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Protein hydrolysates from yeast and pig blood as alternative raw materials in microdiets for gilthead sea bream (*Sparus aurata*) larvae

Enric Gisbert [a,*], Ali Skalli [a,b], Ignacio Fernández [a], Yannis Kotzamanis [c], Jose Luis Zambonino-Infante [d], Rogelio Fabregat [e]

[a] IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR). Ctra. Poble Nou, Km 5.5, 43540 Sant Carles de la Rápita, Tarragona, Spain
[b] TECNOVIT, Tecnologia & Vitaminas, Polígono Industrial Les Sorts, Parcela 10, 43365 Alforja, Tarragona, Spain
[c] Hellenic Centre for Marine Research, Institute of Aquaculture, Hellinikon 16777, Athens, Greece
[d] Ifremer, PFOM Department, Fish Nutrition Laboratory, 29200 Plouzané, France
[e] PROALAN, S.A., Polígon Industrial Congost, Camí de Can Ninou, 16, 08403 Granollers, Barcelona, Spain

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ABSTRACT

In this study, we have evaluated the incorporation of two types of protein hydrolysates at 9 and 12% levels of inclusion, one from yeast (*Saccharomyces cerevisiae*, YPH) and another one from pig blood (PBPH), in microdiets for gilthead sea bream (*Sparus aurata*) larvae, and compared these results to a microdiet containing fish protein hydrolysate and another group only fed with enriched live prey (rotifers and *Artemia*). The trial consisted in substituting up to 75% (wt/wt) the enriched *Artemia* with the experimental microdiets from 15 to 40 days post-hatch, whereas larvae were exclusively fed on microdiets from 40 to 55 dph. Protein hydrolysates used in the present study were obtained from different raw materials (yeast, pig blood and fish protein concentrate) and differed in their amino acid (AA) profile and in their molecular weight distribution. YPH and PBPH were mainly composed by free amino acids (FAA) (44%, MW<200 Da), di- and tripeptides (50%, 200<MW<500 Da) and 6% of larger polypeptides (500<MW<2500 Da); whereas the fish protein hydrolysate (FPH) did only contain a minor quantity of FAA (1.5%) and was mainly composed of di- and tripeptides (36.5%) and larger polypeptides (51.4%, 500<MW<2500 Da). The contents in FAA and di- and tripeptides in the microdiet containing FPH were 0.2 and 4.4%, respectively. FAA levels in microdiets including YPH and PBPH at 9 and 12% were 4.0 and 5.3%, whereas levels of di- and tripeptides were 4.5 and 6.0%, respectively. Results revealed that FPH in microdiets for marine fish larvae may be replaced by alternative protein hydrolysates obtained from yeast and pig blood, as fish fed with those diets performed, in terms of growth, survival, level of maturation of the enterocytes (activity of cytosolic and brush border enzymes) and incidence of skeletal deformities, as well as those larvae fed with only enriched live preys (rotifers and *Artemia*). Using YPH and PBPH, the inclusion level of protein hydrolysate in microdiets might be reduced to 9% (3% lesser to actual practices using fish protein hydrolysates) without affecting larval performance. Present results suggested the importance of leucine, valine and phenylalanine in fish larval skeletogenesis and in the appearance of skeletal disorders.

1. Introduction

One of the main objectives in marine larviculture has been, for the last three decades, the replacement of live preys, normally rotifers and *Artemia*, by inert formulated diets (Cahu and Zambonino Infante, 2001; Engrola et al., 2009; Kolkovski, 2001, 2008). The development of high-quality artificial microparticulate diets may potentially ameliorate water quality and overcome some disease problems, as well as reduce the high cost of live feed production, since rotifers and brine shrimp production and their enrichment procedures require considerable space, manpower and labor. In contrast, microdiets have a high and constant nutritional value, they are easier to maintain and have lower production costs. These advantages have significant implications for the future sustainability of marine fish larvae production (Kolkovski, 2008). Although the formulation and manufacturing of microdiets have been improved during the last years and several commercial microdiets exist in the market (Holt et al., 2011), artificial diets still led to poor larval performance compared to live preys and their successful replacement has only been fully or partially achieved in a very limited number of marine fish species (Cahu and Zambonino Infante, 2001; Fernández-Díaz et al., 2006; Koven et al., 2001; Kvåle et al., 2009; Yúfera et al., 2005; Zambonino-Infante et al., 1997). One of

* Corresponding author. Tel.: +34 977 745427; fax: +34 977 744138.
E-mail address: enric.gisbert@irta.cat (E. Gisbert).

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the strategies for improving the formulation of microdiets for fish larvae is the inclusion of specific nutrients like fish protein hydrolysates that enhance the digestibility and nutritional value of the microdiet (Kolkovski, 2008).

Protein hydrolysates are promising as core materials in microdiets as they typically consist of low molecular-weight peptides resulting from protein pre-digestion, which are more likely to be absorbed by enterocytes compared to high-molecular-weight macromolecules (Önal and Langdon, 2009). In this sense, different types of experimental and commercial protein hydrolysates differing on their original raw material (i.e. casein, krill, squid, shrimp, mussel, fish meal), their production system (i.e. silage, enzymatic digestion, fermentation, among others) and their biochemical characteristics (i.e. amino acid profile, molecular weight of peptides) have shown that protein hydrolysates enhanced larval and fry growth and/or survival performance in several freshwater and marine species, such as common carp *Cyprinus carpio* (Carvalho et al., 1997), rainbow trout *Oncorhynchus mykiss* (Dabrowski et al., 2003), Atlantic salmon *Salmo salar* (Berge and Storebakken, 1996), European sea bass *Dicentrarchus labrax* (Cahu et al., 1999; Zambonino-Infante et al., 1997), Atlantic cod *Gadus morhua* and Atlantic halibut *Hippoglossus hippoglossus* (Kvåle et al., 2009). In contrast, high levels of protein hydrolysate inclusion in microdiets may not or negatively affect larval growth as it has been reported in rainbow trout (Stone et al., 1989), European sea bass (Cahu et al., 1999), turbot *Scophthalmus maximus* (Oliva-Teles et al., 1999), gilthead sea bream *Sparus aurata* (Kolkovski and Tandler, 2000), Atlantic halibut (Kvåle et al., 2002) or common carp (Carvalho et al., 2004). These studies can be hardly compared since the molecular structures of the peptidic chains of the protein hydrolysates were not always well characterized. Yet, this is a crucial factor explaining the positive role of the protein hydrolysates on larval development. Furthermore, protein hydrolysates also act as feed attractants as they contain digested protein components such as free amino acids (FAA) and peptides, thus enhancing the palatability and acceptance of the feed (Carvalho et al., 1997; Kasumyan and Døving, 2003). In addition, protein hydrolysates have been reported to likely enhance the immune response of European sea bass (Kotzamanis et al., 2007) and Atlantic halibut (Hermannsdottir et al., 2009) larvae, and to promote normal skeletogenesis (Zambonino-Infante et al., 1997).

The positive effect of the protein hydrolysates on fish larval development is well recognized nowadays, and most of commercial microdiets designed and manufactured for marine fish larvae include a moderate level of protein hydrolysate in their formulations (Holt et al., 2011); however, it is necessary to characterize the effect of each new potential raw material sources of protein hydrolysates that could be used in larval feeds. In consequence, the objectives of the present study were to evaluate the effects on growth performance, survival, and incidence of skeletal deformities in gilthead sea bream larvae of two new sources of protein hydrolysates, such as those obtained from yeast and pig blood replacing fish protein hydrolysates.

2. Material and methods

2.1. Experimental design, larval rearing and diets

Newly hatched gilthead sea bream larvae were obtained from a Spanish private hatchery (Tinamenor SA, Spain) and shipped to the *Institut de Recerca i Tecnologia Agroalimentaries* (IRTA)—Sant Carles de la Ràpita facilities. After their acclimation (3 h) in a 500 l-tank, larvae were distributed (initial density: 100 larvae l^{-1}; 10,000 larvae $tank^{-1}$) in 18 cylindrical fiberglass tanks (100 l) connected to a water recirculation unit (IRTAmar®; Carbó et al., 2002). Water conditions were as follows: 18.6 ± 0.4 °C, 34.5 ± 0.5 ppt salinity, pH 8.0 ± 0.15 (mean ± SD), 20% of daily water exchange and with gently aeration and oxygenation (>5 mg l^{-1}). Photoperiod was 12L:12D, and light intensity was 500–600 lx at water surface.

The experimental design was conceived to study the effect of total substitution in microdiets of fish protein hydrolysate (FPH; a fish protein concentrate obtained by grinding and enzymatic hydrolysis of fish, whole or canning byproducts, commercially named CPSP-90™, SoproPêche, France) by different sources and levels of protein hydrolysates obtained from yeast (YPH; *Saccharomyces cerevisiae*; NORLAN LV™; PROALAN SA, Spain) and pig blood (PBPH; NORLAN LX™; PROALAN SA, Spain), and measure their effect on growth performance, maturation of the digestive system and larval quality (incidence of skeletal deformities). As gilthead sea bream cannot be fed from the onset of exogenous feeding with inert diets, a co-feeding protocol was used to test their effects on larval performance. For this purpose, six dietary treatments in triplicate were conducted, including a standard live prey feeding regime (enriched rotifer and *Artemia* nauplii and metanauplii), and five co-feeding regimens differing on the type of protein hydrolysate and level of dietary inclusion (9 and 12% for NORLAN microdiets; and 12% for the CPSP-90 microdiet). The levels of dietary inclusion of protein hydrolysates in microdiets were chosen according to Zambonino-Infante and Cahu's (2010) recommendations. The five tested microdiets were formulated (Table 1) and prepared at the *Ifremer—Fish Nutrition Laboratory* facilities as described in Cahu et al. (1999).

The nutritional trial lasted for 55 days, during which enriched *Artemia* was substituted up to 75% (wt/wt) for the five experimental microdiets from 15 to 40 days post-hatch (dph). Since then and until the end of the study, fish were only fed with the experimental microdiets with the exception of the control group which was only fed with enriched live prey. Microdiet ingestion was confirmed by regular observation of the larval digestive tract under a binocular microscope, as microdiets in the gut were visible by transparency. However, the measurement of the microdiet intake rates was not feasible due to methodological issues (Holt et al., 2011). The feeding sequence

Table 1
Composition of the experimental compound microdiets containing different types and levels of protein hydrolysates.

Ingredients[a] (% DM)	12% FPH	9% YPH	12% YPH	9% PBPH	12% PBPH
Fishmeal[1]	50	53	50	53	50
Fish protein hydrolysate[2]	12	–	–	–	–
Yeast protein hydrolysate[3]	–	9	12	–	–
Pig blood protein hydrolysate[4]	–	–	–	9	12
Fish oil[5]	2	2	2	2	2
Soy lecithin[6]	20	20	20	20	20
Vitamin/mineral mix[7, 8]	8/4	8/4	8/4	8/4	8/4
Betaine[9]	1	1	1	1	1
Proximate composition (%)					
Protein	46.2	45.5	45.8	45.5	45.5
Lipids	31.6	30.9	30.4	29.7	30.1
Ash	14.8	15.0	14.9	14.3	14.5
Moisture	7.0	6.9	6.5	7.0	6.9
Gross energy (kJ/kg)[10]	25.9	25.5	25.3	24.9	25.1

[a] All dietary ingredients were obtained commercially: [1]Fishmeal (La Lorientaise, Lorient, France); 77% protein; [2]CPSP-90™ (Soluble Fish Protein Concentrate; Sopropêche, Boulogne sur Mer, France); [3]NORLAN LX™ (PROALAN; Spain); [4]NORLAN LV™ (PROALAN; Spain); [5]fish oil (La Lorientaise, France); [6]soy lecithin (Ets Louis François, St Maur des Fossés, France). Per kg vitamin mixture[7]: choline concentrate 50%, 200 g; vitamin E (500 UI/g), 10 g; vitamin D3 (500,000 UI/g), 500 mg; vitamin B3, 1 g; vitamin B5, 2 g; vitamin B1, 100 mg; vitamin B2, 400 mg; vitamin B6, 300 mg; vitamin C, 20 g; vitamin B9, 100 mg; vitamin concentrate B12 (1 g/kg), 1 g; biotin, 1 g; vitamin K3, 1 g; meso-inositol, 30 g; cellulose, 732.1 g. Per kg mineral mixture[8]: KCl, 90 g; KIO_4, 40 mg; $CaHPO_4 \cdot 2H_2O$, 500 g; NaCl, 40 g; $CuSO_4 \cdot 5H_2O$, 3 g; $ZnSO_4 \cdot 7H_2O$, 4 g; $CoSO_4 \cdot 7H_2O$, 20 mg; $FeSO_4 \cdot 7H_2O$, 20 g; $MnSO_4 \cdot H_2O$, 3 g; $CaCO_3$, 215 g; $MgSO_4 \cdot 7H_2O$, 124 g; NaF, 1 g. [9]Betaine hydrochloride (99%), Sigma; [10]Microdiet gross energy content was estimated as: total carbohydrate × 17.2 J/kg; fat × 39.5 J/kg; protein × 23.5 J/kg.