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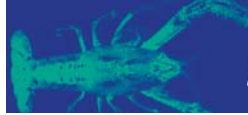
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## A first insight into genotype $\times$ diet interactions in European sea bass (*Dicentrarchus labrax* L. 1756) in the context of plant-based diet use

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### Abstract

This preliminary study assessed genotype  $\times$  diet interaction in late growth of European sea bass (*Dicentrarchus labrax*) fed with either a fish meal (FM)- or a fish oil (FO)-based diet (M) or an all-plant-based (PB) diet. A total of 550 fish from 224 families were reared together and tagged. DNA was sampled and microsatellites were used to assign parentage. When fish weight was  $192 \pm 54$  g, two tanks were fed with M (FM: 100%; FO: 100%) and two others with PB (FM: 0% and FO: 0%). Body weight (BW), fork length (FL) and fillet lipid content (*CorrFat*) were analysed with a linear model and with REML methodology. We observed no significant differences between groups, but a slightly lower ( $P = 0.03$ ) daily growth coefficient in sea bass fed PB than in those fed M. Heritability estimates of BW differed significantly from zero (PB:  $0.37 \pm 0.18$ ; M:  $0.47 \pm 0.24$ ). Sire  $\times$  diet interactions were significant and genetic correlations ranged between 0.51 and 0.87, showing genotype  $\times$  diet interaction for BW and *CorrFat*. For the first time, genetic parameters in the context of total replacement of marine fishery by-products were estimated in European sea bass, showing re-ranking of family performances with extremely contrasted diets.

**Keywords:** genotype by diet  $\times$  environment interaction, European sea bass, plant diet, genetic correlation

### Introduction

Over the past decades, aquaculture has become an animal production sector with the highest annual growth rate (FAO 2008, State of World Aquaculture), thus trying to meet the increasing demands for sea-food supplies coming from economically viable, environmentally friendly and socially acceptable production systems.

To make the production system sustainable, one of the well-known targets in aquaculture is to reduce the reliance on feeds based on ingredients derived from wild fishery resources (Tacon & Metian 2008). Replacement of fish meal (FM) by plant protein sources has hence been a target of many studies and recent works on European sea bass (*Dicentrarchus labrax*) concluded that anywhere from 50% to even 98% of FM-derived proteins can be replaced using plant protein sources, with growth similar to that when fish oil (FO) is used as the lipid source (Oliva-Teles & Gonçalves 2001; Kaushik, Covès, Dutto & Blanc 2004; Messina, Tulli, Messina, Franchin & Tibaldi 2005; Dias, Conceição, Ribeiro, Borges, Valente & Dinis 2009), although in some cases, total replacement of FM by single plant protein sources has been shown to lead to depressed growth (Dias, Alvarez, Arzel, Corraze, Diez, Bautista & Kaushik 2005).

Replacement of FO by vegetable oils (VO) has also been widely studied in European sea bass and replacement of 60% of FO has been shown to have no

negative impact on survival, growth and health of fish (Izquierdo, Obach, Arantzamendi, Montero, Robaina & Rosenlund 2003; Figueiredo-Silva, Rocha, Dias, Silva, Rema, Gomes & Valente 2005; Mourente, Dick, Bell & Tocher 2005; Richard, Mourente, Kaushik & Corraze 2006), whereas growth performances were reduced at higher levels of substitution (80%) (Montero, Robaina, Caballero, Ginés & Izquierdo 2005), contrary to what has been observed in salmonids, where total replacement of FO by VOs has been shown to have no adverse consequences in terms of growth performance (Torstensen, Bell, Rosenlund, Henderson, Graff, Tocher, Lie & Sargent 2005; Richard, Kaushik, Larroquet, Panserat & Corraze 2006). This difference between salmonids and marine teleosts such as the European sea bass is possibly linked to putative differences in fatty acid bioconversion capacities between freshwater and marine finfish (Watanabe 1982; Sargent, Tocher & Bell 2002).

In the general context of reducing the dependence on marine ingredients (FO and FM) for developing aquafeeds, genetic improvement of farmed fish appears as an interesting complementary target (Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Kroghdahl, Nelson, Overturf, Rust, Sealey, Skonberg, Souza, Stone, Wilson & Wurtele 2007), and recent studies on salmonids suggest the existence of genetic variability for utilization of plant-based (PB) diets (Palti, Silverstein, Wieman, Phillips, Barrows & Parsons 2006; Pierce, Palti, Silverstein, Barrows, Halerman & Parsons 2008; Dupont-Nivet, Médale, Leonard, Le Guillou, Tiquet, Quillet & Geurden 2009). Because, in general, selection programmes targeting mainly on growth rate using mostly commercial feeds using hitherto conventional feed ingredients, studying genetic parameters including genotype × diet interactions when using PB diets is a major step forward to address two issues: (1) will the rankings of European sea bass families fed on marine feeds be conserved or not if increased proportions of plant ingredients are used in the feed?; and (2) whether it is possible to specifically select fish for growth on PB diets, thus making it a more environmentally sustainable genotype of interest to future aquaculture?

The existence of genotype × diet interactions has been studied in Rainbow trout *Onchorynchus mykiss* (Blanc 2002, Palti *et al.* 2006; Pierce *et al.* 2008; Dupont-Nivet *et al.* 2009) and European whitefish *Coregonus lavaretus* (Quinton, Kause, Koskela & Ritola 2007; Quinton, Kause, Ruohonen & Koskela 2007). Significant interactions only appeared in the studies of Pierce *et al.* (2008) and of Dupont-Nivet *et al.*

(2009), both of which had the highest replacement rate (37.2 and 0% for FO and 93.75 and 100% for FM respectively), while the other works performed only partial replacement [FO: 11.4%, FM: 87% for early stage and 100% later as in Palti *et al.* (2006); FO: 0%, FM: 73.3% in Quinton, Kause, Ruohonen *et al.* (2007)]. For European sea bass, a leading aquaculture species in the Mediterranean, no such studies exist, but bear significance as this species is reported to show a very high fish in/fish out ratio (Naylor, Goldburg, Primavera, Kautsky, Beveridge, Clay, Folke, Lubchenco, Mooney & Troell 2000). Besides, high genetic gains for growth are possible in this species (Vandeputte, Dupont-Nivet, Haffray, Chavanne, Cenadelli, Parati, Vidal, Vergnet & Chatain 2009) using conventional feeds rich in FM and FO.

The present work focused on weight, length and lipid content. Known as a valuable trait in this species, fat deposition is known to be affected by dietary carbohydrate levels as well as protein sources (Dias *et al.* 2005). Saillant, Dupont-Nivet, Sabourault, Haffray, Laureau, Vidal and Chatain (2009) demonstrated that the quality of prediction of lipid content with Distell Fish Fatmeter, previously used on rainbow trout (Douirin, Haffray, Vallet & Fauconneau 1998; Quillet, Le Guillou, Aubin & Fauconneau 2005; Quillet, Le Guillou, Aubin, Labbé, Fauconneau & Médale 2007), was accurate enough to assess genetic parameters in sea bass, with a moderate heritability ( $0.28 \pm 0.12$ ) for this trait. We measured and followed this trait during our trial.

We focused on growth of juvenile sea bass, where feed consumption is the highest, and chose to compare a marine ingredients-based diet with a totally PB diet, free of any marine ingredient (FO: 0%, FM: 0%), in order to exacerbate any possible differences. All families were mixed in tanks to avoid confusion of genetic and environmental effects and pedigrees were redrawn *a posteriori* with microsatellite markers. In this study based on a total replacement of marine products, diet effect, heritability and genotype × diet interactions are assessed and analysed.

## Materials and methods

### Experimental design

This study was conducted in the Ifremer aquaculture station (Palavas-les-flots, France) with wild-caught West Mediterranean European sea bass broodstock. A full factorial mating design was used to cross eight dams and 41 sires in order to obtain 328 families.

Sperm was collected as described in Fauvel, Suquet, Dreanno, Zonno and Menu (1998). After hormonal induction of ovulation ( $10 \mu\text{g kg}^{-1}$  luteinizing-releasing hormone, Sigma D-TRP6LHRH Chesnes, France), females were manually stripped and the same volume of eggs were collected, mixed and divided into 41 aliquots. Each aliquot of eggs was then fertilized with sperm of one single sire to avoid sperm competition, as described by Saillant, Chatain, Fostier, Przybyla and Fauvel (2001). All fertilizations were carried out within a 30-min period and eggs were pooled in conical-bottom incubators. Floating (live) and sinking (dead) eggs were separated at 48-h post fertilization ( $14^\circ\text{C}$ ) by decanting at a salinity of 48‰ (Chatain 1994). Viable eggs (50 mL) were transferred in a  $0.5 \text{ m}^3$  incubator (in triplicate) containing all families and hatched. Until day 68, larvae were kept in the same tank and the temperature increased from  $14$  to  $20^\circ\text{C}$ . Fish were fed *Artemia* for 40 days and then weaned on artificial feed.

At 306 day post fertilization (dpf), 2000 randomly chosen fish (mean weight:  $48.5 \pm 16.0$  g, mean standard length:  $13.9 \pm 1.4$  cm) were tagged using a Passive Integrated Transponder (AEG-Id, Ulm, Germany) and individual fin clips were stored in 100% ethanol for DNA extraction, genotype analysis and parentage assignment. Until day 588, fish were used in a feed deprivation trial to assess heritability of weight loss as detailed in Grima, Chatain, Ruelle, Vergnet, Launay, Mambrini and Vandeputte (2010). On day 588, 785 of them were then randomly sampled, distributed in four  $5 \text{ m}^3$  tanks (196 fish per tank) and fed with a commercial feed for 135 days to avoid potential effect of the previous study. Water temperature was  $21^\circ\text{C}$  on average, salinity was 38‰ and the system was recirculated (10–30% renewal per day). The feeding trial, described in this paper, started with fish aged 588 days and weighing  $192 \pm 54$  g.

### Feeding trial and experimental diets

Two tanks received a marine (M) experimental diet containing FM and FO and the other two received a PB experimental diet devoid of FM and FO. Both diets were manufactured using a twin-screw extruder (Cletral BC45, Cletral, Firminy, France) at the INRA experimental facilities (Donzacq, France). Ingredients and proximate composition of diets are presented in the Table 1. Diet M contained a blend of FM, corn gluten meal, wheat gluten and extruded wheat

of corn gluten meal, soybean meal, wheat gluten, extruded wheat and white lupin. In order to obtain a total replacement of fish products, only linseed oil (9.4%) was used in the PB diet, whereas diet M contained FO (8.5%). Linseed oil is rich in 18:3 n-3 (50% of total fatty acids), whose incorporation was thus intended to supply high levels of 18:3 n-3 but no long-chain n-3 poly-unsaturated fatty acids (PUFA) such as the EPA or DHA in the PB diet and to verify the potential bioconversion capacities of 18 carbon fatty acids in this euryhaline species. The fatty acid composition of the two feeds is provided in Table 2. Diet PB also contained an attractant mix to increase the palatability (Table 1), which contained only non-essential amino acids (alanine, glycine), a physiological amino acid (taurine) and betaine. The nitrogen contents of these were also taken into account in formulating the feeds to be comparable between the two feeds. Both diets were isoproteic (digestible protein: 43% of dry matter), isolipidic (fat: 13% of dry matter) and isoenergetic ( $17 \text{ MJ kg}^{-1}$ ). Fish were fed one daily meal until apparent satiation (determined when uneaten pellets started to be collected in the faecal

**Table 1.** Ingredients and proximal composition of the experimental diets

Diets	M	PB
Ingredients ( $\text{g kg}^{-1}$ )		
Fish meal	380	0
Corn gluten meal	180	200
Soybean meal	0	182
Wheat gluten	72	20
Extruded wheat	253	72
White lupin	0	140
Fish oil	85	0
Linseed oil	0	94
Soy lecithin	0	10
L-lysine	0	27
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	0	30
Binder (sodium alginate)	10	10
Attractant mix*	0	15
Min. premix†	10	10
Vit. premix‡	10	10
Chemical composition		
Dry matter (DM) (%)	88.9	94.5
Crude protein (%DM)	49.4	49.6
Crude fat (%DM)	14.4	14.1
Ash (%DM)	7.0	6.1
Nitrogen-free extract (NFE) (%DM)‡	18.1	24.7

\*Attractant mix contained ( $\text{g kg}^{-1}$  feed) taurine (3), betaine (3), glycine (2), alanine (2) and glucosamine (5).

†As in Guillaume *et al.* (2001).

‡NFE = dry matter – crude protein – crude fat – ash.

M: fish meal and fish oil diet, PB: all fish meal and fish oil replaced by plant products.

**Table 2** Fatty acid composition (% of total fatty acids) of the two diets used for growing European sea bass

Fatty acid	M	PB
8:0	0.02	0.02
10:0	0.68	0.81
12:0	0.08	0.01
14:0	7.01	0.09
15:0	0.70	0.04
16:0	19.26	8.19
17:0	0.46	0.11
18:0	3.07	3.07
20:0	0.16	0.21
14:1	0.07	–
16:1	5.79	0.16
17:1	0.03	0.04
18:1	15.70	21.72
20:1	5.74	0.59
22:1	4.53	0.21
16:2 n-4	1.09	–
16:3 n-4	0.85	–
16:4 n-1	1.26	–
18:2 n-6	7.51	21.97
18:3 n-6	0.19	–
20:2 n-6	0.20	0.04
20:3 n-6	0.06	–
20:4 n-6	0.47	–
22:2 n-6	0.15	0.10
18:3 n-3	0.99	41.31
18:4 n-3	1.43	0.01
20:3 n-3	0.11	–
20:4 n-3	0.58	–
20:5 n-3 (EPA)	7.41	–
21:5 n-3	0.27	–
22:5 n-3	1.29	–
22:6 n-3 (DHA)	8.78	–
Sum saturates	31.43	12.54
Sum monoenes	31.85	22.72
Sum n-6 fatty acids	8.58	22.11
Sum n-3 fatty acids	20.85	41.33
Sum fatty acids	96.14	98.79

M: fish meal and fish oil diet; PB: all fish meal and fish oil replaced by plant products.

traps), and all uneaten pellets were counted. The meal duration was between 1 h and 1 h 30 min every day and the trial lasted 262 days.

### Data collection

The feeding trial was divided into six periods flanked by seven measures at 588, 605, 647, 679, 736, 787 and 850 dpf in order to get a measure approximately every 40 days. Fish weights and standard lengths were measured at each date and lipid content was indirectly measured with a Fish Fatmeter (FM692, Distell, Fauldhouse, UK) at the same days using the

SEABASS-1 calibration (mean of four measurements, two on each side of the fish, in percentage fresh matter). The daily growth coefficient (DGC)<sub>60</sub> =  $(W_6^{1/3} - W_0^{1/3}) / (t_6 - t_0)$  was also calculated (with  $W_6$  being the weight at the end of period 6,  $W_0$  being the weight at the end of period 0,  $t_6 - t_0$  being the duration in days between dates).

### Parentage assignment

Rapid DNA extractions were performed from the fin clips, using Chelex resin as described in Estoup, Largiadier, Perrot and Chourrout (1996). Parents and offspring were assayed at five or six microsatellite loci as described in Chistiakov, Hellemans and Volckaert (2006) and García de León, Dallas, Chatain, Canonne, Versini and Bonhomme (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 was performed by exclusion using VITASSIGN (Vandeputte, Mauger & Dupont-Nivet 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, 99.2% of the fish sampled (778 of 784) had been successfully and unambiguously assigned to their parents.

### Statistical analyses

Traits followed at the end of each period were the body weight (BW), the standard length and the mean muscle fat content assessment divided by the decimal logarithm of BW of each period (*CorrFat*) as bigger sea bass are known to be fatter.

Fish were sexed by visual inspection of the gonads on slaughtered fish at the end of the experiment, as sex is known to have a highly significant effect on the growth of European sea bass. Because of data collection problems, sex could only be recorded on 554 individuals. Parentage analyses showed that some fish belonged to under-represented half-sib families. Offspring from half-sib families with less than five fish were not used for further statistical analyses. Finally, 550 individuals from seven dams and 32 sires were used (224 full-sib families).

Preliminary analyses indicated that the sire × dam effect was never significant for any trait and was therefore not included in the models. To determine the significance of fixed effects (sex, diet) and to evaluate the significance of genotype × diet inter-

actions, data were first analysed using SAS-GLM (SAS Institute, Cary, NC, USA) with the following model:

$$Y_{ijklmn} = \mu + \text{diet}_i + \text{sex}_j + \text{tank}(\text{diet})_{k(i)} + \text{sire}_l + \text{dam}_m + \text{sire} \times \text{diet}_{il} + \text{dam} \times \text{diet}_{im} + e_{ijk(i)lmn} \quad (1)$$

where  $Y_{ijklmn}$  is the performance of individual  $n$ ,  $\mu$  is the general mean,  $\text{diet}_i$  is the fixed effect of the diet,  $\text{sex}_j$  is the fixed effect of sex  $j$  (1 = male, 2 = female),  $\text{tank}_{k(i)}$  is the random effect of the tank  $k$  ( $k = 1, 2$ ) nested within diet  $i$ ,  $\text{sire}_l$  is the random effect of sire  $l$ ,  $\text{dam}_m$  is the random effect of dam  $m$ ,  $\text{sire} \times \text{diet}_{il}$  the random interaction between sire  $l$  and diet  $i$ ,  $\text{dam} \times \text{diet}_{im}$  the random interaction between dam  $n$  and diet  $i$  and  $e_{ijk(i)lmn}$  is the random residual.

Genotype × diet interactions were also quantified through genetic correlations between the trait of interest with diet M and the same trait with diet PB, considered as two different traits in the analysis. In this case, G × E (genetic × environment) interaction is the difference between 1 and the genetic correlation, and the closer to 1 the correlation, the smaller the G × E interaction. To estimate this correlation as well as trait heritability, the following model was used in ASREML (Gilmour, Gogel, Cullis *et al.* 2006):

$$Y_{ijklm} = \mu + \text{sex}_i + \text{tank}_j + \text{sire}_k + \text{dam}_l + e_{ijklm} \quad (2)$$

where  $Y_{ijklm}$  is the performance of individual  $m$ ,  $\mu$  is the general mean,  $\text{sex}_i$  is the fixed effect of sex  $j$  (1 = male, 2 = female),  $\text{tank}_j$  is the fixed effect of tank  $j$  ( $j = 1, 2$ ; two tanks per diet),  $\text{sire}_k$  is the random effect of sire  $k$ ,  $\text{dam}_l$  is the random effect of dam  $l$  and  $e_{ijklm}$  is the random residual. The sire component of variance ( $\sigma_s^2$ ) was used to estimate the additive genetic variance ( $\sigma_A^2$ ) through the formula  $\sigma_A^2 = 4\sigma_s^2$  (Becker 1984) and heritability was estimated as the ratio  $h^2 = \sigma_A^2/\sigma_P^2$  with  $\sigma_P^2$  being the estimate of phenotypic variance.

## Results

### Diet effect

After 9 months of the growth trial, the total mortality was 0.3% (0.6% in PB, 0.0% in M). Descriptive statistics of studied traits at the end of the study are given in Table 3. Linear model estimates of the mean weight show a 3.1-fold increase with the M diet and a 2.7-fold increase with the PB diet. Body weight of fish fed the PB diet was never significantly lower than that of fish fed diet M throughout the entire experimental period

(Table 4). However, differences between diets increased from period 1 (+11.1%) to period 6 (+16.4%) where it became suggestive ( $P < 0.06$ , see Fig. 1). No differences were observed between fish fed with the two diets for muscle lipid content (*CorrFat*,  $P > 0.51$ , see Table 4). The daily growth coefficient calculated between the last (6) and the first (0) sampling dates was smaller in fish fed diet PB ( $P < 0.03$ ).

### Linear model, heritability estimates and genetic correlations

Diet effects or dam × diet effects were not significant on BW and *CorrFat* at any sampling point (Table 4). Sire × diet interactions on BW and *CorrFat* were

**Table 3** Effect of total replacement of fish oil and fish meal (M) by plant products (PB) on body weight (BW), fork length (FL), estimates of lipid content divided by the weight (*CorrFat*) and daily growth coefficient (DGC =  $\text{FBW}^{1/3} - \text{IBW}^{1/3}$ )/duration with IBW (g) and FBW (g)

Traits	M	PB	F	P-value
BW (g)	647.1 ± 10.8	556.1 ± 10.7	14.34	0.06
FL (mm)	313.9 ± 1.9	299 ± 1.9	5.19	0.15
<i>CorrFat</i>	3.1 ± 0.06	3.1 ± 0.06	0.02	0.89
DGC	1.08 ± 0.01	0.92 ± 0.01	31.71	0.03

IBW, initial body weight; FBW, final body weight.

**Table 4** P-values for diet, tank (diet), sex, and interactions sire × diet and dam × diet effects for body weight (BW) and estimates of lipid content divided by the weight (*CorrFat*) for each date

	Diet	Tank (diet)	Sex	Dam × diet	Sire × diet
BW					
(588) 0	0.57	0.47	<0.0001	0.17	0.15
(605) 1	0.31	0.01	<0.0001	0.14	0.05
(647) 2	0.31	<0.0001	<0.0001	0.30	<0.01
(679) 3	0.25	<0.0001	<0.0001	0.35	<0.01
(736) 4	0.20	<0.0001	<0.0001	0.30	<0.01
(787) 5	0.14	<0.01	<0.0001	0.21	<0.01
(850) 6	0.06	0.08	<0.0001	0.16	0.01
<i>CorrFat</i>					
(588) 0	0.65	0.08	<0.0001	0.15	0.88
(605) 1	0.84	<0.01	0.22	0.34	0.64
(647) 2	0.65	0.1	<0.0001	0.83	0.66
(679) 3	0.63	<0.01	<0.0001	0.99	0.29
(736) 4	0.59	0.24	<0.0001	0.99	0.05
(787) 5	0.51	<0.0001	<0.0001	0.97	0.01
(850) 6	0.89	<0.0001	<0.0001	0.96	<0.01

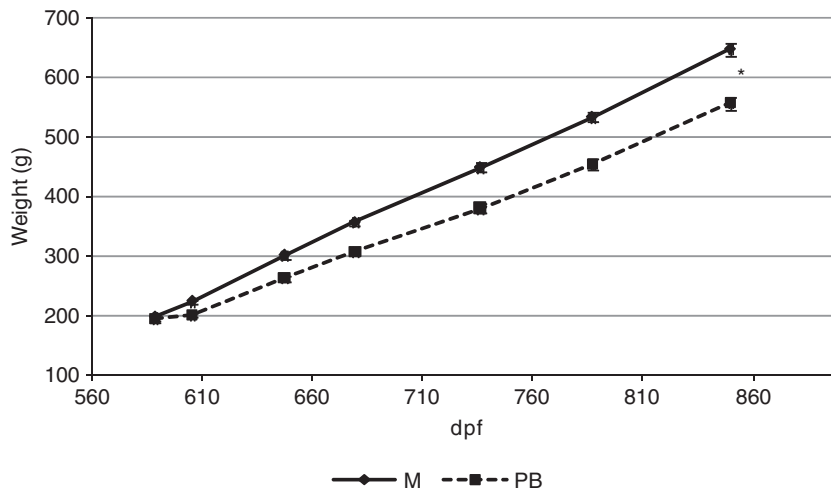
In column 1, the ages of fish (in dpf) are indicated within parentheses before the sampling time.  
dpf, days post fertilization.

significant, showing a significant re-ranking of the sire families between diets for these traits. The multiple measures and individual tagging enabled us to observe an increase of significance of the sire × diet interaction during the trial.

Heritability estimates of BW differed significantly from zero ( $\pm 1.65$  SE for a 90% certainty of rejecting the null hypothesis), whether both diets were combined ( $BW_6$ :  $0.30 \pm 0.13$ ) or considered separately [ $BW_6$  (PB):  $0.37 \pm 0.18$ ;  $BW_6$  (M):  $0.47 \pm 0.24$ ], except for date 3 in the PB batch (Table 5). The highest heritability estimates were obtained for *CorrFat* at measure 3 ( $0.78 \pm 0.21$ ). Two heritability values for *CorrFat* were over 1, probably due to estimation problems resulting from the small size of the dataset.

Heritability estimates were always higher with diet M and tended to decrease over time for both diets, although no significant differences could be shown because of the high values of standard errors (Table 5).

Genetic correlations calculated using model (2) are shown in Table 5. They are all comprised between 0.51 and 0.99, lower values suggesting the existence of genotype × environment interactions. Between dates 3 and 6, the genetic correlation reduced from 0.81 to 0.51 for BW and from 0.99 to 0.83 for *CorrFat*. Standard errors of estimated genetic correlations are too high to show a significant difference between them and it does not allow one to conclude that the decreasing trend at the end of each period is significant (Fig. 1).



**Figure 1.** Evolution of the mean weight (g) of European sea bass fed diet M or plant-based diet PB over 37 weeks. \*Significant difference ( $P < 0.1$ ); dpf, days post fertilization.

**Table 5** Genetic correlations between diets and heritability ( $\pm$  standard errors) for plant-based, marine and both diets

	Genetic correlation	$h^2$ (PB) ( $\pm$ SE)	$h^2$ (M) ( $\pm$ SE)	$h^2$ (total) ( $\pm$ SE)
<b>BW</b>				
(588) 0	$0.99 \pm 0.00$	$0.25 \pm 0.03$	$0.44 \pm 0.04$	$0.34 \pm 0.14$
(605) 1	$0.99 \pm 0.00$	$0.07 \pm 0.00$	$0.52 \pm 0.04$	$0.31 \pm 0.13$
(647) 2	$0.99 \pm 0.00$	$0.14 \pm 0.00$	$0.52 \pm 0.04$	$0.32 \pm 0.13$
(679) 3	$0.81 \pm 0.38$	$0.21 \pm 0.16$	$0.54 \pm 0.24$	$0.31 \pm 0.13$
(736) 4	$0.64 \pm 0.35$	$0.29 \pm 0.17$	$0.51 \pm 0.24$	$0.31 \pm 0.13$
(787) 5	$0.51 \pm 0.34$	$0.36 \pm 0.18$	$0.48 \pm 0.24$	$0.30 \pm 0.13$
(850) 6	$0.51 \pm 0.34$	$0.36 \pm 0.18$	$0.47 \pm 0.24$	$0.30 \pm 0.13$
<b>CorrFat</b>				
(588) 0	$0.99 \pm 0.00$	$0.46 \pm 0.1$	$0.60 \pm 0.08$	$0.63 \pm 0.19$
(605) 1	NE	NE	NE	$0.63 \pm 0.19$
(647) 2	$0.99 \pm 0.00$	$0.19 \pm 0.01$	$0.19 \pm 0.11$	$0.61 \pm 0.18$
(679) 3	$0.99 \pm 0.09$	$0.70 \pm 0.22$	$1.02 \pm 0.29$	$0.78 \pm 0.21$
(736) 4	$0.87 \pm 0.16$	$0.59 \pm 0.22$	$0.83 \pm 0.27$	$0.60 \pm 0.18$
(787) 5	$0.80 \pm 0.16$	$0.57 \pm 0.22$	$1.10 \pm 0.29$	$0.65 \pm 0.19$
(850) 6	$0.83 \pm 0.17$	$0.56 \pm 0.22$	$0.94 \pm 0.23$	$0.65 \pm 0.19$

Ages (in dpf) of the fish are indicated before the period number.

NE, nonestimated due to lack of model convergence.

## Discussion

The objectives of this preliminary study were to evaluate genotype × diet interactions in European sea bass in the case of total replacement of fish products in the diet, together with the estimation of heritabilities of BW, length and fat content in both feeding contexts.

### Diet effect

A 16.4% difference in final weight between fish fed the marine (M) diet and fish fed the PB diet was found after 262 days of trial, but it was not statistically significant. Kaushik *et al.* (2004) have shown previously that an almost total replacement of FM does not have any adverse effect on the growth of European sea bass. The present results clearly proved that it is possible to go further as the total replacement of FO and FM can lead to good performances under similar conditions. Because the feeds were extruded, the putative presence of anti-nutritional factors from plant protein sources were reduced, as also demonstrated by the more or less equivalent growth performance of the two groups. This lack of significant effects could also be due to the experimental design, which was probably not optimal to detect feed effects (only two replicate tanks per feed, with significant tank effects for some dates), because the primary goal of this study was to detect genotype by feed interactions.

The growth rate recorded was lower than those reported in earlier studies (Dias, Corraze, Arzel, Alvarez, Bautista, Lopez-Bote & Kaushik 1999; Kaushik *et al.* 2004). A smaller growth rate has also been reported with sea bass in the case of partial FM or FO replacement (Dias *et al.* 2005; Montero *et al.* 2005). The daily growth coefficient calculated on the entire period takes into account the initial weight of each individual and is higher in the M batch, contrary to weight. The fact that PB fed fish took up to 2 weeks to feed normally after the initiation of the experiment, while M fish ate normally after 2–3 days, supports the hypothesis of a low initial palatability of the PB diet and the need for a period of adaptation.

Estimates of muscle lipid contents did not show differences between fish from the two dietary groups, which is consistent with results obtained in Atlantic salmon fed vegetable-oil-based feeds (Torstensen *et al.* 2005; Nanton, Vegusdal, Rørå, Ruyter, Baeverfjord & Torstensen 2007). Indeed, dietary lipid sources are known not to affect lipid deposition in either liver or muscle in sea bass, but inclusion of VOs in fish diets

modifies the body fatty acid profile (Izquierdo *et al.* 2003).

To our knowledge, this is the first ever study with European sea bass fed with a complete marine ingredients-free diet. Even if a VOs blend can mimic the fatty acid profile of FO for total saturated fatty acids, monounsaturated fatty acids and PUFA (Torstensen *et al.* 2005; Francis, Turchini, Jones & De Silva 2007), deficiency in n-3 long-chain PUFA can still occur (Turchini, Bente, Torstensen & Ng 2009). Moreover, it is generally recognized that marine fish have a lower ability to convert 18C fatty acids into longer PUFAs (Watanabe 1982) and will exhibit direct requirements for HUFA (Sargent *et al.* 2002). Previous works have reported the necessity of 1.0% of n-3 HUFA to fulfil essential fatty acid requirements in sea bass (review by Turchini *et al.* 2009). The total absence of such fatty acids in diet PB was probably partially compensated by fish reserves inherited from common feeding past, although the final BW was almost three times the initial BW. Muscle fatty acid profiles on a subsample of the same fish have shown differences between the two dietary groups reflecting dietary fatty acid profiles but still maintaining non-negligible levels of the long-chain w3 fatty acids (Geay, Santigosa, Culi, Corporeau, Boudry, Dreano, Corcos, Bodin, Vandeputte, Zambonino-Infante, Mazurais & Cahu 2010).

### Genetic parameters

Sire × diet interaction became significant for both traits (BW and *CorrFat*) during the growth trial, indicating that a general re-ranking of the sire families occurred in response to the diet challenge. A statistical procedure (GLM procedure in SAS) allowed us to check whether one of the effects is significant inside each level of the other effect of the interaction. Known as simple effects (Winer 1971), it confirms that significance of diet effect on BW exists for 10 of the 32 sire families ( $P < 0.05$ ).

One interesting finding of the present study is the existence of sire × diet interactions for both BW and muscle lipid content measured with fatmeter in European sea bass in case of total replacement of marine products in the diet in a late growth context. In the whitefish, Quinton, Kause, Koskela *et al.* (2007) found only weak genotype × diet interaction for growth (genetic correlation for daily gain:  $0.99 \pm 0.13$ ). Therefore, they predicted that changing from FM-based to soybean meal-based diets should have only a minor influence on fish breeding programmes. Palti



*et al.* (2006) found no family × diet interactions for growth in rainbow trout fed with FM or gluten-based diet. In our experiment, total substitution leads to clear interactions for BW and brings results close to the conclusions of Pierce *et al.* (2008) and Dupont-Nivet *et al.* (2009) in rainbow trout. Analysis of the evolution of sire × diet interaction shows that it became significant very soon in the trial (after 17 days) for BW and much later for *CorrFat* (after 199 days). Same pattern of changes is observed with genetic correlation (after 91 days for BW and 148 days for *CorrFat*) showing that both calculations are consistent. Considering that weight measurement is accurate on fish of such size, increase of interaction with time could be due to the amplification in differences between families who differ in genetic values (Saillant, Dupont-Nivet, Haffray & Chatain 2006). This would indicate that variability appears fast and remains or increases under these conditions.

Estimates of BW heritability when M and PB diets are combined were almost all significantly higher than 0, but lower than or equal to those found previously in the European sea bass: 0.60 for logarithm of BW (Saillant *et al.* 2006); 0.34–0.46 (Dupont-Nivet, Vandeputte, Vergnet, Merdy, Haffray, Chavanne & Chatain 2008); 0.63 (Saillant *et al.* 2009); and 0.34–0.60 (Vandeputte *et al.* 2009). Notwithstanding the moderate sample size of the present study, heritability estimates can be considered moderate to high for BW and confirm that European sea bass is a good candidate for selection on these traits (Dupont-Nivet *et al.* 2008; Vandeputte *et al.* 2009). The main conclusion is that sea bass fed on PB diet are also good candidates for selection and that heritability estimates are not significantly different between diets.

High heritabilities of *CorrFat* confirm that rapid genetic gain is also possible for this quality trait, as already shown on rainbow trout (Quillet *et al.* 2005), common carp (Kocour, Mauger, Rodina, Gela, Linhart & Vandeputte 2007), European sea bass (Haffray, Pincent, Dupont-Nivet, Vandeputte, Merdy, Chavanne & Chatain 2007) and European whitefish (Kause, Quinton, Ruohonen & Koskela 2009). Selection can be efficient on this trait, whatever be the diet (M or PB). Fat content of the muscle is a valuable trait for industry, but impact of selection on the fatty acid profiles should also be studied in the context of lesser availability or increasing cost of FO. Further studies are warranted to check whether fatty acid profiles can present a genetic variability. The source of global ability to grow better on diets less dependent on wild marine fishery resources could be diverse: a better

HUFA conversion, a better endurance of HUFA restriction or a better appetite to PB diets. We should add that this ability can express itself to a smaller extent possibly even with less extreme feeds.

## Conclusion

Our results allow us to expect that total replacement of marine products in late growth of European sea bass could be performed with moderate effect on BW and lipid content. The significance of genotype × diet interaction in the linear model and moderate genetic correlations between dietary groups for BW and muscle fat content estimated by Fish Fat-meter correspond to a big step towards the consideration of feed products origin in future fish selection. In this trial, we demonstrate that the performance ranking of the families for these traits is not similar with both diets. Extreme contrasts in diet composition as used here are not likely to be encountered in the industry today and a lower interaction may then be observed with commercial diets. However, these diets were chosen to give rise to an increased genotype × diet interaction. The existence of such interactions and of the high genetic variability on diet PB suggests that it is possible to identify fish with a global ability to grow better on the future feeds.

This study proves that genetic improvement can be impacted by extreme changes in diet and the use of PB products. Still, this has to be confirmed in larger experiments, over a longer time period.

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