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Selection of fatty acid composition in muscle of Atlantic salmon

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Summary

The replacement of fish oil and fishmeal with plant ingredients in the diet of farmed Atlantic salmon has reduced the levels of the health-promoting long chain omega-3 fatty acids (n-3 LC-PUFAs) EPA and DHA of their filets. Previous studies have shown that there is potential in using selective breeding as a tool to increase levels of n-3 LC-PUFAs in salmon tissues, but there is a lack of knowledge on the genetic parameters of individual muscle fatty acids and their relationships to other traits. In this study, genetic parameters of muscle fatty acid composition were estimated with the aim of evaluating the selection potential for increased n-3 LC-PUFA in Atlantic salmon muscle. The results showed that individual muscle FAs differed in heritability and correlations to other traits. The heritability of DHA was high (0.26), while EPA was lower (0.09). The content of EPA and DHA in the muscle was connected to body fat deposition, but in different ways; EPA had a positive correlation to muscle fat, while DHA had a high positive genetic correlation to visceral fat (0.61). DHA is the most abundant n-3 LC-PUFA in the salmon muscle, and was the FA with the highest heritability. An increased amount of DHA can be achieved by selecting for increased absolute content of DHA or DHA in percentage of total FAs, but they both have undesirable genetic correlations to different lipid related traits that must be considered.

Keywords: Atlantic salmon, omega-3 fatty acids, selection

Introduction

Farmed Atlantic salmon (*Salmo salar* L.) was traditionally fed diets rich in fish oil and fishmeal. Limited and decreasing availability of raw materials from wild fisheries has led to replacement of a large portion of the marine ingredients with plant based ingredients in aquaculture feed (FAO 2016). This has resulted in reduced levels of the health-promoting omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acids (DHA; 22:6n-3) of their filet, because of the low levels of these fatty acids in plant oils (Ytrestøyl et al., 2014).

Salmonids are capable of converting the shorter-chained fatty acid alpha-linolenic acid (ALA; 18:3n-3), commonly found in many plant oils, into EPA and DHA (Tocher, 2003), and there is evidence that genetics affect muscle FA composition in Atlantic salmon (Bell et al., 2010). Leaver et al. (2011) found high heritability of total n-3 LC-PUFA content of salmon

muscle. Selection for increased expression of genes encoding enzymes in this bioconversion pathway led to increased levels of DHA in liver of salmon (Berge et al., 2015). Hence, there is potential in using selective breeding as a tool to increase levels of n-3 LC-PUFAs in Atlantic salmon muscle. However, there is a lack of knowledge on the genetic parameters of muscle fatty acid composition, and their relationships to other traits of the breeding goals.

The objective of this study was to evaluate the selection potential for increased n-3 LC-PUFAs in Atlantic salmon muscle.

Material and methods

The data was based on recordings on 668 slaughter-sized Atlantic salmon from the commercial breeding population of SalmoBreed. The fish was fed a commercial broodstock feed with a fish oil ratio of 70 %. The fish came from 194 full sib families, originating from 92 sires and 194 dams.

The following recordings were made at the slaughter test: bodyweight (g), length (cm), sex, muscle pigment (by NIR imaging), liver fat (on a scale from 1-5), visceral fat (on a scale from 1-5).

Muscle samples (Norwegian Quality Cut, NQC) were collected at harvest, frozen and stored at −20 °C. Total lipids were extracted from homogenized muscle samples of individual fish (Folch et al., 1957). The FA composition of total lipids was analyzed (Mason & Waller, 1964). The proportional amount of each FA was expressed as a percentage of the total amount of FAs in the analyzed sample. The absolute content of DHA was expressed as grams per 100 grams of muscle.

Data from sea lice challenge tests of siblings of the analyzed fish was obtained from SalmoBreed AS. Lice density was calculated as lice count / bodyweight$^{2/3}$, following (Gjerde et al., 2011).

Data analysis

Variance and covariance components were estimated by residual maximum likelihood procedures using the ASReml Package (Gilmour et al., 2009). A univariate analysis was performed to estimate heritabilities for all traits. Bivariate analyses were performed to estimate (co)variances used to estimate genetic correlations. Variance components were estimated using the bivariate animal model: $\mathbf{Y} = \boldsymbol{\mu} + \mathbf{U} + \mathbf{E}$ (Henderson, 1984), where **Y** was a matrix of phenotypic records for individuals i=1, 2, …, n and traits j=1, 2 , $\boldsymbol{\mu}$ was a vector of the overall means for the two traits, **U** was a matrix containing the random effects of animal i on trait j, with variance $\mathbf{V} \otimes \mathbf{A}$, where **A** was the relationship matrix between individuals and **V** was a genetic variance – covariance matrix. **E** was random deviations, assuming to have a variance of .

For all FAs, pigment, visceral fat, liver fat and muscle fat, bodyweight and sex were included as fixed effects. For lice density, counting person and day were included as fixed effects, while cage was included as random effect.

Four generations of pedigree information on direct ancestors of the fish in the tests was available (n=11801).

Results and discussion

The fish in this study had an average body weight of 3.6 kg, and NQC fat content of 19 %. The average liver fat score was low/normal (2.13). The major FAs in the muscle were 18:1n-9

(30.5 ±0.7 %) and 18:2n-6 (9.7 ±0.7 %), as well as the saturated FA 16:0 (11.7 ±0.7 %). The mean content of EPA and DHA in the muscle was 5.42 ±1.01 % and 6.75 ±0.51 % of total FAs, respectively. These levels were quite high and reflected the high content of these FAs in the feed. The muscle content of individual fatty acids was approximately normally distributed (Data not shown).

The heritability of the n-3 FAs ranged from 0.09 to 0.26, showing that there is additive genetic variation (Table 1). In agreement with other studies (Bell et al., 2010; Leaver et al., 2011), the content of n-3 LC-PUFAs in the salmon fillet can thereby be increased through selective breeding.

Table 1. Heritability and genetic and phenotypic correlations for proportional content (% of total muscle FAs) of fatty acids in the bioconversion pathway.

Fatty acid	18:3n-3	20:3n-3	20:5n-3	22:5n-3	22:6n-3
18:3n-3	***0.26 (0.08)***	0.21 (0.04)	*	-0.15 (0.04)	-0.56 (0.03)
20:3n-3	-0.03 (0.28)	***0.18 (0.08)***	0.40 (0.03)	0.43 (0.03)	-0.06 (0.04)
20:5n-3	*	0.01 (0.42)	***0.09 (0.05)***	0.69 (0.02)	0.23 (0.04)
22:5n-3	-0.44 (0.23)	0.30 (0.25)	0.42 (0.26)	***0.22 (0.07)***	0.32 (0.04)
22:6n-3	-0.28 (0.22)	0.33 (0.28)	0.16 (0.34)	0.41 (0.21)	***0.26 (0.08)***

Heritability on the diagonal. Phenotypic correlations on the upper triangle. Genetic correlations on the lower triangle. Standard errors in brackets. *Parameters not converged.

The estimated heritability found in the current study were lower than that found by Leaver et al. (2011). This may be explained by differences in the age of the fish and diet. The fish in the current study was close to slaughter size and had been fed a high-FO diet, factors that reduce bioconversion (Tocher et al., 2003). However, the negative phenotypic correlation (-0.563) between ALA and DHA, as well as the positive correlations between FAs close in the bioconversion pathway, indicated active bioconversion of ALA to DHA (Table 1).

While comparable studies have looked at genetic parameters for the sum of several n-3 LC-PUFAs, our study showed that individual n-3 LC-PUFAs differ in heritability and correlations to other traits, and should therefore not be grouped together in a breeding program. EPA had favorable genetic correlations to body fat deposition; a higher proportion of EPA in the muscle was concurrent with higher amount of fat in the muscle (r_G=0.60 ±0.28), but less in liver (r_G=-0.31 ±0.41). DHA, on the other hand, showed no genetic correlation to muscle fat (r_G=0.01 ±0.21), and a positive genetic correlation to visceral fat (r_G=0.61 ±0.37). Although the genetic correlations have large standard errors, we see a pattern that contrasts with the corresponding correlations for the 18-carbon FAs.

From a breeder's perspective, the goal is to increase the content of n-3 LC-PUFA in the salmon fillet. EPA was the FA showing the most beneficial correlations to other traits and would therefore be the preferred FA to select for. However, the heritability of EPA was low (0.09). The many metabolic roles of EPA in the body may cause highly variable muscle content of this FA (Glencross et al., 2014), which in turn may explain the low heritability observed.

DHA was the most abundant n-3 LC-PUFA in the muscle, and showed the highest heritability (0.26). DHA therefore proved to be a better selection trait. An increased amount of DHA can be achieved in two ways;

1. Increase the absolute content of DHA. This would imply increasing muscle fat content, due to a genetic correlation between this trait and muscle fat of 0.90 ±0.01. The absolute amount of DHA is an important trait from the consumer perspective, and muscle fat is already another trait in the breeding goal, and can be dealt with similarly to other correlated traits.

2. Increase the proportional content of DHA. This trait was not correlated to muscle fat content, but highly correlated to visceral fat. However, visceral fat is also part of the breeding goal and can be dealt with similarly to other correlated traits.

Selection for higher proportion DHA in the muscle may cause disproportionally high distribution of DHA to the muscle, and may therefore affect the distribution of DHA to other tissues and organs. On the other hand, it may also lead to improved bioconversion capacity. Further analysis of enzyme activity, gene expression and FA composition of the other organs should provide greater understanding of the metabolic consequences of selection.

Conclusions

There is additive genetic variation in the FA-composition of salmon muscle, making it possible to change the muscle FA composition through selective breeding. Individual FAs play different roles in muscle lipid metabolism; they vary in heritability and correlations to other traits, and should therefore not be grouped together in breeding program. Two different selection strategies will increase DHA content in salmon muscle, but they both have undesirable genetic correlations to different lipid related traits that must be considered.

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