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Genotype by diet interactions on growth and processing traits in rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and meagre (*Argyrosomus regius*) fed diets with almost complete substitution of both fish meal and fish oil by vegetal ingredients

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ABSTRACT: With oceanic resources depletion, limitation of marine ingredients in aquafeed is a major challenge for the industry. Our study estimated and compared genotype by diet interactions (G*D) on production traits in 4 major fish species reared in temperate and Southern Europe (rainbow trout, sea bass, sea bream, meagre). DNA-assigned sibs were challenged with a diet containing marine ingredients (MB) or with a plant-based diet (PB) containing only 2% of fish meal and 2% of fish oil (FOM). Similar to higher heritabilities were estimated in PB condition. Limited G*D was estimated for all traits. However, sea bream stopped growing and genetic correlation on growth decreased. Moreover, 90% of the meagre fed with PB died after a sudden drop of water temperature. It was hypothesized that marine fishes mostly lived on their initial reserves acquired before the challenges and that expected non limiting FOM concentrations may be detrimental.

Key words: Aquaculture, rainbow trout; sea bass, sea bream; meager; genotype by diet interactions (G*D)

Introduction

Over the last decades, fish aquaculture has faced dramatic changes in feed formulation (Barrows et al. (2009)). Indeed, aquaculture development led to greater demand in fish oil and fish meal, contributing to increase pressure on wild fish stocks (Naylor et al. (2000)). In order to mitigate this environmental pressure and to balance production costs it became urgent to identify alternative feed ingredients sources. Among them, vegetal ingredients, either as a source of proteins or lipids at different substitution rates constitute a major change for predatory fish (Médale and Kaushik (2009)).

The recent, fast and independent co-evolution of feed composition and breeding programs raises issues about the consequences of changes in feed composition and potential interactions between feed and selection. Indeed, changes in diets could impair the efficacy of selection on growth or processing traits. Inversely, selection of fish on ability to perform on more and more substituted feeds may also offer innovative approaches to limit the environmental footprint of fish aquaculture.

In farmed fish species, preliminary experiments investigated genotype by diet interactions (G*D) with partial and limited replacement of fish meal or fish oil (Blanc (2002); Palti et al. (2006); Quinton et al. (2007); M. Dupont-Nivet et al. (2009); Pierce et al. (2008)). The effect of total replacement of both sources of nutrients was also considered (Le Boucher et al. (2011ab and 2012)). However these last pioneering experiments were not consistent with the industry practices that replace gradually both marine proteins and lipids.

This presentation compares for the first time high, but not total, combined fish meal and fish oil (FOM) replacement in selection schemes of four major fish species reared in temperate and southern Europe: a Salmonid, the rainbow trout (*Oncorhynchus mykiss*); two Perciforms the European sea bass (*Dicentrarchus labrax*) and the sea bream (*Sparus aurata*) and a Sciaenid the meagre (*Argyrosomus regius*). For each species, families were challenged either with commercial feed (MB) or with plant-based diet (PB) containing only 2% of fish meal and 2% of fish oil. Genetic parameters of growth and processing traits were estimated within and between diets. Results are expected to provide clues to optimize selective breeding programs.

Materials and Methods

In each species, partly factorial mating designs were created using artificial fertilization. These designs permit to get large number of families and parents represented among the offspring, which leads to sound estimates of genetic parameters (Dupont-Nivet et al. (2002)). 100 sires and 96 dams were used in rainbow trout, 45 sires and 15 dams in sea bass, 79 sires and 21 dams in sea bream, 13 sires and 3 dams in meagre.

The spawn of each dam was incubated separately. For marine fishes, equal number of hatched larvae were mixed and reared in a single larval tank to avoid tank effect. In rainbow trout, each spawn was managed according to Haffray et al. (2012a) to avoid potential non-genetic maternal bias prior to gathering all families in a single tank.

Table II. Mean \pm standard deviation ($\mu \pm \sigma$) and number of fish (n) measured at slaughtering for the different traits listed below in trout, sea bass and sea bream, either in the marine (MB) or in the plant-based (PB) batch

Trait	Rainbow trout				European sea bass				Gilthead sea bream			
	MB		PB		MB		PB		MB		PB	
	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n
BW	1212 \pm 235	1598	1112 \pm 280	1682	905 \pm 205	966	774 \pm 226	948	414 \pm 72	1744	253 \pm 58	1610
L	458 \pm 26	1580	417 \pm 35	1680	391 \pm 28.2	960	370 \pm 32	934	262 \pm 13	1734	227 \pm 15.0	1610
K	1.26 \pm 0.12	1573	1.50 \pm 0.12	1668	1.49 \pm 0.14	960	1.50 \pm 0.15	929	2.29 \pm 0.20	1734	2.12 \pm 0.22	1608
Fat	-	-	-	-	11.2 \pm 4.2	964	13.1 \pm 4.8	947	13.9 \pm 2.1	1739	7.6 \pm 3.0	1612
CY	87.6 \pm 1.4	1580	86.9 \pm 1.8	1672	89.7 \pm 1.9	787	89.3 \pm 1.9	775	91.8 \pm 1.4	1711	94.0 \pm 1.4	1587
HGY	78.7 \pm 1.6	1567	78.4 \pm 1.8	1663	68.2 \pm 1.8	855	66.6 \pm 2.3	853	72.3 \pm 1.6	1708	70.0 \pm 2.2	1589
HY	8.5 \pm 0.7	1582	7.9 \pm 0.8	1657	19.3 \pm 1.7	852	20.1 \pm 2.2	849	19.0 \pm 1.4	1714	23.5 \pm 2.1	1588
FY	-	-	-	-	55.8 \pm 2.4	853	54.6 \pm 2.9	843	-	-	-	-

Body weight (BW, g); condition coefficient (K, g/cm³); muscle adiposity indirectly estimated with Torry Fish Fat Meter (Fat, %); carcass yield (CY, %); headless gutted carcass yield (HGY, %); head yield (HY, %); fillet yield (FY, Y); non available (-)

Tableau I. $h^2 \pm$ standard error for each trait in MB and PB batches (h^2_{MB} ; h^2_{PB}) and genetic correlation \pm standard error of the trait between batches at slaughtering (R_g)

Trait	Rainbow trout			European sea bass			Gilthead sea bream		
	h^2_{MB}	h^2_{PB}	R_g	h^2_{MB}	h^2_{PB}	R_g	h^2_{MB}	h^2_{PB}	R_g
BW	0.26 \pm 0.05	0.42 \pm 0.06	0.92 \pm 0.06	0.19 \pm 0.05	0.35 \pm 0.08	0.92 \pm 0.06	0.30 \pm 0.06	0.34 \pm 0.06	0.81 \pm 0.09
K, g/cm ³	0.23 \pm 0.05	0.38 \pm 0.06	0.95 \pm 0.05	0.37 \pm 0.09	0.44 \pm 0.10	0.98 \pm 0.05	0.27 \pm 0.06	0.38 \pm 0.07	0.77 \pm 0.08
Fat, %	-	-	-	0.31 \pm 0.07	0.41 \pm 0.09	0.88 \pm 0.09	0.37 \pm 0.07	0.54 \pm 0.08	0.74 \pm 0.09
CY, %	0.54 \pm 0.06	0.60 \pm 0.07	0.99 \pm 0.02	0.54 \pm 0.10	0.55 \pm 0.10	1.00 \pm 0.00	0.55 \pm 0.08	0.25 \pm 0.05	0.80 \pm 0.08
HGY, %	0.49 \pm 0.06	0.54 \pm 0.06	0.99 \pm 0.03	0.35 \pm 0.08	0.42 \pm 0.09	0.89 \pm 0.08	0.37 \pm 0.07	0.24 \pm 0.05	0.73 \pm 0.10
HY, %	0.28 \pm 0.05	0.38 \pm 0.06	0.89 \pm 0.06	0.32 \pm 0.08	0.35 \pm 0.09	0.94 \pm 0.07	0.26 \pm 0.06	0.31 \pm 0.06	0.62 \pm 0.12
FY, %	-	-	-	0.20 \pm 0.06	0.34 \pm 0.08	0.90 \pm 0.09	-	-	-

Non available (-)

PB diets were formulated to be isolipidic, isoproteic and isoenergetic with MB commercial feed currently used by the industry. Pellets of the size and composition adapted to the age of the animals were automatically distributed in batches. Rations were daily calculated according to each tank biomass.

For each species, fish were randomly divided into 2 tanks to be challenged with PB or MB diets. The animals were individually tagged before the challenge which started at 50g, 190g, 40g and 60g respectively in trout, sea bass, sea bream and meagre. Individuals were *a posteriori* DNA assigned by LABOGENA (Jouy-en-Josas, France) using microsatellites and a combined exclusion and likelihood assignment method. Batches were slaughtered at the same age for sea bass (715 dpf). In rainbow trout, the PB batch was slaughtered one week after the MB batch (557 and 564 dpf). Sea breams were not killed at same age in an attempt to obtain animals of similar weight at slaughtering (434 and 553 dpf). The meagre slaughtering could not be scheduled since the PB batch died (90% mortality) in one-week span during winter after a sudden water temperature drop (from 16.5 to 10.0°C).

At slaughtering, fillet yield was indirectly estimated by headless gutted carcass yield (HGY) as this trait is highly genetically correlated with fillet yield (Haffray et al. (2012b)).

VCE software was used to estimate genetic parameters. Within-batch heritability (h^2) estimation was based on univariate animal model. Genetic correlations

between traits (R_g), either within- or between-batch required the use of bivariate models.

Results

Delay in growth had been observed in rainbow trout (1212g in MB batch vs. 1112g in PB batch at slaughtering), in sea bass (903g vs. 765g) and sea bream (414g vs. 235g). Contrary to trout and sea bass, there was a difference of mortality in sea bream: 12% in the MB batch vs. 18% in the PB batch. In meagre, despite the abortion of the experiment, individual body weight (BW) was recorded in the MB batch on 436 fish (236g mean BW), 9 months after the beginning of the trial, and h^2 of BW reached 0.78 \pm 0.20. The remaining PB fish reached 137g mean BW at the same age. Tables I and II describe phenotypic data and genetic parameters estimates in the other three species.

Discussion

The h^2 estimate for BW obtained in meagre is promising as it is the first one ever reported in this species. However, it is impossible to assess accurately the G*D between the two nutritional conditions due to overmortality in the batch fed PB diet. Nevertheless, this highlights a potential interaction between feed substitution and temperature tolerance in this Sciaenid species. Indeed, PB diet impaired somehow the growth and the immune system of the animals which could not face low winter temperatures like the control batch did.

Whatever the species (except meagre), the traits and the diets, h^2 were in the range of expectations for rainbow trout (Gjedrem (1983); Kause et al. (2003); Pierce et al. (2008); Dupont-Nivet et al. (2009); Le Boucher et al. (2011a), Haffray et al. (2012b)), European sea bass (Saillant et al. (2006); Dupont-Nivet et al. (2008); Vandeputte et al. (2009); Le Boucher et al. (2011b)) and gilthead sea bream (Navarro et al. (2009)). Always above 0.24 in the PB batch, h^2 estimates confirmed previous results of additive genetic variation for all traits when fed PB diet. Therefore genetic improvement of all production traits considered here is achievable. The higher h^2 generally observed in PB diet suggests that this dietary environment may amplify the expression of the fish genetic potential. However, it was not possible to disentangle between a higher additive genetic variation with PB diets and the occurrence of some nutritional deficiency or anti-nutritional factors for some families, even if feed compositions were designed to avoid such bias. Measuring genetic response to selection in the offspring will be the only way to confirm such higher additive genetic variation.

Our results confirmed previous insignificant G*D interactions in rainbow trout (Palti et al. (2006); Le Boucher et al. (2011a)). This was in contradiction with other authors that reported significant G*D (0.73 ± 0.13 for BW at 600g mean weight) (Pierce et al. (2008)) or clones re-ranking during early growth (Dupont-Nivet et al. (2009)).

In sea bass, previous experiments concluded to the presence of G*D interaction for BW (0.51 ± 0.34) but not for the other traits (Le Boucher et al. (2011b)). As feeding challenges started at similar mean BW (192g, 588dpf), this apparent discrepancy between the two experiments could partly be explained by the fact that extreme feeding challenge was used in the previous work with PB diet totally devoid of FOM. Our results with 98 % substitution provide new insight of limited G*D interaction in sea bass in more commercial-like feed conditions. However, the challenge started late and the experimental phase ended when animals were already much heavier than the usual market size of 350g. Thus, different results might have been expected if the feeding challenge had been initiated much earlier, e.g. when growers receive their juveniles from hatcheries.

In sea bream, limited G*D interaction was reported for BW and CY. This means that there are some genotypes more able to perform under PB diet. But it has to be noticed that fishes fed PB diet showed higher mortality rate and stopped growing even if still eating. This is the first time that such phenomena are reported under PB diet conditions, even with 2% of FOM. It could be hypothesized that early fish growth required initial stocks of limiting substances (e.g. poly-unsaturated fatty acids), differences appearing gradually as fishes ran out of these essential nutrients.

To sum up, rainbow trout and sea bass did not exhibit G*D interaction for growth, on the contrary to sea bream. However, the challenge was initiated at larger size

in sea bass than in sea bream. Therefore, higher G*D interactions could have possibly been observed if the challenges had started at smaller BW in sea bass, but also in sea bream. Still, except for CY in sea bream, all the processing traits exhibited high R_g between diets. This means that growth and processing traits are genetic characters with limited genotype by environment interaction, at least in these three species. Adaptation of feeding practices with “finishing” diets complemented in FOM, or succession of highly substituted and non-substituted feeds, may also limit expression of G*D and the necessity to select under highly substituted feed.

Conclusions

Challenging meagre with a diet containing only 2% of both fish meal and fish oil during growth in commercial selective breeding condition resulted in extreme mortality rate under our experimental conditions. Survival was not impaired in rainbow trout nor in sea bass. The growth delay observed in fish fed PB diet should be overcome within a few selection runs, at least in rainbow trout and sea bass. Indeed, only moderate G*D interactions were observed in this experiment and the genetic parameters estimates imply that selection in a PB environment for production traits is possible. It means that selective breeding companies producing rainbow trout could set up selection programs feeding their fish PB diet. Companies selecting sea bass could do likewise, paying special attention to the moment at which they start the feeding challenge. The breeders could choose to feed the fish MB diet to warranty reproductive success, since there is no available data yet regarding the related production traits while fed the whole life cycle PB diet. Concerning sea bream, there is at present too much uncertainty to initiate reasonably such a breeding program relying on PB diet.

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