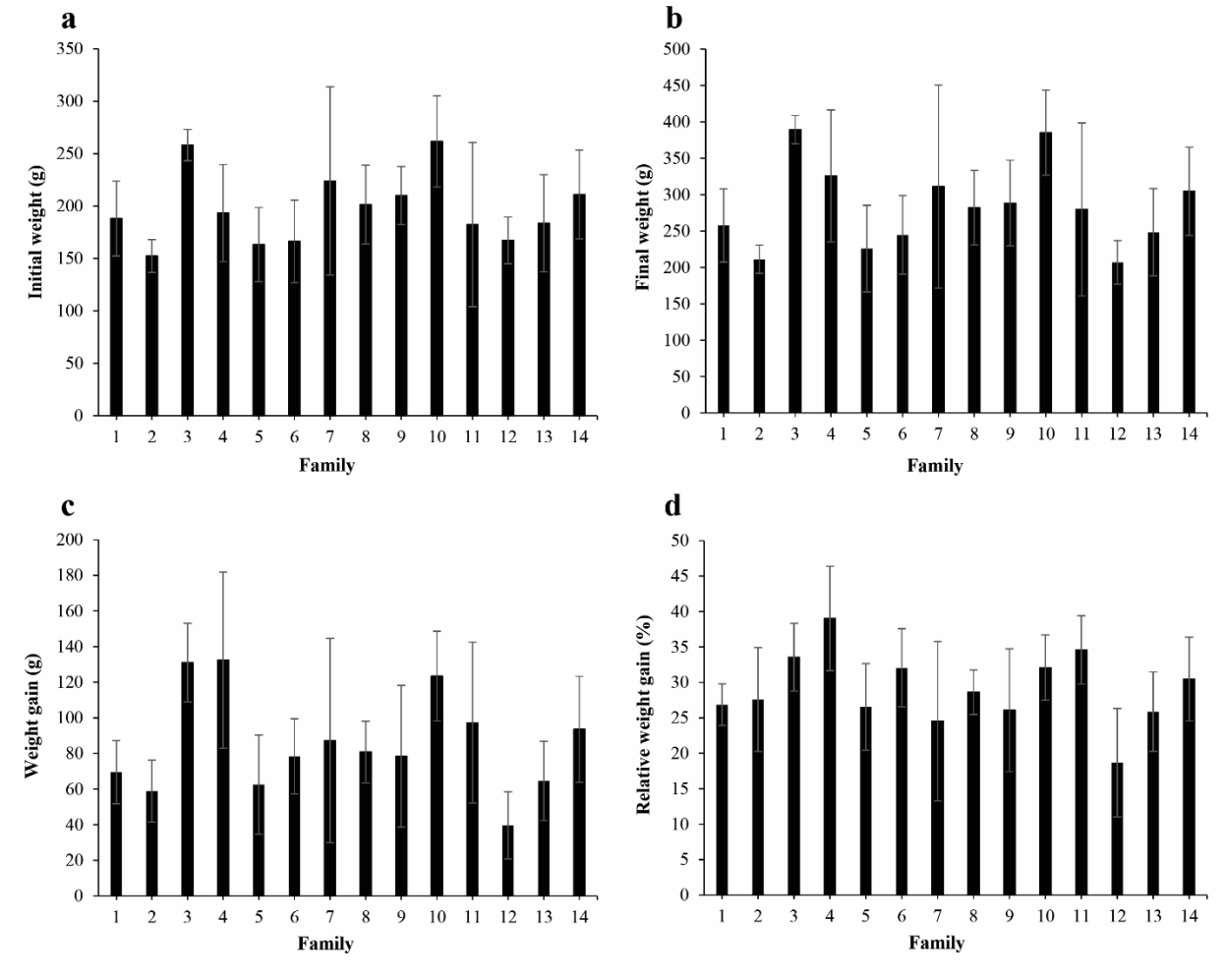
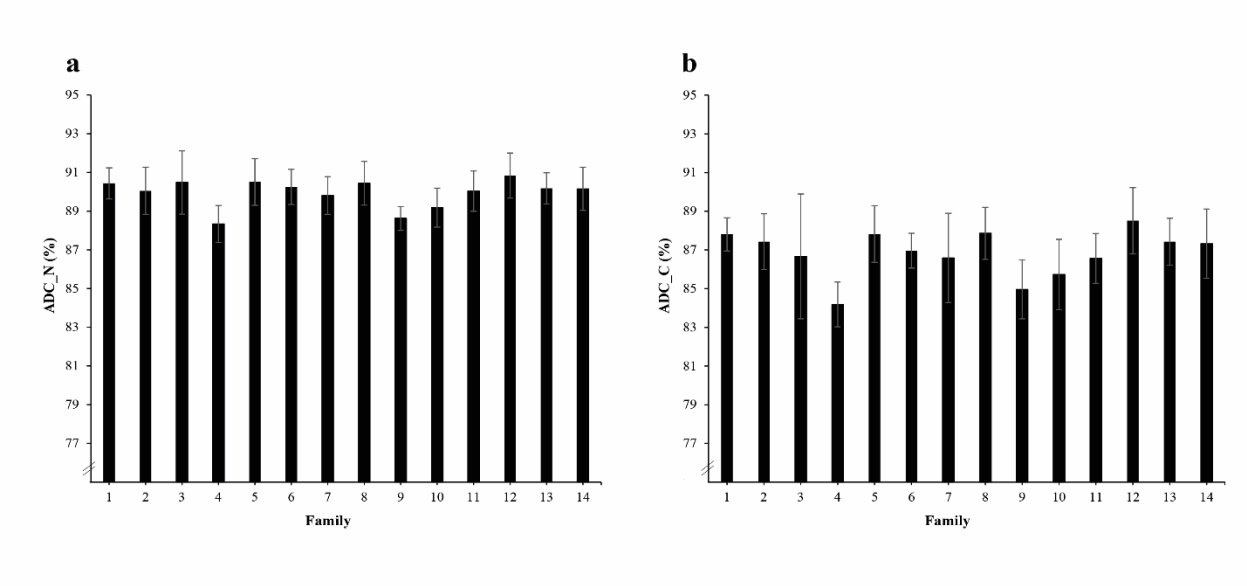
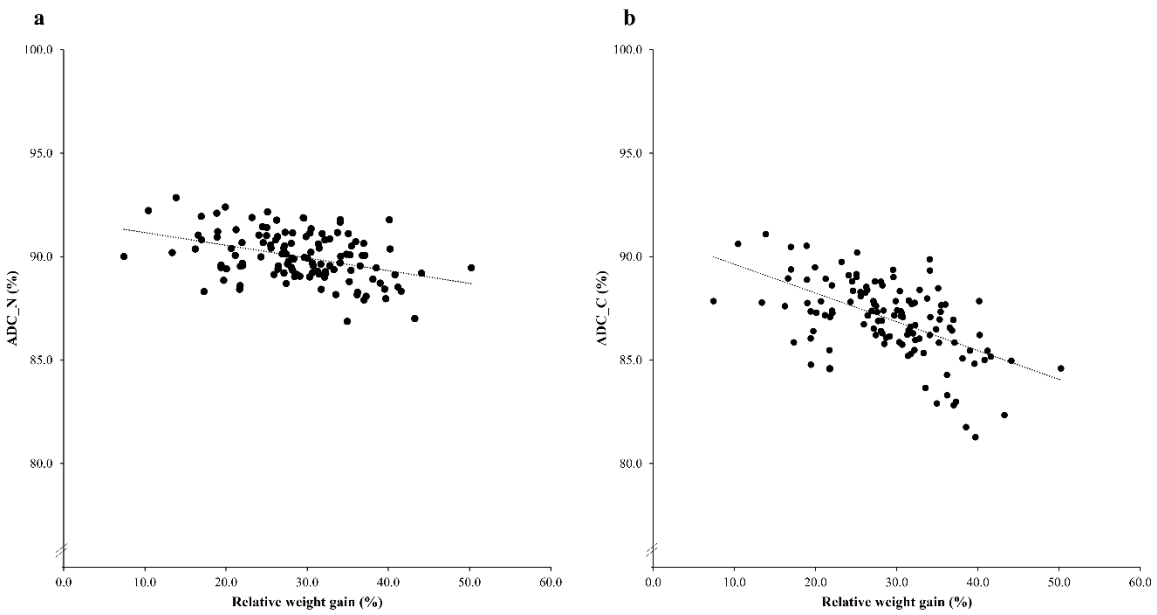

a
Initial weight (g)
Family
b
Final weight (g)
Family
c
Weight gain (g)
Family
d
Relative weight gain (%)
Family

Figure 2.


a
b
ADC_N (%)
ADC_C (%)
95
93
91
89
87
85
83
81
79
77
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Family

538 **Figure 3.**



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