Use of porcine byproducts as fish meal replacement in feed for gilthead seabream on-growing: effects on growth, fillet composition and immune status

J. Firmino[1], E. Gisbert[1], A. Estévez[1], M. Sastre[1], D. Gonzalez-Silvera[2], M. Gyftea[2], M.A. Esteban[2], C. Rodriguez[3], J. Polo[3]

1 IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR), Crta. Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Spain.
2 Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology,, University of Murcia, 30100 Murcia, Spain.
3 APC Europe S.A., Granollers, Barcelona, Spain

Contact: joana.pereira@irta.cat

Introduction

One of the current challenges for aquaculture production is to identify high quality sources of alternative proteins for aquafeeds. In this context, scientific community and aquaculture industry are putting together notorious efforts in the development of functional feeds to optimize animal health condition and performance.

Although still unexploited, it is worldwide recognized that terrestrial animal byproduct meals represent a safe source of animal protein and lipid available for the aquafeed industry. In addition to its use as a source of protein, those blood byproducts had also been recommended in animal diets as an immunological support due to their high levels of immunoglobulins.

In this study, the inclusion of two porcine byproducts (hydrolyzed protein, **PEPTEIVA®** and spray-dried plasma, **APPETEIN®**, APC Europe, S.A.) was used in low FM diets (7%) to substitute 5% fishmeal (FM) for on-growing gilthead seabream (*Sparus aurata* L.) juveniles. Fish growth, nonspecific hematological immune parameters, proximal composition, fatty acid composition and oxidative stress condition were assessed.

Table 1 Experimental diets composition.

Ingredients, %	CTRL %	D2 %	D3 %
Fishmeal LT70 (NORVIK)	7,000	2,000	2,000
Porcine plasma		**5,000**	
Porcine protein			**5,000**
Soy protein concentrate (Soycomil)	21,000	21,000	21,000
Pea protein concentrate	12,000	12,000	12,000
Wheat gluten	12,000	12,000	12,000
Corn gluten	12,000	12,000	12,000
Soybean meal 48	5,000	5,000	5,000
Wheat meal	10,400	10,400	10,400
Fish oil - SAVINOR	15,000	15,000	15,000
Vit & Min Premix PV01	1,000	1,000	1,000
Soy lecithin - Powder	1,000	1,000	1,000
Binder (guar gum)	1,000	1,000	1,000
MCP	2,000	2,000	2,000
L-Lysine	0,300	0,300	0,300
L-Tryptophan	0,100	0,100	0,100
DL-Methionine	0,200	0,200	0,200
Total	**100,000**	**100,000**	**100,000**

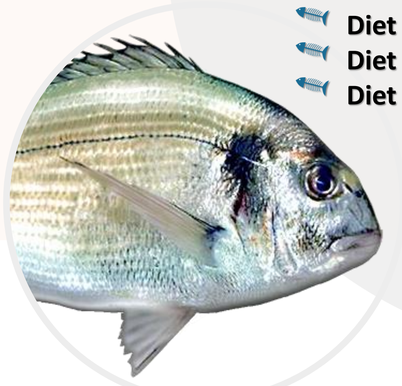
Fish trial

420 seabream initially weighting 26 g were kept in 12 well-aerated 450 L tanks connected to a recirculation system (IRTAmar™) at an initial density of 2 kg/m³ under environmental conditions of photoperiod and water temperature (22–27° C).

Three experimental diets were established (48% crude protein, 17% crude fat, energy: 21.7 MJ/kg feed):

- **Diet 1: Control***
- **Diet 2: Porcine spray dried plasma (APPETEIN®)**
- **Diet 3: Porcine hydrolyzed protein (PEPTEIVA®)**

*diet devoid of the feed additives



The nutritional assay had a duration of **92 days**. Each diet was tested with four replicates.

Results

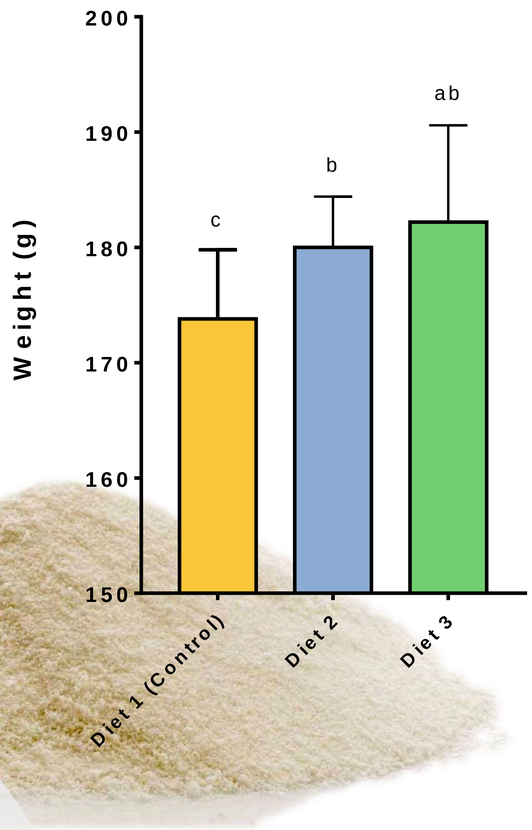
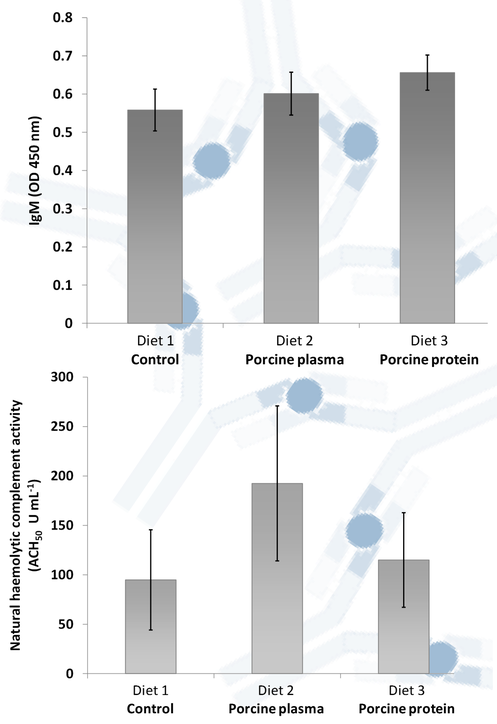


Figure 1
Final weight of juvenile gilthead seabream fed with three experimental diets with different additive inclusion levels. Different letters represent significant differences among groups (p<0.05).

Table 2 Results in proximal (% dry weight) and fatty acid composition (% of total fatty acids) of the fillet at the end of the study, different letters show significant differences (ANOVA, P<0.05).

	Diet 1 Control	Diet 2 Porcine plasma	Diet 3 Porcine protein
Water (%)	70.89±0.49	70.19±0.86	71.71±0.45
Protein (% DW)	62.00±3.01a	61.44±2.54a	69.28±2.81b
Carbohydrates (% DW)	1.33±0.25	1.56±0.14	1.29±0.12
Lipids (%DW)	12.69±1.32	13.90±2.28	12.27±1.78
Ash (%)	2.12±0.23	2.49±0.34	2.53±0.55
Total Fatty acids (mg/g lipids)	735.05±72.65	665.76±12.96	693.32±84.14
Fatty Acid profile (% Total)			
Total Saturated	25.35±0.63	25.60±0.28	25.63±0.65
Total Monounsaturated	30.46±1.47	31.55±1.21	29.04±4.92
20:4n-6 (ARA)	0.82±0.05	0.87±0.16	0.76±0.31
Total n-6 PUFA	10.52±0.20	11.26±0.24	10.78±1.73
20:5n-3 (EPA)	8.00±0.38	8.12±0.32	7.31±1.29
22:6n-3 (DHA)	13.64±0.75	14.14±0.90	12.78±2.56
Total n-3 PUFA	25.96±1.41	26.38±1.02	24.04±3.97
Total PUFA	36.48±1.57	37.64±1.25	34.82±5.65



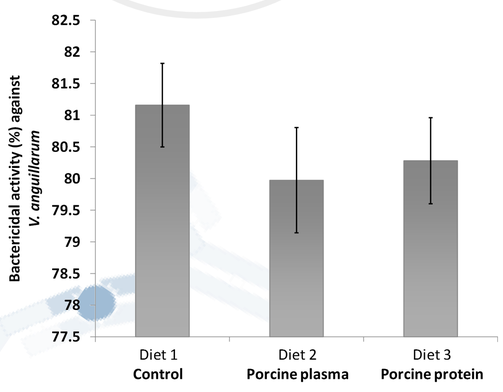


Figure 2 Total immunoglobulin M (IgM) levels and natural haemolytic complement activity were analyzed in seabream serum. Bactericidal activity against an opportunist marine pathogenic, *Vibrio anguillarum*, was also measured. No significant differences were observed between dietary treatments.

Table 3 Lipid peroxidation levels (LOP), activity of antioxidative stress enzymes (Superoxide dismutase, SOD; Glutathione-S-transferase, GST; Glutathione reductase, GR) and total non-enzymatic antioxidant capacity (TAC) in the liver and intestine of gilthead seabream juveniles fed with control and PEPTEIVA® diets at day 92 (end of the trial).

	Liver		*Intestine*	
	Control	PEPTEIVA®	Control	PEPTEIVA®
LOP *(nmol MDA μl^{-1})*	0.0099 ± 0.0024	0.0078 ± 0.0023	0.0134 ± 0.0091	0.0167 ± 0.0152
SOD *(% inhibition)*	88.62 ± 2.46	90.00 ± 2.59	61.85 ± 5.91	58.14 ± 8.66
GST *($\mu mol\ ml^{-1}\ min^{-1}$)*	2.73 ± 1.64	3.39 ± 0.60	2.24 ± 0.22	2.01 ± 0.37
TAC *(nmol μl^{-1} mg $protein^{-1}$)*	0.050 ± 0.034	0.043 ± 0.024	0.032 ± 0.007	0.046 ± 0.014
GR *($mU\ ml^{-1}$)*	68.53 ± 5.85	68.13 ± 16.44	88.96 ± 22.84	81.39 ± 18.19

Conclusions

- **The inclusion of the additives in juvenile seabream feeding had positive effects on growth, promoting a statistically significant increase in body-weight when compared with control group (*Figure 1*).**
- **Contrarily to what it was initially hypothesized, non-specific serological immune parameters (IgM, natural haemolytic complement activity and bactericidal activity) were not affected by the inclusion of the porcine byproducts (*Figure 2*). These results might be due to the high level of fishmeal substitution by soybean protein sources in the basal diet, which can affect the seabream immune capacity and mask the additives potential effects.**
- **Proximal composition analysis indicate a slightly higher content of protein in the fillet of fish fed with the porcine hydrolyzed protein, PEPTEIVA® (*Table 2*). Fatty acid profile seem not to be altered by the porcine byproducts inclusion.**
- **Antioxidative stress enzymes activity appear not to be affected by the additives presence in the diet, since no significant differences were observed between diet treatments (*Table 3*). These results show that the inclusion of the additives does not affect negatively the normal performance of the oxidative stress related metabolism.**
- **The hydrolyzed protein, PEPTEIVA® and spray-dried plasma, APPETEIN®, have shown to be beneficial and affective for gilthead seabream. Feed with only 2% FM can be successfully used when remaining protein content is substituted by porcine byproducts, not only improving fish growth, but also not disturbing the general animal health condition and fillet quality.**

UNIÓN EUROPEA
FONDO EUROPEO MARÍTIMO Y DE PESCA (FEMP)
GOBIERNO DE ESPAÑA MINISTERIO DE AGRICULTURA Y PESCA, ALIMENTACIÓN Y MEDIO AMBIENTE
DIETAplus

Acknowledgements: The Project "Estrategias nutricionales para la mejora en el rendimiento productivo: uso de piensos funcionales y dietas salud en acuicultura (DIETAplus)" was funded by JACUMAR (Spanish Ministry of Agriculture, Fisheries and Environment, MAPAMA) and FEMP (EU) funds.