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In search for indirect criteria to improve feed utilization efficiency in sea bass (*Dicentrarchus labrax*): Part II: Heritability of weight loss during feed deprivation and weight gain during re-feeding periods

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Abstract:

Selective breeding to improve residual feed intake (RFI) in sea bass (*Dicentrarchus labrax*) is a major goal that would optimize economic gain while minimizing the environmental impact of production. Due to the difficulty in accurately measuring individual feed intake, no selective breeding program has yet been started. In a previous study, we identified a criterion phenotypically related to RFI variations: the loss of weight during feed deprivation, FD. Moreover, an additional composite criterion (CC) integrating both FD and weight gain during subsequent re-feeding (RF) was closely related to RFI, even though the relationship was only close to significance ($P = 0.06$). The aim of the present study was to estimate the heritability of these two traits, in order to complete the analysis of their pertinence as indirect criteria for use in a selective breeding program to improve RFI. We set up a full factorial mating design combining 41 sires and eight dams to produce mixed families offspring, which were all raised in the same tank. At 50 g, 1300 individuals were individually tagged and a fin clip was sampled for DNA extraction and parentage reassignment using five to six microsatellite loci, which showed they originated from 261 families. The evolution of individual body weight was recorded during a growth period of three weeks, followed by successive periods of three weeks feed deprivation and three weeks *ad libitum* re-feeding, repeated twice. Phenotypic and genetic correlations between the two feed deprivation periods or the two re-feeding periods were moderate ($r = 0.45–0.51$, and 0.71–0.73 respectively for phenotypic and genetic correlations) indicating that FD and RF are relatively repeatable. FD, RF and CC heritabilities were of the same magnitude (0.23 ± 0.04, 0.19 ± 0.04 and 0.22 ± 0.04, respectively), and sufficient to consider the use of such criteria in a future breeding program.

Keywords: European sea bass; *Dicentrarchus labrax*; Feed efficiency; Heritability; Genetic parameters; Residual feed intake; Feed deprivation; Compensatory growth; Selective breeding

1. Introduction

In fish, improving feed utilization efficiency is a crucial step toward reducing the cost and ecological impact of aquaculture production. Thanks to better knowledge of rearing methodologies and feeding, major progress has been done in the last 40 years in fish feed utilization efficiency. However, the possibility of enhancement by genetic means only starts to be explored (Kause et al., 2006b; Quinton et al., 2007a; Quinton et al., 2007b), while breeding programs in fish have been quite successful due to the moderate to high heritability of most production traits (Gjedrem, 2000).

In land vertebrates, residual feed intake (RFI) is the criterion commonly used to express feed utilization efficiency performance in breeding programs. It is calculated as the difference between actual and expected intake of an individual over a given weight gain interval. The expected intake is generally predicted from maintenance and production requirements which permit to select individuals using feed efficiently with no loss in production (Crews, 2005). Usually maintenance requirements are estimated from the metabolic body weight, and the production requirements from the body weight gains. This means that RFI is phenotypically independent from these two traits, which enables to compare feed utilization efficiency of individuals diverging for their body weight or their body weight gain. The part of feed utilization which is independent on body weight gain is precisely what we want to access, as all fish breeding programs incorporate selection for growth, which may (Thodesen et al., 1999; Kause et al., 2006b; Quinton et al., 2007b) or may not (Sanchez et al., 2001; Ogata et al., 2002; Mambrini et al., 2004) lead to improved feed utilization, depending on species and experiments. In any case, our objective is to find a trait independent from growth to allow further improvements in feed utilization efficiency. Recent experiments with rainbow trout, measuring the RFI on individually-raised fish (Silverstein et al., 2005), or using radiographically-labelled feed to estimate individual intake (Grima et al., 2008), revealed significant genetic variability in RFI. In fish, the lack of breeding strategies to improve RFI is explained by the difficulties of accurately estimating individual intakes (Kause et al., 2006a) and by the lack of knowledge regarding the genetic basis of residual feed intake.

Although RFI offers a potential new means of improving feed utilization efficiency to form the basis of future selective breeding programmes, indirect criteria must be found that are easily recorded and non-invasive. We recently identified a non-invasive criterion in rainbow trout (Grima et al., 2008) which is genetically correlated with RFI and can be easily measured under normal rearing conditions. It combines the weight loss during a feed deprivation period (FD) and the weight gain during a subsequent re-feeding period (RF). We tested this combined criterion because the underlying assumption is that its variation reflects both those of maintenance requirement (FD) and of protein and lipid turnover rates (RF). In sea bass, we constituted four divergent groups of fish according to their performance for this criterion. We estimated the RFI of these groups using the metabolic body weight and the body weight gain to predicted group's intake. These four groups presented phenotypic differences of RFI close to significance ($P = 0.06$ - Grima et al., 2010), but phenotypic differences of RFI between groups of fish constituted according to their FD divergences were significant ($P<0.05$). These two indirect criteria are therefore interesting candidates for selective breeding programs on feed utilization efficiency, provided that they are stable and heritable. The aim of the present study was to validate the relevance of FD and CC as indirect criteria by testing their heritability, their repeatability, and their suitability for use in breeding programs previously based on body weight.

Accurate estimation of heritabilities and genetic correlations is possible by the use of genomic markers for *a posteriori* parentage assignment, which enables the use of factorial designs producing a large number of families and the rearing of all the fish in a common environment from hatching, thus avoiding any environmental effect common to full-sibs (Vandeputte et al., 2001). Microsatellite markers are reliable tools to perform parentage assignment, since assignment rate higher than 95 % have been reached in several species

such as Atlantic salmon, common carp and sea bass (Dupont-Nivet et al., 2008; Norris and Cunningham, 2004; Vandeputte et al., 2004).

2. Materials and methods

2.1. Animals

The study was carried out on a full factorial mating design combining eight dams and 41 sires, with 328 possible full-sibs families. The breeders were caught from the wild (West Mediterranean). Sperm was collected in advance and cryopreserved in 250 ml straws according to the method described in Fauvel et al. (1998). Egg release was obtained by hand stripping following hormonal induction of ovulation (10 µg/kg luteinizing hormone-releasing hormone, Sigma, D-TRP6LHRH). A fin clip was collected from all parents and kept in 90% ethanol for DNA analyses and parentage assignment. Fertilization was performed as soon as the eggs were stripped. Eggs from all dams were pooled by mixing an identical volume of eggs from each dam (180 ml), and the pooled eggs were then divided into 41 aliquots of 20 ml each. Each aliquot was individually fertilized by a single sire. Five minutes after fertilization, eggs were pooled for incubation (48 hours at 14°C), after which 50 ml of viable eggs (~ 40,000) were collected and placed in a single 0.5 m^3 incubator containing all families. Standard rearing conditions were used, with temperature gradually increased from 14°C to 20°C over the first 68 days, after which fish were transferred into a 5 m^3 fiberglass tank until the start of the experiment. Salinity varied between 24 and 39 $g.l^{-1}$ during this pre-growing phase.

2.2. Measurements of indirect criteria

At day 306 post fertilization, 2,000 randomly chosen fish were individually tagged with a Passive Integrated Transponder (AEG-Id, Germany) and placed in a common fiberglass tank (volume: 5 m^3) within a recirculated system, where water temperature was recorded daily and maintained around 20°C. The salinity was recorded daily as well, and was 37 $g.l^{-1}$ on average. After an initial four weeks period of basic growth (BG: from day 341 to day 370), fish were submitted to two successive periods of three weeks feed deprivation (FD1: from day 371 to day 392, and FD2: from day 415 to day 436, respectively), alternated with two successive periods of three weeks re-feeding (RF1: from day 393 to day 414, and RF2: from day 437 to day 458, respectively). During basic growth and re-feeding periods, fish were fed *ad libitum* using a 24h-access self feeder with a standard commercial diet (Neogrower, Le Gouessant, France) containing 45% proteins and 17% lipids. Fish were individually weighed at the beginning and end of each experimental period. Before each body weight measurement, all fish were starved for 24 hours. The fish were re-fed the day after the measurements, except during the two periods of feed deprivation.

2.3. Calculation of indirect criteria

Different calculation methods might be used to characterize the loss of body weight during feed deprivation periods, FD, and the gain of weight during re-feeding periods, RF. In the present we used i) the thermal growth coefficient (TGC), this growth rate offering a standardized measure of growth that is unaffected by live weight, time interval and water temperature (Iwama and Tautz, 1981) ii) the corrected values of TGC used in the companion paper to study the relationship between RFI, FD and RF (Grima et al 2010). Calculation details of these two criteria are given below.
TGC values were calculated using the following equation:

Thermal growth coefficient (TGC) = $\frac{(W_f^{1/3} - W_i^{1/3})}{\sum T} \times 100$

where W_f and W_i are the final and initial body weight, of the period considered, and $\sum T$ is the sum of day degrees during this period. Growth rates for the different periods will be referred to as TGC_{BG}, TGC_{FD1}, TGC_{RF1}, TGC_{FD2}, and TGC_{RF2}.

Body weight gain and loss during feed deprivation and re-feeding periods are closely linked to individual growth performances. Because we want to characterize the part of the relationship between RFI and FD or RF which is independent of growth performance, we corrected TGC_{FD} and TGC_{RF1} values by growth performance (Grima et al. 2010). Moreover, as fish lose proportionally less weight during deprivation periods than they gain during re-feeding periods, the corrected TGC values were standardized (mean = 0; SD = 1) to give as much importance to weight loss as to weight gain. In practice, TGC_{FD} and TGC_{RF1} (for time schedule reasons, TGC_{RF2} could not be included in the calculations) were each regressed on TGC_{BG} and BW_{341} in a multiple linear regression. The residuals of the regressions were standardized and their values (FD_{corr} and RF_{corr}) were used to used to select fish for RFI studies described in Grima et al. (2010).
We also calculated a composite criterion (CC), by subtracting RF_{corr} to FD_{corr} ($CC = RF_{corr} - FD_{corr}$), so that the most divergent fish for CC were also the most divergent ones for RFI according to the results of the companion paper (Grima et al, 2010).

We considered both uncorrected (TGC_{FD1}, TGC_{RF1}, TGC_{FD2}, and TGC_{RF2}) and corrected (FD_{corr} RF_{corr}, and CC) values of TGC as potential indirect criteria for RFI. In futher parts of the paper, unless otherwise stated, FD refers to all criteria describing weight loss during feed deprivation, namely TGC_{FD1}, TGC_{FD2} and FD_{corr}, while RF refers to all criteria describing growth during re-feeding, TGC_{RF1}, TGC_{RF2} and RF_{corr}, while CC always refers to $RF_{corr} - FD_{corr}$.

2.4. Parentage assignment

After measuring the indirect criteria, 1,300 fish out of the 2,000 were randomly chosen to be assigned to their respective parents through microsatellite DNA analysis. A fin clip was taken from each of these 1,300 individuals.
Rapid DNA extractions were made from the fin clips, using Chelex resin as described in Estoup et al. (1996). Parents and offspring were assayed at five or six microsatellite loci as described in Chistiakov et al. (2005) and Garcia De Leon et al. (1995). Four loci were combined in two multiplex panels (multiplex 1: DLA0016 and DLA0014; multiplex 2: Labrax 8 and Labrax 17) while the locus Labrax 29 was assayed singly. Parentage assignment was performed by exclusion using VITASSIGN (Vandeputte et al., 2006). When more than one parent pair was found per individual, the locus Labrax 3 was used to discriminate the potential families. At the end of this process, 99.5% of the fish sampled (1,294/1,300) had been successfully and unambiguously assigned. The 1,294 assigned fish belonged to 261 out of the 328 possible full-sib families. The number of offspring per sire varied between three and 83, and was 31 on average. The number of offspring per dam varied between 10 and 410, and was 162 on average.

2.5. Statistical analyses

The variance components of all TGCs, BW_{340}, FD_{corr}, RF_{corr} and CC were assessed on the 1,294 assigned fish using VCE5.0 software Kovac and Groeneveld, 2003, fitting animal models (without fixed effects) to estimate heritabilities and additive genetic correlations. In order to ensure that maternal effect did not strongly affect genetic parameters estimations, we first tried animal models with a random maternal effect in VCE, but they did not converge.

Then, we performed analyses of variance on all the corrected and uncorrected TGCs with sire and dam as random effect, and we estimated the maternal effect using $m^2=(\sigma^2_d-\sigma^2_s)/\sigma^2_P$, with σ^2_d the dam component of variance, σ^2_s the sire component and σ^2_P the phenotypic variance. Although these estimates cannot be considered reliable as the number of dams (8) is low, their value was low enough (0.01- 0.05) to decide that they could be excluded from the analysis. Variance components of BW_{340}, TGC_{BG}, TGC_{FD1}, TGC_{RF1}, TGC_{FD2}, and TGC_{RF2} were tested in the same multi-trait animal model, to take into account the fact that they were issued from repeated body weight measurements of the same fish. Heritabilities of FD_{corr}, RF_{corr} and CC were estimated using single-trait animal models. To estimate genetic and phenotypic correlations between those three traits and basic growth, body weight and the uncorrected TGCs, we used mutiple-traits animal models. However, not all of the uncorrected TGCs could be included in a single model together with FD_{corr}, RF_{corr} and CC because of convergence problems. Therefore, different combinations of traits ensuring convergence were used.

3. Results

By the end of the experiment, mortality was only 2%, meaning that fish underwent food shortage with no particular problems.

3.1. Correlations between indirect criteria

The two periods of feed deprivation were genetically and phenotypically correlated, as were the two periods of re-feeding. All the correlations were moderate ($r_g = 0.73$; $r_p = 0.45$ and $r_g = 0.73$; $r_p = 0.51$ for the feed deprivation periods and the re-feeding periods, respectively).
Genetic and phenotypic correlations of CC with FD_{corr} or with RF_{corr} were of comparable magnitude ($r_g = -0.83$ with FD_{corr}, 0.75 with RF_{corr} and $r_p = -0.82$ with FD_{corr} and 0.78 with RF_{corr}), showing that CC integrates these two traits to the same degree (Table 1). Genetic and phenotypic correlations between CC and the uncorrected TGCs were high to moderate, negative for TGC_{FD1} and TGC_{FD2}, and positive for TGC_{RF1} and TGC_{RF2}. Then genetic correlation between TGC_{FD1} and TGC_{RF1} was moderate (-0.32), and the genetic correlation between TGC_{FD2} and TGC_{RF2} was low (-0.19), while the genetic correlation between FD_{corr} and RF_{corr} was in-between ($r_g = -0.25$).

3.2. Heritability of indirect criteria

Estimates of additive genetic and environmental variances are presented in Table 2, with the heritabilities of the traits. The heritability estimated for BW_{341} was moderate ($h^2 = 0.39$). Heritability estimates for uncorrected TGC were low to moderate, ranging from 0.13 for TGC_{FD1} to 0.38 for TGC_{RF2}. TGC measured during feed deprivation periods displayed lower heritability than TGC measured during re-feeding periods. Heritabilities of TGC were slightly lower during the first round of feed deprivation / re-feeding (TGC_{FD1} and TGC_{RF1}) than during the second round of feed deprivation / re-feeding (TGC_{FD2} and TGC_{RF2}). This did not result from a decrease in the residual variation (σ^2_E remained stable between the two feed deprivation periods and increased from 0.0035 to 0.0040 between the first and the second re-feeding periods), but from an increase in genetic variance. The standard errors of all estimated heritabilities were low (0.03-0.04), demonstrating the accuracy of the estimates and the relevance of the data set.

3.3. Genetic and phenotypic correlations between body weight and indirect criteria

Genetic and phenotypic correlations of the indirect criteria with the body weight are presented in Table 1. Almost all the TGCs were phenotypically correlated with BW_{341} (except

TGC_{FD2} for which r_p = -0.08). In addition, TGC_{RF1} and TGC_{RF2} were significantly genetically correlated with BW_{341} (r_g = 0.57 and 0.56, respectively).
Phenotypic correlations of BW_{341} with FD_{corr}, RF_{corr} and CC were very close to zero, as expected from adjusting BW_{341} in the calculation of FD_{corr} and RF_{corr}. However, genetic correlations of BW_{341} with FD_{corr} (0.26) and RF_{corr} (0.29) were significantly different from zero, while the genetic correlation of BW_{341} with CC was equal to zero.

4. Discussion

In fish, despite the important economic and environmental potential impact of improved residual feed intake (RFI), no selection program to improve RFI has started yet because this trait is highly sensitive to environmental variations and because individual feed intake is difficult to measure. Indirect criteria on which selection could be targeted are therefore of highest interest for the initiation of a future breeding program for this trait. In previous studies carried out on rainbow trout (Grima et al., 2008) and on sea bass (Grima et al., 2010) we highlighted that the loss of weight during feed deprivation (FD), alone or as part of a composite criterion (CC) integrating FD and the gain of weight during re-feeding (RF), is significantly related with RFI variations. The aim of the present study was to estimate the heritability of these two traits, in order to complete the analysis of their pertinence as indirect criteria for use in a selective breeding program to improve RFI. We used a common tank to raise offspring of Mediterranean sea bass produced from a full factorial mating design with wild breeders (eight dams and 41 sires). Among the 328 possible families, 261 were present in the sample genotyped and were therefore genetically analyzed. Two types of criteria were used to characterize FD and RF. First, we expressed the loss of weight during feed deprivation periods and the subsequent gain of weight during re-feeding periods using the thermal growth coefficient (TGC). Second, we used the corrected values of TGC (corrected by the body weight and the growth performances, and standardized (mean = 0, SD= 1)) calculated in the companion paper (Grima et al. 2010), because these were the ones we used to demonstrate the significant relationship between RFI and FD and CC.
All traits presented low to moderate, but significant, heritability. These heritability values mean that both corrected and uncorrected TGC values present enough genetic variability to be used in a breeding program. The heritability of the CC was also significantly different from zero. In addition, the detection of substantial heritability of $TGC_{FD1\&2}$ and $TGC_{RF1\&2}$ provided the first evidence of significant heritability in FD and RF in fish. Genetic variability of these traits had already been demonstrated in rainbow trout, but with an experimental design that did not allow heritability estimations (Grima et al., 2008). Both in rainbow trout and sea bass, FD presented lower genetic variance than RF. With our experimental design (eight dams crossed with 41 sires), it was not possible to reliably estimate maternal effects variance, due to the limited number of dams used, and then we only studied the additive genetic variance. However, we verified that there was no evidence for large maternal effects, and additionally it has been shown previously that, in marine fish, maternal effect have little influence on growth (Saillant et al., 2006; Dupont-Nivet et al., 2008), especially when fish are getting older (Doupe and Lymbery, 2005).
As there are no heritability estimates for FD and RF in the literature, we could not compare our heritability estimates with previous data. Nevertheless, we did compare them with existing heritability estimates for growth. Our heritability estimations for FD varied between 0.13 and 0.24, which is in the lower end of heritabilities for growth rates found in the literature: 0.04 to 0.26 in Atlantic Salmon (Gjerde et al., 1994), 0.26 ± 0.12 in rainbow trout (Kinghorn, 1983), and on average 0.35 in turbot (Gjerde et al., 1997). Our heritability estimates for RF varied between 0.19 and 0.38 and are therefore more in the same range as those found in the literature. Surprisingly, the heritability of initial growth (TGC_{BG}) in our study was found to be lower (h^2 = 0.19 ± 0.03) than the estimates for TGC_{FD2}, TGC_{RF1} and TGC_{RF2}. One explanation could be that growth was recorded over a too short period, preventing

accurate measurement of its heritability. The body weight heritability estimated here was moderate (0.39), which is in the range of what is found in the literature: between 0.38 and 0.44 in sea bass (Dupont-Nivet et al., 2008), 0.35 ± 0.10 in Atlantic salmon (Rye and Refstie, 1995), 0.25 ± 0.10 in chinook salmon (Winkelman and Peterson, 1994), 0.52 ± 0.26 in Atlantic cod (Gjerde et al., 2004), 0.21-0.26 ± 0.03 in rainbow trout (Kause et al., 2003), 0.52 ± 0.17 in arctic char (Nilsson, 1992), and 0.33 ± 0.07 in common carp (Vandeputte et al., 2004). It should be noted that the heritabilities calculated here may be a little upward biased if maternal effects are present, which is quite unlikely (see material and methods) but cannot be formally ruled out.

The significant genetic and phenotypic correlations between TGC_{FD1} and TGC_{FD2}, and between TGC_{RF1} and TGC_{RF2}, indicate that FD and RF performances are relatively stable and that individual differences remain rather consistent across time, which is an asset for their use in a breeding program. However, the correlations estimated were significantly lower than unity, which suggests that performances exhibited during the first and the second period of feed deprivation / re-feeding may involve different physiologic processes. This hypothesis is supported by the fact that TGC_{FD1} and TGC_{FD2}, as well as TGC_{RF1} and TGC_{RF2}, had different genetic determinism (*e.g.* different heritabilities as well as different additive genetic variance). These results underline the need to integrate at least two periods of feed deprivation to accurately characterize FD performances, as well as two periods of re-feeding to characterize RF performances. We also showed that the two successive periods of feed deprivation and re-feeding were moderately correlated, demonstrating that selection made solely on FD performances would moderately influence the RF performance variations and *vice versa*. When choosing a criterion integrating both FD and RF performances to predict RFI variations, CC is a good compromise as we have seen that this trait reflects the average performance of both FD and RF. Practically speaking, the FD and RF performances studied here are valid for early growth (80 to 120 g BW), but we demonstrated that they were phenotypically correlated with FD and RF performances at a later stage (350g, Grima et al 2000X), for which we could not estimate heritabilities, due to a smaller sample size. Early evaluation of FD and RF is more practical, but a later evaluation could also be more representative of the stage where fish eat the most. The final choice would need heritabilities and genetic correlations to be estimated at the two stages.

We estimated the genetic correlations of body weight with the different indirect criteria to assess whether our indirect criteria fit with breeding programs previously based on body weight. We found a moderate positive genetic correlation of FD_{corr} with body weight, meaning that selection for high $FD_{corr,}$ which is expected to decrease RFI (Grima et al. 2010) should increase body weight at the same time. Selection for CC, which may also reduce RFI (Grima et al 2000X), would have no impact on body weight. The other uncorrected criteria are either positively genetically correlated with body weight (TGC_{RF1}, TGC_{RF2}) or not significantly correlated with body weight (TGC_{FD1}, TGC_{FD2}). This indicates that all these traits could be easily introduced in selection breeding program based on body weight, with no adverse effect.

We calculated the expected genetic response after individual selection for FD and CC. Based on our results, a selection intensity of 1 would improves FD_{corr} of 0.23 phenotypic standard deviations (SD) per generation, and of 0.33 phenotypic SD per generation for CC value. Knowing that mean FD_{corr} values of fish from the FD^{-} group tested in Grima et al (2010) is 1.15 phenotypic SD, and that these fish eat in average 3.12 % less than the average population for the same weight gain, we can predict that a selection for increased FD_{corr} with a selection intensity of 1 (equivalent to a proportion selected of 38%) will lead to 0.62 % feed saving per generation. Of course this prediction is subject to caution since we only know the phenotypic relation between RFI and FD. Using the same type of calculations, a selection for CC with a selection intensity of 1 should lead to 0.36 % feed saving per generation.

While a selection for FD or for CC appears to be a promising approach to improve RFI and feed efficiency, three other strategies have been proposed to indirectly improve feed utilization efficiency in fish i) selection for growth (Kause et al., 2006b; Quinton et al., 2007b)

ii) simultaneous selection for growth and against feed intake (Kause et al., 2006b; Quinton et al., 2007b) iii) simultaneous selection for growth and against lipid content (% of wet weight). Authors predicted that selection intensity of 1 for growth would lead to 8.4 % and to 0.49 % increase in feed efficiency per generation respectively in rainbow trout (Kause et al., 2006b) and in European whitefish (Quinton et al., 2007b). In rainbow trout simultaneous selection for growth and against feed intake would not improve genetic response of feed efficiency compared with selection solely for growth (Kause et al., 2006b), while in European whitefish the same strategy would double the genetic gain (1.06 %) compared with selection solely for growth (Quinton et al., 2007b). Finally, the third strategy is expected to lead to 0.73 % increase in feed efficiency in European whitefish (Quinton et al., 2007b). When comparing results obtain from these two studies, it appears that expected genetic gain may be species or experimental protocol (experimental diet, etc..) dependent. It seems therefore very delicate to evaluate indirect criteria relevance from different studies, and then we cannot precisely compare our indirect criteria with those presented in literature. Nevertheless, in European whitefish (when expected genetic gains are weak), combining different indirect criteria conducted to an enhancement of the expected genetic gain. Hence, combining FD and CC with other indirect criteria could be of interest to improve the response of selection. Because we took a particular attention to work with indirect criteria independent from growth performances, it is likely that FD and CC are indirect criteria complementary to growth which could be advantageously combined to this trait in a breeding program to improve feed utilization efficiency. Regarding the use of fat content as indirect criteria our results contrast with those presented by Quinton et al. (2007b), as while our results predict that the most efficient fish are also the fatter ones (Grima et al. 2010), it seems that in European whitefish selection for leaner individual leads to more efficient fish. These differences may be explained either by the fact that we used two different species, or by the fact that we used different criteria to characterize the fat content, lipid percentage base on wet weight in Quinton et al. (2007b) study and percentage of fat content in dorsal muscle in the present study. As it has been shown to be significantly related to feed utilization efficiency, carcass lipid content could be included as an indirect criterion in a breeding program to improve feed utilization efficiency in combination with growth and our indirect criteria. Nevertheless, the relationship between fat content and feed utilization efficiency need further investigation before including fat content as indirect criteria in breeding program in sea bass, as our results pointed out that fish using feed efficiently were also those presented the highest muscle fat content.

Conclusion

We validated the relevance of FD and of the composite criterion CC, two traits previously shown to be correlated with RFI variations (Grima et al 2008, 2010), as indirect criteria for breeding programs. Indeed, i) FD and CC are heritable, ii) FD performances are stable, as are RF performances (the second component of CC), and iii) FD and CC could be easily introduced in breeding programs previously based on body weight.
Selective breeding to constitute two divergent lines for the CC (FD^-/RF^- and FD^+/RF^+) and for FD (FD^- and FD^+) will be carried out in our experimental facilities, in order to investigate the correlated response to selection using our indirect criteria on traits of agronomic interest (RFI, fat percent and carcass yield).

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Tables

Table 1. Phenotypic correlations (above diagonal) and genetic correlations ± S.E (below diagonal) between body weight at day 341 (BW_{341}), basic growth (TGC_{BG}) and 7 potential indirect criteria for RFI in sea bass. TGC_{FD1} and TGC_{FD2} correspond to body weight loss during the first and second feed deprivation periods, respectively, and $TGC_{RF1+ \text{ and}}$ TGC_{RF2} to body weight gain during the first and the second re-feeding periods, respectively. FD_{corr} and RF_{corr} are the standardized residuals of the regressions on TGC_{BC} and BW_{341} of mean $TGC_{FD1\&2}$ and $TGC_{RF1,}$ respectively; CC (composite criterion) was calculated with the following equation = RF_{corr} - FD_{corr}. 1241 < sample size< 1294

Trait	BW_{341}	TGC_{BG}	TGC_{FD1}	TGC_{RF1}	TGC_{FD2}	TGC_{RF2}	FD_{corr}	RF_{corr}	CC
BW_{341}		0.12	-0.19	0.30	-0.08	0.46	0.00	-0.02	-0.02
TGC_{BG}	0.12 ± 0.09		-0.37	0.45	-0.26	0.33	-0.01	-0.07	-0.04
TGC_{FD1}	-0.14 ± 0.11	-0.34 ± 0.09		-0.28	0.45	-0.22	0.75	-0.07	-0.55
TGC_{RF1}	0.57 ± 0.07	0.52 ± 0.07	-0.32 ± 0.09		-0.32	0.51	-0.19	0.84	0.65
TGC_{FD2}	-0.16 ± 0.10	-0.27 ± 0.10	0.71 ± 0.05	-0.25 ± 0.08		-0.30	0.86	-0.21	-0.70
TGC_{RF2}	0.56 ± 0.05	0.25 ± 0.07	-0.22 ± 0.08	0.73 ± 0.04	-0.19 ± 0.09		-0.16	0.27	0.27
FD_{corr}	0.26 ± 0.06	0.00 ± 0.04	0.76 ± 0.04	-0.06 ± 0.10	0.93 ± 0.02	-0.07 ± 0.11		-0.22	-0.82
RF_{corr}	0.29 ± 0.07	0.24 ± 0.12	-0.17 ± 0.15	0.87 ± 0.03	-0.24 ± 0.11	0.64 ± 0.08	-0.25 ± 0.15		0.78
CC	0.00 ± 0.09	0.15 ± 0.14	-0.66 ± 0.09	0.60 ± 0.08	-0.79 ± 0.05	0.46 ± 0.09	-0.83 ± 0.05	0.75 ± 0.07	

* : $P < 0.05$; ** : $P < 0.01$; *** : $P < 0.001$

Table 2. Mean, additive, and environmental variance estimates and heritability of body weight at day 341 (BW_{341}), basic growth (TGC_{BG}) and 7 potential indirect criteria for RFI in sea bass. TGC_{FD1} and TGC_{FD2} correspond to body weight loss during the first and second feed deprivation periods, respectively, and $TGC_{RF1+ and}$ TGC_{RF2} to body weight gain during the first and the second re-feeding periods, respectively. FD_{corr} and RF_{corr} are the standardized residuals of the regressions on TGC_{BC} and BW_{341} of mean $TGC_{FD1\&2}$ and $TGC_{RF1,}$ respectively; CC (composite criterion) was calculated with the following equation = RF_{corr} - FD_{corr} 1241 < sample size < 1294

Trait	Mean	σ^2A	σ^2E	Heritability
BW_{341}	48.26	91	141	0.39 ± 0.03
TGC_{BG}	0.08	0.0027	0.011	0.19 ± 0.03
TGC_{FD1}	-0.04	0.00053	0.0035	0.13 ± 0.03
TGC_{RF1}	0.12	0.0055	0.012	0.31 ± 0.03
TGC_{FD2}	-0.04	0.0013	0.0040	0.24 ± 0.03
TGC_{RF2}	0.10	0.0073	0.012	0.38 ± 0.02
FD_{corr}	0	0.21	0.70	0.23 ± 0.04
RF_{corr}	0	0.15	0.64	0.19 ± 0.04
CC	0	0.45	1.60	0.22 ± 0.04