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The effects of dietary marine protein hydrolysates on the development of sea bass larvae, *Dicentrarchus labrax*, and associated microbiota

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Abstract:

Protein hydrolysate is an essential component of dry starter diets for fish larvae, as promoting healthy development. Peptides are also suitable substrates for many intestinal microbes. Five experimental diets were compared to a control diet (CONT) supplemented with a commercial fish protein hydrolysate. Each diet contained one marine protein hydrolysate, which differed by the proportion of di- and tri-peptides, and by raw materials. Two diets (HYD4 and HYD5) stimulated larval growth compared with CONT. Two other diets (HYD1 and HYD2) yielded inferior growth. HYD1 was detrimental for survival, which was associated with an up-regulation of genes involved in inflammation and antioxidative responses, while the gene coding for osteocalcin was down-regulated in this group. The relative activity of enzymes in the brush border membrane of enterocytes was significantly stimulated with diets HYD3 and HYD4. Gut microbiota were influenced by the diet, but the bacterial community profiles observed with HYD3 and HYD4 were the only ones that were not significantly dissimilar. Groups HYD1 and HYD2 showed the most dissimilar microbiota. It was concluded that the nature of the marine protein hydrolysate is important for larval development and health, but a high proportion of small peptides is not a sufficient criterion to assess dietary value.

Keywords: compound diets ; denaturing gradient gel electrophoresis ; digestive enzymes ; gene expression ; gut maturation ; peptides

1. Introduction

Sea bass, *Dicentrarchus labrax*, is among the main species exploited in southern European aquaculture. The hatchery production has benefited from the ability of sea bass larvae to start to feed at mouth opening on microbound diets, whose composition still needs to be refined. Cahu & Zambonino-Infante (2001) emphasized the interest of incorporating di- and tri-peptides in starter diet to improve fish larval development. Suitable sources of such peptides include marine protein hydrolysates, which are increasingly used for their bioactive properties (Harnedy & FitzGerald, 2012). The protein source and the degree of hydrolysis affect the dietary efficiency of protein hydrolysates for sea bass and sea bream larvae, and the optimum level of incorporation is around 10% of the dry diet (Kotzamanis *et al.* 2007; Gisbert *et al.* 2012). The healthy development of sea bass larvae is dependent on associated microbiota, and the composition of starter diets should be also aimed at controlling bacterial colonisation in the gut (Dimitroglou *et al.*, 2011). Short peptides are used as substrate by many intestinal microbes, and Swiatecka *et al.* (2012) considered pea protein hydrolysate as a tool to modulate the interaction between bacteria and host's enterocytes. Kotzamanis *et al.* (2007) showed the effect of fish hydrolysates on culturable bacteria associated with sea bass larvae, especially *Vibrio* spp. Peptide-enriched live feed stimulated the immune response in first feeding Atlantic halibut, but not the associated microbiota (Hermannsdottir *et al.* 2009).

In the light of these results, the present experiment evaluated several pilot hydrolysates of marine origin as feed component for sea bass larvae, with particular attention to gut maturation, developmental marker genes, and gut microbiota. Gut maturation and the onset of digestive enzymes are critical to assess fish larval development (Cahu & Zambonino-Infante, 2001). The expression level of the gene coding for osteocalcin appeared as a good descriptor of bone mineralization and harmonious development in sea bass larvae (Darias *et al.*, 2010; Lamari *et al.*, 2013). Interleukin-1β is a pleiotropic cytokine involved in the regulation of innate and adaptive immune responses, inflammation and haematopoiesis. The transcript level of the corresponding gene was used to document the resistance of sea bass against vibriosis (Chistiakov *et al.*, 2010), and the immune status of sea bass larvae (Picchietti *et al.*, 2009). The production of reactive oxygen species is essential for the microbial killing capacity of macrophages in case of infection, while it requires the activation of antioxidant enzymes to detoxify fish tissues (Reyes-Becerril *et al.*, 2011). The transcript level of catalase, glutathione peroxidase and superoxide dismutase genes were thus estimated in sea bass larvae, according to Tovar-Ramírez *et al.* (2010). RT-PCR-DGGE appeared as a convenient tool to evaluate the influence of dietary manipulations on the active bacterial community associated with fish larvae (Lamari *et al.*, 2013).

2. Materials and methods

Sea bass larvae were allotted at 4 dph (day post hatch) in 24 tanks of 35 L (3000 larvae/tank), and reared in the general conditions described by Lamari *et al.* (2013). The 24 tanks were divided in six groups of four tanks, which were fed the compound diets from 8 dph onwards. The six diets differed by their supply in marine animal protein hydrolysate.

The control (CONT) corresponded to the commercial fish protein hydrolysate CPSP G, used in previous experiments (Kotzamanis et al., 2007). Three French private companies (Aquativ, Elven; Copalis, Boulogne-sur-Mer; PhosphoTech, Saint-Herblain) provided five experimental hydrolysates made with marine animals (fish, molluscs, or shrimps), which were tested in separate diets (HYD1-HYD5; Table 1). The amounts of the hydrolysates were adjusted to bring almost the same level of nitrogen to the diet, and fish oil was added to diets HYD2, HYD3, and HYD5 to homogenize the levels of fatty acids.

The degree of hydrolysis of the marine proteins was evaluated with HPLC/UV (Agrobio, Vézin le coquet, France; Fig. 1). HYD2 and HYD3 featured almost the same profile of molecular masses as CPSP G (CONT), while the three other hydrolysates contained a high proportion of di- and tri-peptides (500>M>200).

The expression levels of functional genomic markers in the whole larvae were measured by real time PCR at 24, 42, and 60 dph, according to the method of Mazurais *et al.* (2008; two samples of 100 mg larvae in each tank at each sampling date). For gene expression analysis, Ef1 (elongation factor 1 α) was used as reference gene since it did not exhibit significant variation between samples (relative standard deviation<5%). At 25 dph, two sets of 50 larvae were sampled in each tank to assess the maturation level of the digestive system by measuring the indicators of two consecutive steps in the maturation process: (1) the level of secretion of pancreatic amylase and trypsin, and (2) the relative activity of enzymes from the brush border membranes of enterocytes that correspond to the differentiation of these cells, according to the methods cited by Kotzamanis *et al.* (2007). At 36-37 dph, between 500 and 1200 larvae were removed from the tanks, depending on survival, with a view to maintain the biomass at a convenient level for optimal growth. The larvae were regularly sampled in three of the four replicates per diet (at 24-25, 42 and 59-60 dph), and the fourth tank was used to estimate final survival at 60 dph.

The bacterial community associated with sea bass larvae was analysed at 59 dph. At this stage, the fish were big enough for hasty dissection in sterile Petri dishes on ice. The head and the dorso-posterior section were separated from the abdominal section, which was retained for microbial analysis. A particular attention to the active microbiota was done by focusing on ribosomal RNA. To this end, the abdominal sections of five individuals were sampled in each tank at 59 dph (15 individuals per treatment), and the samples were fixed in Extract-All® (Eurobio) immediately after dissection. The RT-PCR-DGGE was performed as described by Lamari *et al.* (2013). After DGGE, some bands corresponding to Operational Taxonomic Units (OTUs) were picked up from the gels, and treated as described previously by Silva *et al.* (2011). The final PCR products were cleaned up with GenElute (Sigma), and sequenced by MilleGen Biotechnologies (Labège, France). A series of six gels with 25 RT-PCR products each were successively analysed, five gels for binary comparisons between each experimental group compared to CONT, and the sixth with samples from groups HYD3, HYD4, HYD5, and CONT. The bacterial community profiles (BCPs) observed on the six gels were aligned with the replicated samples. For these replicates, the mean abundance of each OTU and the mean diversity indices were computed to obtain one mean profile per sampled larva. The diversity indices were computed with the Paleontological Statistics Software Package (PAST; Hammer *et al.* 2001), and compared with ANOVA or Kruskal-Wallis. The similarity of BCPs was studied among the six dietary groups with the Bray-Curtis indices, which were compared by analysis of similarities (ANOSIM; Clarke 1993). The most dominant bands were classified

depending on their contribution to dissimilarity with similarity percentage (SIMPER; Clarke 1993). The overall average dissimilarity between two groups was computed as 1 minus the average of the inter-group Bray-Curtis similarity indices, multiplied by 100.

3. Results

3.1. Growth and survival

The survival was estimated in one tank per treatment (Table 2). Though the statistical comparison was not possible, the low survival measured with diet HYD1 was observed in the three other tanks of this group. The survival rates of the other groups were in the normal range generally obtained in hatcheries. At 30 dph, the groups HYD2, HYD3, HYD5 and CONT had individual mean weights that were not significantly different among them (circa 3mg, Table 2), while significantly higher and lower growth rates were obtained with diets HYD4 and HYD1, respectively. At 60 dph, there were still significant differences in growth. The lowest mean weights corresponded to diets HYD1 and HYD2, and the highest ones were obtained with HYD4 and HYD5. The intermediary level corresponded to group HYD3, the mean weight of which was not significantly different from that of the control group.

3.2. Gut maturation

The level of secretion of pancreatic enzymes (Table 3) was generally not different between groups at 25dph, except the inferior proportion of amylase and trypsin that was secreted in groups HYD1 and HYD2, respectively.

The relative activity of enzymes in the brush border membrane of enterocytes indicates the degree of maturation reached by the digestive system in metamorphosing larvae at 25 dph. The relative activity of amino-peptidase N showed that groups HYD3 and HYD4 were more mature than the other groups at 25 dph (Table 3). This was confirmed by the relative activity of alkaline phosphatase, and group HYD4 appeared as the most advanced in this regard.

3.3. Relative gene expression of markers of the development

Interleukin 1-ß was used as a marker of the immune system, and the corresponding gene was up-regulated at 24 dph in group HYD1, compared to groups HYD2, HYD4 and HYD5 (Fig. 2). At the same age, the gene coding for catalase was overexpressed in group HYD1 compared to group HYD2. The expression levels of two other genes coding for antoxidative enzymes, glutathione peroxidase and superoxide dismutase, were not significantly affected by the diet (data not shown). The fastest ossification process seemed to occur in group HYD5, as the gene coding for osteocalcin was up-regulated in this group, compared to groups HYD1 and HYD3 at 24 dph, and compared to groups HYD1 and CONT at 42 dph. At 60 dph, this gene was under-expressed in group HYD1, compared to the control group.

3.4. Gut microbial community at the end of the experiment

At 59 dph, The profiles of the active microbiota assessed by RT-PCR-DGGE were dissimilar between groups (ANOSIM; R= 0.479, p<0.0001), and all the pairwise comparisons indicated highly significant Bonferroni-corrected p values, except that between groups HYD3 and HYD4, whose mean BCPs were not significantly dissimilar (p=0.24). A high overall dissimilarity of 75% was observed between all dietary groups with SIMPER analysis. The paired comparisons of overall dissimilarities showed that the bacterial profiles of groups HYD1 and HYD2 were the most dissimilar from the profiles of every other group (Table 4). The main OTUs that contributed to the overall dissimilarity between tanks were generally not identified, but three were particularly abundant in group HYD5. Enterobacteriaceae AK (98% identical with Genbank ID KC255196) was the main contributor of dissimilarity between HYD5 and the control group (4.5% of the overall dissimilarity). Moraxellaceae AE (99% identical with Genbank ID KC502909) accounted for 3.4% of the overall dissimilarity between groups HYD5 and HYD2. Methylobacteriaceae C (99% identical with Genbank ID KC776527) accounted for 2.4% of the overall dissimilarity between groups HYD5 and HYD4. *Staphylococcus* sp. AD (100% identical with Genbank ID JX861157) were less abundant, and its main contribution to dissimilarity was between groups HYD3 and HYD4 (1.1%). The bacterial community associated with group HYD2 was dominated by two predominant unidentified OTUs, which caused inferior diversity indices, compared to the relatively more equitable distribution in the other groups (Table 5).

4. Discussion

The experimental hydrolysates showed different capacities to stimulate growth in comparison with the commercial fish hydrolysate. The best growth performances were obtained with a high supply of di- and tri-peptides in diets HYD4 and HYD5, but this criterion did not appear sufficient to qualify the dietary value, as inferior growth and survival were obtained with a third hydrolysate that was similarly rich in short peptides (HYD1). The raw materials and/or the mode of preparation were likely critical to allow the best growth-promoting efficiency. In summer flounder larvae fed microparticulate diet, Lian *et al.* (2008) observed a decreased dietary value of the hydrolysate when squid protein was blended with 30% herring protein.

A proteomic study on Atlantic cod larvae showed that early energy metabolism and muscular development was stimulated with the passive ingestion of fish protein hydrolysate at mouth opening, while the trypsin level was not increased (Sveinsdottir & Gudmundsdottir 2010). A dietary supply of fish protein hydrolysate compensated for the detrimental effect of trypsin inhibitor on growth, survival and enzymatic activities after metamorphosis in spotted wolffish fed compound diet from mouth opening onwards (Savoie *et al.* 2011). In the present experiment on sea bass, five of the six hydrolysates induced a similar level of secretion of pancreatic enzymes in the intestine at metamorphosis. This level seemed to correspond to an optimum, which was higher than the level reached by one of two diets that gave inferior growth performances, HYD1 and HYD2, with respect to amylase and trypsin, respectively. Zambonino-Infante et al. (1997) reported that larval growth and survival were directly related to timely gut maturation in sea

bass larvae fed diets supplemented with di- and tri-peptides. The relative activity of alkaline phosphatase in the brush border membranes of enterocytes was used as an indicator of advanced gut maturation, and the highest score corresponded to the highest mean weight at 30 dph in the group fed HYD4. However there was no clear relationship between gut maturation and body growth, as the relative activity of amino-peptidase N was significantly stimulated by the diet HYD3, unlike growth compared with that in the control group.

The group fed HYD4 featured constantly the most advanced state of gut maturation at 25 dph, one of the highest body growth scores at 30 and 60 dph, and osteocalcin gene up-regulation at 24, 42, and 60 dph. The group fed HYD5 appeared less advanced at metamorphosis, in terms of gut maturation and body growth, but there was a final recovery towards the best growth level by the end of the experiment, likely related to up-regulation of the osteocalcin gene at 42 dph. Lamari *et al.* (2013) noted that ontogenetic chronology should be considered beyond growth, and further investigation should be necessary to discriminate the best dietary value between the two hydrolysates HYD4 and HYD5.

Kotzamanis *et al.* (2007) related the dietary value of two dietary fish protein hydrolysates to the ability of sea bass larvae to resist bacterial disease, and they observed differences between associated microbiota. Significant dissimilarities appeared also between the bacterial fingerprints in the present experiment. At the end of the experiment, the active gut microbiota was dissimilar among all the dietary groups, except between groups HYD3 and HYD4, which both showed the most advanced state of gut maturation at 25 dph in terms of relative activity of amino-peptidase N. It would be worth investigating whether an early maturation of the digestive system may induce the concomitant maturation of gut microbiota, as a possible direction for improving bacterial management. Reciprocally, there are scarce indications that the bacterial community could stimulate and contribute to digestion in fish larvae (Dimitroglou *et al.*, 2011). The two groups with the lowest final mean weights, HYD1 and HYD2 had the most dissimilar bacterial profiles, indicating either a delay in ‘microbial maturation’ or some disruption in bacterial homeostasis. Such disruption may be suspected in group HYD1 due to high mortality, and to particular trends in the expression of marker genes. The overexpression of interleukin-1β and catalase genes in this group at 24 dph may suggest an anti-infective response. Picchietti *et al.* (2009) stressed the pro-inflammatory role of the cytokine in sea bass larvae. Reyes-Becerril *et al.* (2011) noted the up-regulation of the catalase gene after bacterial infection in leopard grouper. The ossification process was also likely retarded in group HYD1 during the experiment, and a link between impaired bone mineralization and inflammation was already hypothesised (Gil-Martens, 2010; Lamari *et al.*, 2013). However, transcript levels of marker genes are not sufficient to validate such hypotheses.

More generally, the interaction between microbiota and sea bass larvae was markedly influenced by the nature of dietary protein hydrolysates, but the mechanism behind this influence is not clear. The development of new tools to study the functional microbiome should help to understand which characteristics are essential. The supply of specific bioactive peptides at the right dose may be involved, but the non-peptidic compounds that are brought by the raw material may also play an important role in the dietary value, such as marine phospholipids.

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References

Cahu, C. & Zambonino Infante, J. (2001) Substitution of live food by formulated diets in marine fish larvae. *Aquaculture*, **200**, 161-180.

Chistiakov, D.A., Kabanov, F.V., Troepolskaya, O.D., & Tischenko, M.M. (2010) A variant of the interleukin-1β gene in European sea bass, *Dicentrarchus labrax* L., is associated with increased resistance against *Vibrio anguillarum*. *J. Fish Dis.*, **33**, 759-767.

Clarke, K.R. (1993) Non-parametric multivariate analysis of changes in community structure. *Aust. J. Ecol.*, **18**, 117-143.

Darias, M.J., Mazurais, D., Koumoundouros, G., Glynatsi, N., Christodoulopoulou, S., Huelvan, C., Desbruyeres, E., Le Gall, M.M., Quazuguel, P., Cahu, C.L. & Zambonino-Infante, J.L. (2010) Dietary vitamin D3 affects digestive system ontogenesis and ossification in European sea bass (*Dicentrachus labrax*, Linnaeus, 1758). *Aquaculture*, **298**, 300-307.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Güroy, D. & Davies, S.J. (2011) Microbial manipulations to improve fish health and production - A Mediterranean perspective. *Fish Shellfish Immunol.*, **30**, 1-16.

Gil-Martens, L. (2010) Inflammation as a potential risk factor for spinal deformities in farmed Atlantic salmon (*Salmo salar* L.). *J. Appl. Ichthyol.*, **26**, 350-354.

Gisbert, E., Skalli, A., Fernández, I., Kotzamanis, Y., Zambonino-Infante, J.L. & Fabregat, R. (2012) Protein hydrolysates from yeast and pig blood as alternative raw materials in microdiets for gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture*, **338**, 96-104.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, **4**, 9 pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm. Accessed 28 May 2013

Harnedy, P.A. & FitzGerald, R.J. (2012) Bioactive peptides from marine processing waste and shellfish: A review. *J. Funct. Foods*, **4**, 6-24.

Hermannsdottir, R., Johannsdottir, J., Smaradottir, H., Sigurgisladottir, S., Gudmundsdottir, B.K. & Bjornsdottir, R. (2009) Analysis of effects induced by a pollock protein hydrolysate on early development, innate immunity and the bacterial community

structure of first feeding of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Fish Shellfish Immunol.*, **27**, 595-602.

Kotzamanis, Y.P., Gisbert, E., Gatesoupe, F.J., Zambonino Infante, J. & Cahu, C. (2007) Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae. *Comp. Biochem. Physiol.*, **147A**, 205-214.

Lamari, F., Castex, M., Larcher, T., Ledevin, M., Mazurais, D., Bakhrouf, A. & Gatesoupe, F.J. (2013) Comparison of the effects of the dietary addition of two lactic acid bacteria on the development and conformation of sea bass larvae, *Dicentrarchus labrax*, and the influence on associated microbiota. *Aquaculture*, **376**, 137-145.

Lian, P., Lee, C.M. & Bengtson, D.A. (2008) Development of a squid-hydrolysate-based larval diet and its feeding performance on summer flounder, *Paralichthys dentatus*, larvae. *J. World Aquacult. Soc.*, **39**, 196-204.

Mazurais, D., Darias, M.J., Gouillou-Coustans, M.F., Le Gall, M.M., Huelvan, C., Desbruyères, E., Quazuguel, P., Cahu, C., Zambonino-Infante, J.L. (2008) Dietary vitamin mix levels influence the ossification process in European sea bass (*Dicentrarchus labrax*) larvae. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **294**, R520-R527.

Picchietti, S., Fausto, A.M., Randelli, E., Carnevali, O., Taddei, A.R., Buonocore, F., Scapigliati, G. & Abelli, L. (2009) Early treatment with *Lactobacillus delbrueckii* strain induces an increase in intestinal T-cells and granulocytes and modulates immune-related genes of larval *Dicentrarchus labrax* (L.). *Fish Shellfish Immunol.*, **26**, 368-376.

Reyes-Becerril, M., Tovar-Ramírez, D., Ascencio-Valle, F., Civera-Cerecedo, R., Gracia-López, V., Barbosa-Solomieu, V. & Esteban, M.Á. (2011) Effects of dietary supplementation with probiotic live yeast Debaryomyces hansenii on the immune and antioxidant systems of leopard grouper Mycteroperca rosacea infected with Aeromonas hydrophila. *Aquac. Res.*, **42**, 1676-1686.

Savoie, A., Le François, N.R., Lamarre, S.G., Blier, P.U., Beaulieu, L., & Cahu, C. (2011) Dietary protein hydrolysate and trypsin inhibitor effects on digestive capacities and performances during early-stages of spotted wolffish: Suggested mechanisms. *Comp. Biochem. Physiol.*, **158A**, 525-530.

Silva, F.C.P., Nicoli, J.R., Zambonino, J.L., Kaushik, S. & Gatesoupe, F.J. (2011) Influence of the diet on microbial diversity of faecal and gastrointestinal contents in gilthead sea bream (*Sparus aurata*) and intestinal contents in goldfish (*Carassius auratus*). *FEMS Microbiol. Ecol.*, **78**, 285–296.

Sveinsdottir, H. & Gudmundsdottir, A. (2010) Proteome profile comparison of two differently fed groups of Atlantic cod (*Gadus morhua*) larvae. *Aquacult. Nutr.*, **16**, 662-670.

Swiatecka, D., Markiewicz, L.H. & Wroblewska, B. (2012) Pea protein hydrolysate as a factor modulating the adhesion of bacteria to enterocytes, epithelial proliferation and cytokine secretion - an in vitro study. *Centr. Eur. J. Immunol.*, **37**, 227-231.

Tovar-Ramírez, D., Mazurais, D., Gatesoupe, J.F., Quazuguel, P., Cahu, C.L. & Zambonino-Infante, J.L. (2010) Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (Dicentrarchus labrax) larvae. *Aquaculture*, **300**, 142-147.

Zambonino-Infante, J.L., Cahu, C.L., Peres, A., Quazuguel, P. & Legall, M.M. (1996) Sea bass (*Dicentrarchus labrax*) larvae fed different *Artemia* rations: Growth, pancreas enzymatic response and development of digestive functions. *Aquaculture*, **139**, 129-138.

Zambonino-Infante, J.L., Cahu, C.L. & Peres, A. (1997) Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. *J. Nutr.*, **127**, 608-614.

Tables

Table 1 Composition of the experimental diets.

Ingredients (%, dry matter basis)	CONT	HYD1	HYD2	HYD3	HYD4	HYD5
Fish meal	55	55	55	55	55	55
CPSP G (fish)	10	-	-	-	-	-
Hydrolysate 1 (mollusc)	-	15	-	-	-	-
Hydrolysate 2 (mollusc)	-	-	10	-	-	-
Hydrolysate 3(fish)	-	-	-	10	-	-
Hydrolysate 4 (shrimp)	-	-	-	-	11	-
Hydrolysate 5 (mollusc)	-	-	-	-	-	14
Fish oil	0	0	1	1	0	1
Soy lecithin	20	20	20	20	20	20
Vitamin mix	8	8	8	8	8	8
Mineral mix	4	4	4	4	4	4
Cellulose	5	0	4	4	4	0
Theoretical composition						
Crude protein (%, dry matter basis)	42.0	41.3	43.1	42.4	41.3	41.3
Hydrolysed/crude protein ratio (%)	16.2	14.9	18.5	17.0	14.8	14.9
Lipids (%, dry matter basis)	28.1	27.2	27.4	27.7	28.4	27.2
EPA+DHA (%, dry matter basis)	1.51	1.36	1.44	1.51	1.56	1.43

Table 2 Growth and survival rate of sea bass larvae fed the experimental diets. Superscript letters indicated significant differences between mean weights (mg ± SE) of dietary groups at the same date (post-hoc test: Student-Newman-Keuls after ANOVA).

Diet	HYD1	HYD2	HYD3	HYD4	HYD5	CONT
Mean weight (mg)						
30 dph	1.3^{c} ± 0.1	2.0^{b} ± 0.2	3.7^{b} ± 0.2	4.5^{a} ± 0.3	3.4^{b} ± 0.3	2.9^{b} ± 0.2
60 dph	50^{c} ± 4	42^{c} ± 5	82^{b} ± 5	112^{a} ± 6	124^{a} ± 7	73^{b} ± 5
Survival (%)*	25.7	65.2	75.3	72.2	44.5	58.9

*no statistics available: the estimates were done after one tank per diet, where there was not sampling for analysis during the experiment.

Table 3 Intestinal maturation at 25 dph, expressed as percentage of the secretion of activity of released pancreatic enzymes (trypsin and amylase; percent activity in the intestinal segment related to total activity in the pancreatic and intestinal segment; Zambonino-Infante *et al.*, 1996), and as relative activity of brush border membrane enzymes (amino-peptidase N, and alkaline phosphatase). Superscript letters indicated significant differences between means (± SE) of dietary groups at the same date (post-hoc test: Student-Newman-Keuls after ANOVA).

Diet	HYD1	HYD2	HYD3	HYD4	HYD5	CONT
Pancreatic enzymes secretion (%)						
Trypsin	40^{ab} ± 9	31^{b} ± 9	49^{a} ± 7	49^{a} ± 2	43^{ab} ± 4	50^{a} ± 6
Amylase	53^{b} ± 6	65^{a} ± 7	60^{ab} ± 7	66^{a} ± 5	69^{a} ± 1	71^{a} ± 3
Relative activity of enzymes in the brush border membrane of enterocytes*						
Amino-peptidase N	4^{b} ± 1	5^{b} ± 1	13^{a} ± 1	14^{a} ± 2	8^{b} ± 5	5^{b} ± 2
Alkaline phosphatase	9^{c} ± 2	14^{c} ± 4	30^{b} ± 4	40^{a} ± 6	19^{c} ± 9	13^{c} ± 4

*100 × (ratio to the activity of cytosolic leucine-alanine peptidase)

Table 4 Overall average Bray-Curtis dissimilarities (%) between the RT-PCR-DGGE profiles of each dietary group, computed after SIMPER

Diet	HYD1	HYD2	HYD3	HYD4	HYD5
HYD2	83.3				
HYD3	78.2	82.6			
HYD4	77.8	80.4	65.5		
HYD5	81.4	88.0	75.5	70.5	
CONT	71.1	77.6	65.4	61.9	71.7

Table 5 ANOVA on diversity indices of bacterial communities in the abdominal section of the six dietary groups at the end of the experiment (± SE). Superscripts a and b indicate the significant differences between diets (post-hoc test: Tukey after ANOVA and Dunn's test after KW: Kruskal-Wallis).

Diet	Dominance	Shannon	Equitability	Fisher-α
CONT	$0.14^{ab} \pm 0.01$	$2.2^{ab} \pm 0.1$	$0.88^{ab} \pm 0.01$	$4.2^{ab} \pm 0.4$
HYD1	$0.13^{ab} \pm 0.01$	$2.2^{ab} \pm 0.1$	$0.88^{ab} \pm 0.01$	$4.3^{ab} \pm 0.5$
HYD2	$0.19^{a} \pm 0.01$	$1.9^{b} \pm 0.1$	$0.85^{b} \pm 0.02$	$2.8^{b} \pm 0.3$
HYD3	$0.13^{ab} \pm 0.01$	$2.3^{ab} \pm 0.1$	$0.88^{ab} \pm 0.01$	$4.3^{ab} \pm 0.4$
HYD4	$0.14^{ab} \pm 0.01$	$2.3^{ab} \pm 0.1$	$0.87^{ab} \pm 0.02$	$4.8^{a} \pm 0.5$
HYD5	$0.12^{b} \pm 0.01$	$2.3^{a} \pm 0.1$	$0.92^{a} \pm 0.01$	$4.1^{ab} \pm 0.4$
p	0.01* (KW)	0.03* (KW)	0.02* (KW)	0.03* (ANOVA)

Figures

Figure 1 Molar mass repartition of the hydrolysates incorporated in the experimental diets (% of the total extract).

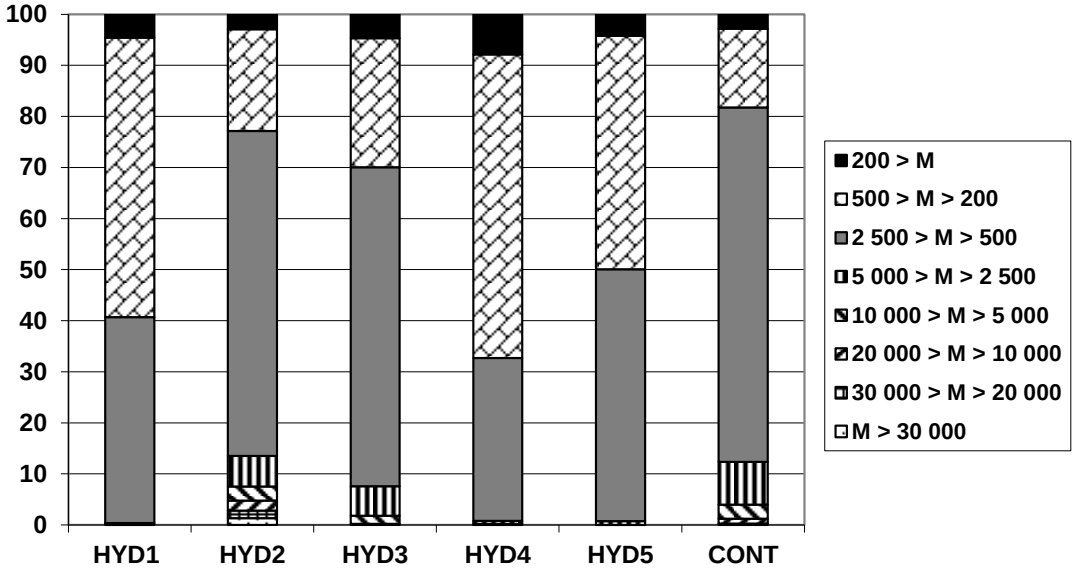

Figure 2 Relative transcript levels of marker genes in sea bass larvae either at 24, 42 or 60 dph, using Ef1 as housekeeping gene (relative expression in arbitrary units). The significant differences are indicated by different superscript letters after the means in the same row (± standard error).