Genotype by diet interactions in European sea bass (*Dicentrarchus labrax* L.): Nutritional challenge with totally plant-based diets

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Genotype by diet interactions in European sea bass (*Dicentrarchus labrax* L.): Nutritional challenge with totally plant-based diets[1]

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ABSTRACT: Aquaculture of carnivorous species has strongly relied on fish meal and fish oil for feed formulation; however, greater replacement by terrestrial plant-based products is occurring now. This rapid change in dietary environment has been a major revolution and has to be taken into consideration in breeding programs. The present study analyzes potential consequences of this nutritional tendency for selective breeding by estimating genetic parameters of BW and growth rates estimated by the thermal growth coefficient (TGC) over different periods with extremely different diets. European sea bass (*Dicentrarchus labrax* L.) from a factorial cross (1,526 fish) between 25 sires and 9 dams were used to estimate heritabilities and genotype by diet interaction. Starting 87 d after fertilization (2.5 g), one-half of the sea bass were fed a diet containing marine products (M), and the other one-half were fed a totally plant-based (PB) diet (without any fish meal or fish oil). The fish were individually tagged, reared in a recirculated system, and genotyped at 13 microsatellites to rebuild parentage of individuals. Body weight and TGC were measured for 335 d until fish fed the M diet reached 108.3 g of BW. These traits were significantly less in fish fed the PB diet ($P < 0.05$) in the very first stages after the dietary shift, but the difference in TGC between diets rapidly disappeared ($P > 0.1$). Survival was significantly less in fish fed the PB diet (PB = 64.7%, M = 93.7% after 418 d, $P < 0.05$). This work identified moderate heritabilities (0.18 to 0.46) for BW with both diets and high genetic correlations between diets (0.78 to 0.93), meaning low genotype by diet interactions, although diets were extremely different. Heritabilities of TGC (0.11 to 0.3) were less than for BW as well as genetic correlations between diets (0.43 to 0.64). Using such extremely different diets, predicted BW gains in different scenarios indicated that selecting fish for growth on a marine diet should be the most efficient way to increase growth on plant-based diets, meaning that, in this case, indirect selection should be more efficient than direct selection.

Key words: European sea bass, genetic correlation, genotype by diet interaction, genotype by environment interaction, plant-based diet

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INTRODUCTION

With the decline of fisheries worldwide, aquaculture now supplies an increasing proportion of aquatic products for human consumption. Farmed fish are supposed to cover the increase in demand in the next 20 yr (Food and Agriculture Organization, 2008) when fisheries will not be able to meet the demand (Tacon and Metian, 2008; Naylor et al., 2009). Carnivorous species, like the European sea bass, were identified for their high use of fish meal (**FM**) and fish oil (**FO**) from small pelagic species. The pressure to reduce dependency on such marine resources is high (Naylor et al., 2009), and greater substitution rates with plant-based products have been tested in the diets (Powell, 2003).

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In the late 1990s, the first breeding programs started with European sea bass and strong progress for growth (+23% to 42% per generation) was demonstrated to be feasible (Vandeputte et al., 2009) as heritability estimates (h^2) were estimated to be medium to high (Saillant et al., 2006; Dupont-Nivet et al., 2008). However, recent trials showed that genotype by environment interactions could impact growth when fish were reared in different culture conditions (Dupont-Nivet et al., 2010). By extension, diet composition is projected to be highly variable, and estimates of genotype by diet interactions are now needed to know how fingerlings will perform in different dietary environments. Most published results on this issue have been obtained for salmonids. Some found high genetic correlation between diets (0.97 ± 0.21; Quinton et al., 2007a,b) but did not find significant genotype by environment interactions (Paltiet al., 2006; Quinton et al., 2007a,b). However, others found moderate genetic correlation between diets (0.73 ± 0.13; Pierce et al., 2008) and concluded that significant interactions were present (Pierce et al., 2008; Dupont-Nivet et al., 2009). Starting at early stages (2.5 g), we assessed heritabilities of growth traits and genetic correlations between and within diets comparing a totally plant-based diet and a diet containing marine products.

The objective of the present study was to assess the potential efficiency of a breeding program when fish had been selected using a given diet (diets containing FM and FO or not) and then transferred to a different diet.

Table 1. Ingredients and analytical composition of the experimental diets (DM basis)

	Diet[1]	
Item	M	PB
Ingredients, g/kg		
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
$CaHPO_4$, $2H_20$	0	30
Binder (sodium alginate)	10	10
Attractant mix[2]	0	15
Mineral premix[3]	10	10
Vitamin premix[3]	10	10
Chemicals composition		
DM, %	92.3	93.7
CP, % DM	50.6	49.6
Crude fat, % DM	15.3	14.3
NFE,[4] % DM	23.0.1	19.3
Energy, kJ/g DM	23.0	23.3

[1]M = fish meal and fish oil diet; PB = all fish meal and fish oil replaced by plant products.

[2]Attractant mix contained (g/kg feed) taurine (3), betaine (3), glycine (2), alanine (2), and glucosamine (5).

[3]As in Guillaume et al. (2001).

[4]Nitrogen-free extract (NFE) = DM − CP − crude fat − ash.

MATERIAL AND METHODS

All experiments were conducted in accordance with the European directive 86/609/CEE regarding the protection of animals used for experimental and other scientific purposes. The experiments have been designed and performed by reseachers (RLB, MV, BC) having level 1 agreement for the design and performance of experiments on animals. All technicians in the experimental facilities hold a level 3 agreement for animal experiments, and the Ifremer facility of Palavas is itself approved for animal experiments by the French Veterinary Services (agreement B 34-192-6).

Experimental Diets

Two experimental diets were formulated by INRA to be isoproteic, isolipidic, and isoenergetic. Plant-based (**PB**) and marine (**M**) diets were very different regarding the proportion of marine products. The M diet contained both FM and FO, but the PB diet was totally devoid of marine products (Table 1). Both diets contained mineral and vitamin premixes to meet European sea bass nutritional requirements according to NRC (1993). A first batch of feed was processed in INRA facilities (Donzacq, France) and distributed until 344 d of age, and a second batch of feed was processed by Biomar (Denmark) and distributed until the end of the trial. Analytical compositions of the 2 batches were identical (Table 1). Pellet size was adjusted according to mean BW of fish, and diets based on estimated biomass (1% to 2% of the biomass) were distributed using self-feeders.

Fish

This study was conducted in the Ifremer aquaculture station (Palavas-les-Flots, France) with wild-caught west Mediterranean European sea bass brood stock. A factorial cross between 9 dams and 25 sires was done to obtain 225 families. Sperm had been collected 1 yr earlier and cryopreserved. After hormonal induction of ovulation (10 µg/kg luteinizing releasing hormone, Sigma D-TRP6LHRH; Sigma-Aldrich, Saint-Quentin Fallavier, France), females were manually stripped, and the same volume of eggs from each female was collected,

mixed, and divided into 25 aliquots of 40 mL. Each aliquot of eggs was then fertilized by thawed sperm from a single male to avoid sperm competition. All fertilizations were performed within 30 min. After anesthesia (phenoxyethanol, 300 mg/L), fin clips were collected on the parents and stored in ethanol pending DNA extraction. In this experiment, the age of the fish is expressed in days postfertilization. Until 68 d, larvae were all kept in the same tank, and the temperature was increased from 14°C to 20°C (Chatain, 1994). Fish were fed on *Artemia* nauplii for 40 d, then weaned on a commercial diet that contained both marine ingredients and plant products (Marin Start, Le Gouessant, Lamballe, France). A high sensitivity of sea bass juveniles to plant-based products was reported in preliminary trials, but little is known about the origin of this sensitivity. This is the reason why a commercial diet rather than a 1:1 mixture of experimental diets was used before the beginning of the trial, which would have been another possible option. At 83 d, 9,600 fish (2.5 g) were distributed in 6 conical-bottom 1.5-m^3 tanks (3 tanks per diet). Recirculated water with a regulated temperature (mean ± SD: 20.8°C ± 1.1°C; range: 16.9°C to 25.3°C) was supplied at a temperature below the optimum temperature for the maximal growth of European sea bass, which is about 27°C (G. Lemarié, Ifremer, personal communication). Experimental diets were loaded in self-feeders (87 d), and active feeding for both diets started 3 d later. When the fish reached approximately 20 g (mean ± SD: M (224 d) = 23.3 ± 8.1 g, PB (266 d) = 17.9 ± 7.6 g), fish were individually tagged with passive integrative transponders (AEG-Id, Ulm, Germany). After anesthesia (phenoxyethanol, 300 mg/L), all fish were transferred (360 d, mean ± SD: M = 70.3 ± 1.6 g and PB = 36.6 ± 2.06 g) to 6 tanks (5 m3) to keep the same batches and maintain low fish densities (<20 kg/m3). During the transfer, fin samples were collected from each fish after anesthesia (phenoxyethanol, 300 mg/L) and were kept into absolute ethanol.

Data Collection

To estimate early growth (before tagging), random samples of 50 fish per tank were measured at 4 dates (83, 116, 151, and 224 d) for BW (in grams) and standard length (**SL**, in millimeters). After tagging, BW and SL were individually measured monthly (266, 298, 326, 361, 389, and 418 d), and survival was monitored daily. Fish shape was characterized by computing Fulton's condition factor [$K = (BW/SL^3) \times 100$]. Between the second and the third measurements (300 d), a technical problem in the oxygen circuit led to massive mortality in 1 tank of the PB batch without involving the other tanks. Consequently, only 2 PB tanks were further considered for analyses. At the end of the trial (418 d), DNA samples of 1,526 randomly chosen fish (3 × 272 in M and 2 × 355 in PB) were analyzed for parentage assignment, and the corresponding fish were slaughtered and dissected to determine their sex. The trial ended when M fish reached approximately 100 g, which is one-third of their commercial size (300 to 450 g).

The thermal unit growth coefficient (**TGC**) was chosen as a standardized measure of growth (Dumas et al., 2007) that is assumed to be unaffected by BW, time interval, and water temperature (Iwama and Tautz, 1981), as long as temperature is below the optimum temperature for growth, which was the case in the present study. For a period starting on day n and ending on day m, it is calculated as

$$TGC_{nm} = 1000 \times \frac{BW_m^{(1/3)} - BW_n^{(1/3)}}{\sum_{i=n}^{m} (T_i - 10)},$$

where BW_n and BW_m were fish BW (g) on days n and m, respectively, and Ti (°C) was the daily temperature.

Parentage Assignment

Parents and offspring were assayed at 13 microsatellite loci described in Chistiakov et al. (2006) and García de León et al. (1995). Rapid DNA extraction and genotyping were done by LABOGENA (Jouy-en-Josas, France). Parentage assignment was performed by exclusion allowing a maximum of 2 allelic mismatches using VITASSIGN (Vandeputte et al., 2006). At the end of this process, 98.7% of the fish sampled had been successfully and unambiguously assigned to their parents. Some families were poorly represented, and only half-sib families containing more than 10 individuals per diet were kept for the analyses, meaning that the offspring analyzed in both diets were finally issued from the same factorial cross between 9 dams and 22 sires. Similar sizes of half-sib families were obtained in the M diet (mean ± SD: 37.1 ± 22.0 for sire half-sib families; range = 12, 95, 90.8 ± 78.0 for dam half-sib families; range = 25, 247) and the PB diet (33.2 ± 19.5 for sire half-sib families; range = 13, 85, 81.1 ± 75.5 for dam half-sib families; range = 21, 248). In the same way, half-sib families were also evenly distributed among tanks.

Statistical Analyses

For measurements collected before individual tagging, the significance of diet and tank effects was tested with this model in the MIXED procedure (SAS Inst. Inc., Cary, NC) using the REPEATED command of SAS to account for variance heterogeneity between diets:

$$Y_{ijk} = ¼ + Diet_i + tank(diet)_{j(i)} + e_{ijk}, \quad [1]$$

where Y_{ijk} is the individual performance, μ is the overall mean, $Diet_i$ is the fixed effect of the diet (i = 1, 2), $tank_{j(i)}$ is the random effect of the $tank_j$ (j = 1, 2, 3 for M batch and 1, 2 for PB batch) nested within diet i, and eijk is the random residual. At this stage, fish were not yet identified, and sex, dam, and sire were not known. The containment method was used to define the degrees of freedom as the denominator.

For measurements collected after tagging, the significance of diet, sex, tank, sire, and dam effects as well as sire-diet and dam-diet interactions were tested with this model in the MIXED procedure of SAS using the REPEATED command of SAS to account for variance heterogeneity between diets:

$$\begin{aligned} Y_{ijklmn} = {} & ¼ + Diet_i + tank(diet)_{j(i)} + Sex_k \\ & + sire_l + dam_m + sire \times Diet_{il} \\ & + dam \times Diet_{im} + e_{ijklmn}, \end{aligned} \quad [2]$$

where Y_{ijklmn} is the performance of individual n, Sex_k is the fixed effect of sex_k (1 = male, 2 = female), $sire_l$ is the random effect of $sire_l$, dam_m is the random effect of dam_m, sire × $diet_{il}$ is the random interaction between $sire_l$ and $diet_i$, dam × $diet_{in}$ is the random interaction between dam_n and $diet_I$, and e_{ijklmn} is the random residual. The containment method was used to define the degrees of freedom as the denominator. Preliminary analyses indicated that the sire-dam interaction was never significant ($P > 0.05$) for any trait, and it was therefore not included in the models. Preliminary analyses also showed that K was influenced by BW, which could lead to biased estimates when comparing fish fed different diets. Body weight was tested as a covariate, but as there was a significant interaction between BW as covariate and diet (heterogeneous slopes), log (BW) for which slopes were homogeneous was preferred and added as a covariate in model, so that the comparison of K between diets was corrected for the phenotypic effect of BW.

The CV (SD/mean in %), skewness (**Skew**), and kurtosis (**Kurt**) were calculated for each tank within each diet using the MIXED procedure of SAS. Skewness and Kurt were considered significantly different from 0 when their absolute value was greater than twice the SE [$\sqrt{}$(6/N) for Skew and $\sqrt{}$(24/N) for Kurt].

The effect of diet on BW variance was studied by comparing models with homogeneous (MIXED procedure of SAS) or heterogeneous (REPEATED command in MIXED procedure of SAS) variances in each diet. Chi-squared tests (1 df, $P < 0.05$) were computed between Akaike's information criterion (**AIC**) of both models to determine when heterogeneous variances were useful to describe the model. When this was the case, CV of BW were considered to be different in each diet. To analyze the effect of diet on survival, numbers of live and dead individuals were computed in a generalized linear mixed model setting conditional distribution of the data as binomial. The Lmer procedure with family set to binomial (Vazquez et al., 2010) was used in the R software (Ihaka and Gentleman, 1996) and its lme4 library (Bates and Sarkar, 2006).

The ASREML software (Gilmour et al., 2008) was used to estimate heritabilities (using univariate models) of the traits in both diets, as well as genetic correlations (using bivariate models) between and within diets according to the following model:

$$\begin{aligned} Y_{ijklmno} = {} & Diet_{ij} + Sex_{ik} + tank(diet)_{il(ij)} \\ & + an_{im} + dam_{in} + e_{ijklmno}, \end{aligned} \quad [3]$$

where i is the trait, Yi_{jklmo} is the ith performance of individual m (i = 1 for univariate models and i = 1, 2 for bivariate models), $Diet_{ij}$ is the fixed effect of $diet_j$ on $trait_i$, Sex_{ik} is the fixed effect of sex_k (1 = male, 2 = female) on $trait_i$, $tank_{il(ij)}$ is the fixed effect of tank l (l = 1, 2, …, 5) nested within $diet_k$ on $trait_i$, an_{im} is the random additive genetic effect of trait i on animal m, dam_{in} is the random effect of dam_n on trait i (accounting for the nongenetic maternal effect), and $e_{ijklmno}$ is the random residual error for trait i.

To analyze family rankings evolution over time within each diet, genetic correlations between different measurements were estimated (1 per measurement pair); the closer to 1 the correlation was, the more consistent the family rankings were. To analyze the genotype by diet interaction, genetic correlations between a trait in the M batch and the same trait in the PB batch were estimated. In this case, diet effect was removed and environmental covariance was set to 0; the closer to 1 the correlation was, the smaller the genotype by diet interaction was. To know how the genotype by diet interaction evolved with time, these correlations were also computed between nonsimultaneous dates (for instance, between BW in M fish at 265 d and BW in PB fish at 361 d).

Heritabilities and genetic correlations of BW and TGC were used to predict the potential relative BW gain (% of BW, TGC) for 1 generation of individual selection (Falconer, 1952). Direct response expected for BW on diet X was given by

$$R_X = i_X h^2_X \sigma_X,$$

where R_X is the direct response when fish were fed a diet X for an ix intensity of selection, h^2_X is the heritability of BW in diet X, and σ_X is the phenotypic SD of BW fed

diet X. The correlated response expected for BW on diet Y when selection pressure was applied on individuals fed a diet X was given by

$$RC_Y = i_X h_X h_Y r_A \sigma_Y,$$

where RC_Y is the correlated response on BW (g) for fish fed a diet Y for a selection intensity i_x applied on candidates fed a diet X, h_X is the square root of the heritability of BW with diet X, h_Y is the square root of heritability of BW with diet Y, r_A is the genetic correlation of BW between diets X and Y, and σ_Y is the phenotypic SD of BW with diet Y.

Under farming conditions, fish are usually reared until an expected BW; thus, the correlated response was estimated for similar BW instead of similar ages. To be consistent with this choice, corresponding heritabilities and genetic correlations estimates were used. For example, assuming that mean BW of fish fed the M diet at date 3 is similar to BW of fish fed the PB diet at date 6, the correlated response of BW on the PB diet after a selection on the M diet was given by

$$RC_{PB_6} = i h_{M_3} h_{PB_6} r_{3\text{-}6} \sigma_{PB_6},$$

where RC_{PB6} is the correlated response of BW in fish fed a PB diet (based on their BW at date 6) for a selection intensity i applied when fed an M diet at date 3, h_{M3} is the square root of heritability of BW with the M diet (date 3), h_{PB6} is the square root of heritability of BW with the PB diet at date 6, $r_{3–6}$ is the genetic correlation of BW between the M diet at date 3 and the PB diet at date 6, and σ_{PB6} is the phenotypic SD of BW on PB diet at date 6.

RESULTS

Diet Effect

Survival was lower (Table 1; $P < 0.01$) in fish fed the PB diet (83.9%) than in fish fed the M diet (99.3%) at 224 d, and this difference was greater at the end of the trial (418 d), when survival rates were 64.7% in fish fed the PB diet and 93.7% in fish fed the M diet (Table 2).

Before the first feeding of experimental diets, BW were similar ($P = 0.66$) in both the M and the PB samples (Table 3). As early as the second measurement (116 d), fish fed the M diet were significantly heavier ($P < 0.001$) and longer ($P < 0.01$) than fish fed the PB diet, and these differences remained significant throughout the trial ($P < 0.01$). When considering TGC (Table 3), the PB-fed fish grew less than the M-fed fish in the first 2 periods ($P < 0.05$), but the difference disappeared after 151 d (except between 266 and 298 d). The diet effect was significant on the shape of the fish (Table 2), and fish fed the M diet were thinner ($P < 0.05$) than fish fed the PB diet after 266 d. The CV of BW (Table 4) were almost always significantly greater in the PB diet (43.3% to 34.7%) than in the M diet (34.5% to 30.5%) as the model to analyze BW with heterogeneous variances had a lower AIC ($P < 0.05$, χ^2 tests, df = 1) than the model with homogeneous variances. Greater Kurt of BW distribution in fish fed the PB diet indicated that population shape had a sharper peak than in fish fed the M diet. Greater Skew (Table 4) showed that smaller fish were proportionally more present in the PB batch.

Genetic Parameters

Heritability estimates were moderate (between 0.18 and 0.46) for BW, were stable over time in M fish, and tended to decrease in PB fish (Fig. 1). Heritability estimates were greater in M fish at each measurement, but the difference between diets was never significant (less than 2 SE). The genetic correlation for BW calculated between simultaneous measurements (Fig. 1) in both diets was a little less than 1 for the first measurement, decreased slightly during the trial, and stabilized around 0.8.

Figure 2A shows genetic correlations for BW between diets with a time dimension. For synchronous measurements (on the diagonal), the estimates are the same as those presented in Fig. 1. For asynchronous measurements, genetic correlations decreased with the time interval. On both sides of the diagonal, correlations were slightly unbalanced, and evolution can be considered as similar in both diets. The lower diagonal (Fig. 2A, dotted line) indicates a measurement when BW in each diet were similar (Table 3); genetic correlations were then between 0.8 and 0.9 in this case. Within-diet genetic correlations were high (Figs. 2B and 2C), showing a strong consistency for family structures within diets.

Table 2. Means and SD for survival on M (marine) and PB (plant-based) diets and statistical tests for diet effect

			M		PB		Statistical test for diet effect		
Days	Trait	Unit	Mean	SD	Mean	SD	df[1]	F	*P*-value
83	S	%	100.0	0.0	100.0	0.0	4		
116	S	%	99.9	0.1	97.9	0.7	4	4.9	0.0001
151	S	%	99.8	0.2	96.5	0.7	4	4.4	0.0001
224	S	%	99.3	0.5	83.9	7.5	4	2.8	0.0005
266	S	%	98.8	0.6	79.3	9.0	4	2.6	0.0009
298	S	%	98.1	1.4	72.9	9.6	4	2.3	0.0195
326	S	%	98.1	1.4	71.0	9.7	4	2.2	0.0260
361	S	%	93.8	1.5	68.0	9.5	4	2.5	0.0135
389	S	%	93.7	1.4	65.5	10.0	4	2.3	0.0229
418	S	%	93.7	1.4	64.7	9.9	4	2.2	0.0270

[1]Degrees of freedom (df) are denominator.

Table 3. Least square means (Mean) and SE for traits on M (marine) and PB (plant-based) diets and statistical tests for diet effect[1]

			M		PB		Statistical test for diet effect		
Days	Trait[2]	Unit	Mean	SE	Mean	SE	df	F	*P*-value
83	BW[3]	g	2.5	±0.1	2.6	±0.1	4	−0.5	0.6555
116	BW[3]	g	6.9	±0.2	4.8	±0.2	4	9.3	0.0007
151	BW[3]	g	11.4	±0.4	6.6	±0.4	4	7.9	0.0014
224	BW[3]	g	23.5	±0.5	13.5	±0.6	4	11.7	0.0003
266	BW[4]	g	36.1	±1.8	20.5	±1.9	3	8.0	0.0041
298	BW[4]	g	47.6	±2.3	24.8	±2.5	3	8.7	0.0032
326	BW[4]	g	55.9	±2.2	29.5	±2.3	3	12.0	0.0012
361	BW[4]	g	72.1	±2.8	38.3	±2.8	3	12.1	0.0012
389	BW[4]	g	85.3	±2.8	48.4	±2.7	3	14.7	0.0007
418	BW[4]	g	108.3	±3.7	64.6	±3.9	3	11.7	0.0013
83	SL[3]	mm	59.3	±1.0	60.0	±1.0	4	−0.5	0.6535
116	SL[3]	mm	78.9	±0.8	71.7	±0.7	4	6.8	0.0025
151	SL[3]	mm	97.5	±1.4	83.0	±1.4	4	7.3	0.0019
224	SL[3]	mm	126.5	±1.5	105.0	±1.6	4	9.7	0.0006
266	SL[4]	mm	148.7	±2.6	120.2	±2.8	3	11.6	0.0014
298	SL[4]	mm	n.e.	n.e.	n.e.	n.e.			
326	SL[4]	mm	173.4	±2.7	134.8	±2.8	3	16.2	0.0005
361	SL[4]	mm	191.4	±2.5	148.8	±2.6	3	20.0	0.0003
389	SL[4]	mm	197.5	±2.5	154.4	±2.6	3	18.3	0.0004
418	SL[4]	mm	207.8	±2.6	167.3	±2.7	3	15.6	0.0006
83	K[3]		1.17	±0.02	1.15	±0.02	4	0.6	0.5812
116	K[3]		1.37	±0.02	1.26	±0.02	4	3.8	0.0192
151	K[3]		1.18	±0.01	1.10	±0.01	4	4.4	0.0112
224	K[3]		1.12	±0.01	1.10	±0.01	4	0.8	0.4587
266	K[4]		1.0	±0.01	1.12	±0.01	3	−4.2	0.0244
298	K[4]		n.e.	n.e.	n.e.	n.e.			n.e.
326	K[4]		1.02	± 0.01	1.15	±0.01	3	−16.7	0.0005
361	K[4]		0.97	±0.01	1.12	±0.01	3	−9.2	0.0027
389	K[4]		1.05	±0.02	1.28	±0.02	3	−10.8	0.0017
418	K[4]		1.15	±0.01	1.35	±0.01	3	−15.1	0.0006
83	TGC[3]								
116	TGC[3]		1.78	±0.06	1.04	±0.04	4	10.2	0.0005
151	TGC[3]		0.89	±0.08	0.48	±0.11	4	2.9	0.0424
224	TGC[3]		0.98	±0.16	0.98	±0.09	4	0.0	0.9919
266	TGC[4]		0.77	±0.06	0.52	±0.11	4	2.0	0.1121
298	TGC[4]		0.89	±0.05	0.52	±0.06	3	1.0	0.0202
326	TGC[4]		0.63	±0.06	0.52	±0.06	3	0.9	0.4187
361	TGC[4]		0.79	±0.07	0.67	±0.09	3	1.1	0.3502
389	TGC[4]		0.69	±0.07	0.80	±0.06	3	1.0	0.2582
418	TGC[4]		1.24	±0.09	1.28	±0.01	3	−0.3	0.7899

[1]Here n.e. = not estimated.

[2]SL = standard length, K = condition factor, and TGC = thermal growth rate calculated for the previous shortest period.

[3]Analyses were done with model [1]; degrees of freedom (df) are denominator.

[4]Analyses were done with model [2]; degrees of freedom (df) are denominator.

Heritabilities and genetic correlations for TGC are shown in Table 5. When focusing on the longest period (152 d between 266 and 418 d), heritability estimates were low in both diets and significantly less in fish fed the PB diet than in fish fed the M diet (more than 2 SE). For shorter periods (60 d between 266 and 326 d and 92 d between 326 and 418 d), heritability estimates were similar in both diets. Genetic correlations between diets were moderate, ranging from 0.43 for the shortest period to 0.64 for the longest one (Table 5).

Table 4. Coefficients of phenotypic variation (CV), kurtosis (Kurt), skewness (Skew) of BW on M (marine) and PB (plant-based) diets and statistical tests for diet effect

	M			PB			Statistical test for diet effect		
Days	CV	Kurt	Skew	CV	Kurt	Skew	CV[1]	Kurt[2]	Skew[2]
83	30.5	0.0	0.7	34.7	0.8	0.7	*	*	NS
116	30.9	0.4	0.7	29.0	1.6	0.8	*	*	NS
151	32.0	-0.3	0.3	38.9	2.2	1.1	*	*	*
224	34.5	0.4	0.7	41.4	0.9	1.0	*	*	NS
266	32.7	0.6	0.7	40.9	1.8	1.2	*	*	*
298	31.9	0.5	0.7	43.3	2.4	1.3	*	*	*
326	31.3	0.5	0.7	42.9	2.9	1.3	*	*	*
361	31.7	0.4	0.6	42.9	3.7	1.4	*	*	*
389	31.1	0.2	0.6	40.3	2.8	1.2	*	*	*
418	31.1	0.1	0.5	38.3	2.2	1.1	*	*	*

[1]Traits were considered significantly different (*) when the AIC of a model with heterogeneous variances in each diet was lower ($P < 0.05$, χ^2 tests, df = 1) than the AIC of a model with homogeneous variances in each diet for BW analyses.

[2]Traits were considered significantly different (*) when they differed by more than 2 SE.

Expected Genetic Gains

Heritability and genetic correlation estimates of BW were used to predict the expected genetic gain by individual selection on BW, with a proportion of selected animals of 5% (Fig. 3). In a first scenario, selection response was estimated for fish fed the PB diet and selected when they reached approximately 37 g. If progenies were fed the PB diet, an 18.3% gain in BW per generation could be expected. If these progenies were fed the M diet, the expected gain would be reduced to 14.9%. However, there would not be any difference between these strategies if fish were selected at around 46 g (+13.4% vs. +13.1%) or 52 g (+11.8% vs. +11.2%).

In a second scenario, if fish were fed the M diet and selected when they approximately reached 35 g, a 26.3% gain in BW per generation could be expected if progenies were fed the same M diet. If these progenies were fed the PB diet, the expected gain would be reduced to 21.5%. Similar differences between these 2 strategies were observed if fish were selected around 46 g (+23.7% vs. +16.7%) or 54 g (+22.9% vs. 17.8%). Comparing the 2 scenarios, selecting fish fed the PB diet would always give lower gains in BW than selecting fish fed the M diet (Fig. 3), regardless of which diet was fed to the progeny.

Similar strategies were then tested for a selection of TGC calculated for the largest period (152 d between 266 and 418 d of age; Fig. 4). If fish were fed the M diet and selected at 418 d for their TGC, the expected BW gain of their progenies could be +9% if they were fed the same diet. However, if the progenies were fed the PB diet, the expected BW gain would be only +3%. In a second scenario, selection response was estimated for fish selected when they were fed the PB diet. The expected growth gain for their progenies would be very similar whatever diet they were fed (+3% to 4%).

DISCUSSION

Diet Effect on Health

Total replacement of FM and FO by plant-based products led to an important diet effect on survival, with a high final difference between diets (PB = 64.7%, M = 93.7%). Effects of fish oil replacement with plant-based oil on fish health have been reviewed by Montero and Izquierdo (2010) and included stress response, immune status, disease resistance, and physiological mechanisms. However, the same review concluded that evaluation of these effects is a complex subject as it is "very much dependent on the type of both FO and vegetal oil, level of FO substitution, duration of feeding, fish species, fish size and stage in the life cycle, presence and availability of other nutrients and environmental conditions, mainly temperature." In European sea bass, only a few studies focused on the health effects of substitution. Parpoura and Alexis (2001) observed a greater sensibility to pathological symptoms when sea bass are fed with plant-based oil. Piedecausa et al. (2007) found less survival due to sudden postsampling mortality in sharp snout sea bream (*Diplodus puntazzo*) fed a diet containing linseed oil. Immune system disturbances associated with plant-based products are partly due to n-3 highly unsaturated fatty acids deficiencies (Montero and Izquierdo, 2010), but more work is needed on marine fish to understand the link with pathogen sensitivity.

Diet Effect on Growth

In previous studies, partial replacement of FO with plant-based oils [characterized by the FO replacement ratio (**FOr)** = plant-based oils/total oils] produced uneven consequences on growth. Some studies showed similar growth in European sea bass for FOr = 100%

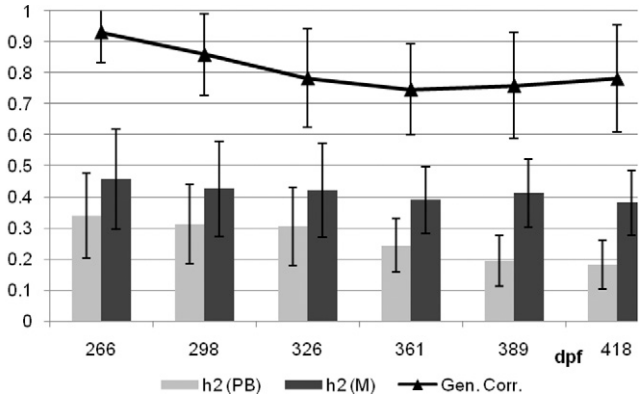

Figure 1. Heritabilities (h2) of BW for both plant-based (PB) and marine (M) diets and genetic correlations (Gen. Corr.) for this trait between diets. Estimates are calculated for each measurement in days postfertilization (dpf) and vertical bars indicate SE.

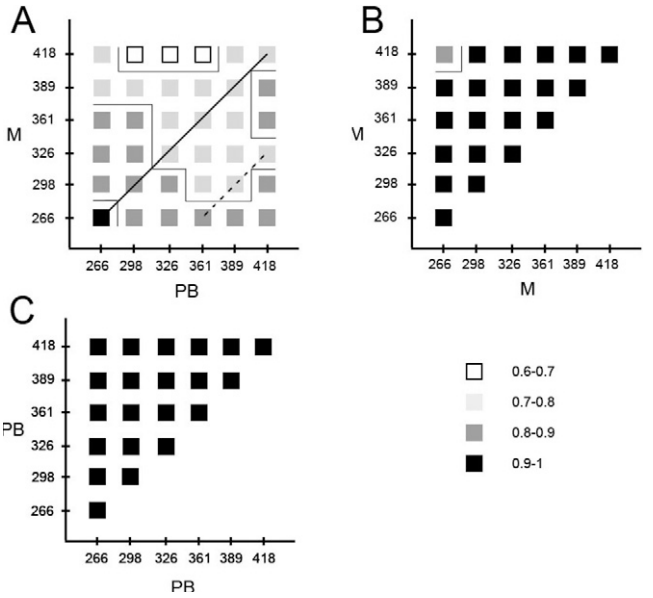

Figure 2. (A) Genetic correlations for BW between fish fed a marine (M) diet or a plant-based (PB) diet; estimates are calculated for each pair of dates. Genetic correlations for BW (B) within the PB batch and (C) within the M batch were also estimated for each pair of dates.

(Parpoura and Alexis, 2001), FOr = 60% (Izquierdoet al., 2003), FOr = 60% (Mourente et al., 2005), and FOr = 83% (Martins et al., 2006), whereas some other studies found less growth for FOr = 60% to 80% (Montero et al., 2005) and FOr = 80% (sea bream, *Sparus aurata* L.; Izquierdo et al., 2005). A review by Turchini et al. (2009) concluded that FO can be completely replaced by plant-based oils when essential fatty acid (**EFA**) requirements are met. Regarding fish meal replacement rate (**FMr** = plant-based meal/total meal), some studies evidenced less growth in marine fish for FMr = 43% (Dias et al., 2005), FMr = 100% (sea bream; Gómez-Requeni et al., 2004), and FMr = 100% (Montero et al., 2005), whereas other studies proved that replacement with plant-based meals could be done without any impact on growth for FMr = 40% (sea bream; Robaina et al., 1995), FMr = 95% (Kaushik et al., 2004), FMr = 69% (Adamidou et al., 2009), FMr = 65% (Oliva-Teles and Gonçalves, 2001), and FMr = 70% (Ballestrazzi et al., 1994).Our choice of extremely contrasted diets aimed at facilitating the observation of contrasted genetic abilities to cope with M or PB diets, and replacement rates were, respectively, FOr = 100% and FMr = 100%. This led to a significant impact on BW and SL from the first days after the dietary transition.

The TGC were less in the PB diet only for the first 151 d after starting experimental diets. After this date, no difference was visible, confirming that the first stages of such a dietary transition induced a delay in growth that remains visible in the final BW difference, as seen in older sea bass by Le Boucher et al. (2011). The initial decreased growth after the dietary transition could be due to reduced feed intake or reduced feed efficiency, and it would be interesting to know more about these traits when fish are faced with a new diet. Deficiencies for some EFA (Navarro et al., 1997), especially for a totally PB diet (Geay et al., 2010), have also been identified as a source of potential reduced growth. The effects of the PB diet in this study

Table 5. Heritabilities (h^2), common environment ratios (c^2), their SE, and variance components of genetic (σ_G), maternal (σ_m), and residual (σ_r) variance for TGC on M (marine) and PB (plant-based) diets and genetic correlations for these traits between diets

	PB							M							Genetic correlation	
TGC	h^2	SE	c^2	SE	σ_G	σ_m	σ_r	h^2	SE	c^2	SE	σ_G	σ_m	σ_r	ρ	SE
TGC[1]	0.11	±0.06	0.0	±0.0	0.5	0.0	2.4	0.33	±0.10	0.0	±0.0	0.7	0.0	1.9	0.64	±0.28
TGC[2]	0.20	±0.08	0.0	±0.0	0.5	0.0	1.8	0.35	±0.13	0.0	±0.1	1.0	0.0	1.8	0.43	±0.27
TGC[2]	0.19	±0.07	0.0	±0.0	0.2	0.0	1.4	0.31	±0.09	0.0	±0.0	0.6	0.0	1.3	0.56	±0.35

[1]TGC = thermal growth coefficients calculated for the longest period [152 d between 266 (date 5) and 418 d (date 10)].

[2]TGC = thermal growth coefficients calculated for intermediary period [60 d between 266 (date 5) and 326 d (date 7].

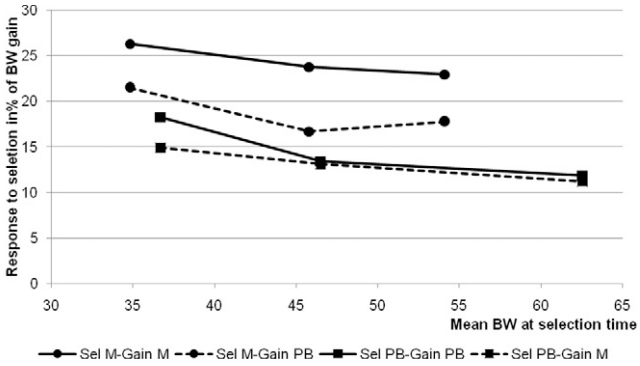

Figure 3. Direct and indirect gains (% of BW) in 4 selection strategies for 5% proportion of selected individuals: offspring of fish selected on the marine (M) diet are fed with the M diet (Sel M–Gain M), offspring of fish selected on the M diet are fed with the plant-based (PB) diet (Sel M–Gain PB), offspring of fish selected on the PB diet are fed with the PB diet (Sel PB–Gain PB), and offspring of fish selected on the PB diet are fed with the M diet (Sel PB–Gain M). Abscissa axis indicates the mean population BW at selection time.

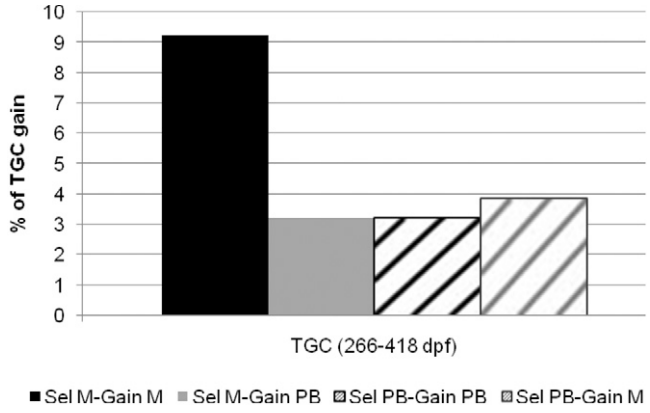

Figure 4. Direct and indirect gains [percent of thermal growth rate (TGC)] in 4 selection strategies for 5% proportion of selected individuals: offspring of fish selected on the marine (M) diet are fed with the M diet (Sel M–Gain M), offspring of fish selected on the M diet are fed with the plant-based (PB) diet (Sel M–Gain PB), offspring of fish selected on the PB diet are fed with the PB diet (Sel PB–Gain PB), and offspring of fish selected on the PB diet are fed with the M diet (Sel PB–Gain M). Selection trait was TGC calculated on the largest period [152 d between 266 and 418 days post-fertilization(dpf)].

could be a combination of confounding factors, and more studies are needed to determine the respective importance of feeding behavior, diet palatability, digestibility, nutritional value (Glencross et al., 2007), or genetic abilities to cope with these effects, especially in the early stage.

The population size distribution was also impacted, as fish fed the PB diet showed a more heterogeneous repartition of BW. The CV has been suggested as an indicator of welfare (Jobling, 1995; North et al., 2006), and high heterogeneity is known to facilitate cannibalism (Baras, 1999; Fessehaye et al., 2004). In our case, greater CV and skewness confirmed that the cohort of fish fed the PB diet faced challenging environmental conditions. When temperature, salinity, and zootechnical conditions are the usual suspects for environment variability, the composition of the feed should clearly be considered as one of the main parameters of this environment.

In the present trial, it is difficult to distinguish the diet effect from the diet switch effect at the beginning of the trial. Both batches received a commercial diet containing marine products and plant products before the beginning of the trial, and the diet switch shock may explain an important part of the observed diet effect. More information is needed to understand the effect of plant-based diets on European sea bass juveniles.

Genetic Parameters for BW

Genetic variability estimates for the ability of fish to grow on a PB diet are needed to know whether genetic improvement on growth can be made using specifically PB diets. Most of the previous results have been obtained on salmonids. Palti et al. (2006) concluded that genetic variability for growth and BW exists in rainbow trout (*Oncorhynchus mykiss* W.) in case of partial substitution (FOr = 11.4%, FMr = 87%). Pierce et al. (2008) and Quinton et al. (2007a) found moderate heritabilities both for growth (0.31 ± 0.07) in rainbow trout (FOr = 37.2%, FMr = 93.7%) and for individual daily BW gain (0.32 ± 0.14) in European whitefish (*Coregonus larvaretus* L., FOr = 0%, FMr = 73.3%). In the present study, heritabilities of BW when fish were fed the M diet were very similar (Saillant et al., 2006; Dupont-Nivet et al., 2010) or slightly less (Dupont-Nivet et al., 2008) than those previously found in European sea bass. The differences between the M and PB diets were never significant, but lower heritabilities for fish fed the PB diet was a tendency that has also been suggested in a previous work (Le Boucher et al., 2011). These results proved nevertheless proved that genetic gains for growth are still possible in fish fed the PB diet (h^2 between 0.18 and 0.36).

Genotype by diet interactions could impact the efficiency of breeding programs initially conducted on mainly marine diets, but previous results are variable. Comparing diets with different proportions of plant-based products, Blanc (2002) and Palti et al. (2006) found nonsignificant interactions for BW in rainbow trout (FOr = 0%, FMr = 47.6% and FOr = 11.4%, FMr = 87%, respectively). In 2007, 2 studies with European whitefish recorded nonsignificant reranking of family performances for whole-body lipid, protein percentages (Quinton et al., 2007a) and growth, feed intake, and feed efficiency (Quinton et al., 2007b; FOr = 0%, FMr = 73.3%) and evidenced high genetic correlations between diets for these traits (0.89 to 1.00). In contrast, significant reranking of the families for growth (Pierce et al., 2008; Dupont-Nivet et al., 2009;

FOr = 0%, FMr = 100%) as well as feed intake and feed efficiency (Dupont-Nivet et al., 2009) were found in rainbow trout, and in this case genetic correlation was moderate between diets (0.73 ± 0.13; Pierce et al., 2008). In European sea bass, total substitution rate was recently tested with older fish, starting at 192 g mean BW (FOr = 100%, FMr = 100%), and low genotype by diet interactions were evidenced for lipid content of the filet and late BW (Le Boucher et al., 2011) as the genetic correlations between diets were never significantly different from 1.

When fish were compared at the same BW, which could be the classical selection condition, the genetic correlation between diets observed in the present study was around 0.7 to 0.8. Standard errors are still too high to observe significant evolution over time, but the greatest genetic correlation was found at the first individual measurement (266 d). It would tend to indicate that family rankings were not modified in the early stages after dietary transition, but this would have to be ascertained using individual tagging of smaller fish.

In the absence of studies about the optimal design for genotype by environment interactions, we chose to use a relatively low number of sires to increase the mean half-sib family size and avoid the risk of having very unbalanced offspring numbers for the same family in both diets (due to a posteriori assignment and potential differential mortalities), which we anticipated would lower the quality of genotype by environment estimates. In the course of the present study, the publication by Sae-Lim et al. (2010) of an optimal design simulation study for genotype by environment estimation proposed that 200 families with 10 individuals per family would be appropriate for a trait of moderate heritability. This is not far from our figures in terms of full-sib families. However the structure they used was a nested design, which implies the use of more sires and dams for the same number of families and hence reasonably suggests that the number of parents that we used was below the optimum and may lower the precision of our estimates.

In the present study, high genetic correlations (0.8 to 0.9) were observed between diets for synchronous measurements of BW. It confirmed that total substitution of FM and FO would not deeply impact family rankings for BW. As a comparison, genotype by environment interactions for BW have been studied in contrasting rearing conditions and production systems in European sea bass (Dupont-Nivet et al., 2008). Dupont-Nivet et al. concluded that genetic correlation for BW was 0.84 ± 0.08 (mean ± SE) comparing a semiclosed recirculation system (30 kg/m3, 20°C to 22°C) and a semi-intensive estuarine system (2 kg/m3, 9°C to 25°C). In the same work, the genetic correlation for BW was 0.70 ± 0.10 comparing a semi-intensive estuarine system (2 kg/m3, 9°C to 25°C) and a floating cage in tropical waters system (4 kg/m3, 22°C to 27°C). This result confirms that dietary environments could impact BW performance in the same range as culture conditions.

Genetic Parameters for Growth Rate

Individual tagging was only possible at 224 d for fish fed the M diet and 266 d for fish fed the PB diet (135 and 177 d after dietary transition, respectively), when BW was already strongly influenced by the diet. The first BW measurement was also influenced by the commercial diet given until 87 d, which was not linked with the experimental diets. Therefore, the consequences of the dietary challenge are indirectly estimated by BW, and TGC gave a more realistic performance estimate than final BW as it is not dependent on the initial BW. Dupont-Nivet et al. (2010) evidenced greater genotype by environment interactions for daily growth rate than for BW comparing rearing conditions and production systems. In our experiment, the genetic correlation between TGC (266 to 418 d postfertilization) in each diet was lower (0.64) than those found for BW at any date (0.74 to 0.93). This result confirmed that the genotype by diet interaction was more important for this trait and thus that families showed different abilities to grow depending on the diet.

Expected Genetic Gains

Increased BW gains for BW would be possible in European sea bass (18.3% for a 5% selection pressure) if selected breeders and their progenies were fed the PB diet. If progenies issued from this strategy were fed the M diet, the gain in BW would similar. This means that selecting fish for their ability to grow on the PB diet would lead to increased genetic BW gains whatever the diet (M or PB) given to the progenies (Fig. 3). The reason is that the greater genetic variation we evidenced with the M diet would compensate for the reduced accuracy of selection with the PB diet and the less than unity genetic correlation between diets.

Greater gains for BW would be possible (26.3% for a 5% selection pressure) if selected breeders and their progenies were fed the M diet because of a greater accuracy of selection. If progenies issued from this strategy were fed a PB diet, the gain in BW would be less (21.5%). To anticipate a transition from the M to PB diet and maximize the genetic gains on BW, it would be more interesting to continue with a selection using the M diet. Expected genetic gains from selection for TGC would lead to similar genetic gains (3%) in progenies fed the PB diet whatever the diet used during selection (Fig. 4). The cumulative effect of a medium genetic correlation (0.64) and a low genetic variance for TGC ($h^2 = 0.11$) in fish fed the PB diet rendered the strategy to select on the M diet less efficient for TGC than for BW.

Expected indirect gains were estimated comparing fish fed the M or PB diet with similar BW rather than at the same age. The objective was to remain close to the commercial conditions where fish are harvested at a (market-driven) fixed mean BW.

We also made the choice to compare noncommercial but extremely contrasting diets, so conclusions can only be used as boundaries to address the current challenges of breeding programs. For instance, the strong effect of a totally PB diet on phenotypic traits may have hindered the expression of essential adaptation mechanisms and created metabolism bottlenecks. The low heritability estimates on the PB diet may partly reflect a reduced health status of fish fed the PB diet and should be considered as a consequence of our extreme choice in diet composition.

In a similar way, the impact of the totally PB diet on global survival may also have biased genetic parameter estimates, and more studies are needed to orient breeding strategies in their adaptation to dietary transition in European sea bass.

Fish farming faces rapid diversification of rearing environments, and proof of genotype sensitivity to environment exists as fish are extremely sensitive to temperature, salinity, and stocking density (Jackson et al., 1998; Streelman and Kocher, 2002; Dupont-Nivet et al., 2010). However, the dietary environment, whether it concerns feed composition, access to rations, or feeding rhythms, has only recently been considered as a potential source of interaction with genotypes. This is why it is very important to have a better knowledge of the effect of these parameters on breeding values to improve on-site efficiency of breeding programs. Our estimation of predicted gains tends to prove that a totally PB diet compared with an M diet would have a negative impact on breeding program efficiency. We showed that selecting European sea bass on their ability to grow on the experimental PB diet would not be more efficient than pursuing a breeding program using the experimental M diet, even if commercial fish are grown on a PB diet. This is consistent with the beneficial selection in an alternative environment described by Falconer (1952). As summarized in Kause et al. (2006), it could be beneficial to select breeders in an environment that is not the production one when the heritability of the trait in this environment and genetic correlation between environments are high. The extreme choice in diet ingredients allowed estimating genotype by diet interactions in a worst-case scenario, but more studies are needed to understand how intermediate substitution rates and product quality would impact selective breeding of sea bass.

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