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Inclusion of Low Levels of Blood and Feathermeal in Practical Diets For Gilthead Seabream (*Sparus aurata*)

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Abstract

Rendered animal protein ingredients, such as feather meal and blood meal, are promising animal protein sources for the replacement of fish meal often proved to combine synergistically. Three practical diets containing similar amounts of PD/ED (22.0 mg/kJ) but differing in the amount of digestible protein were tested in sea bream juveniles of initial body weight 41.81±1.12 g. FBCM diet (40%PD) and FBM diet (42%PD) contained similar percentages of blood and feather meal (10% and 5%, respectively) but differed in the proportion of soybean / rapeseed meal. Although growth performance and feed utilization were very similar in all treatments, chemical composition showed that blood and feather meal supplementation increased significantly whole-body lipid content compared with fishmeal diet (P205A). Liver lipid content was significantly lower in fish fed FBCM diet. Whole-body fatty acids composition was similar between treatments, ranging between 242.57±14.17 mg g^{-1} in FBM diet and 274.62±23.95 mg g^{-1} for FBCM diet. Palmitic acid, oleic acid, linoleic acid; EPA and DHA were the most abundant fatty acids in both polar and neutral lipid fractions of the fish. Economical evaluation indicated that the incorporation of blood and feather meal as a substitute of fish meal decreased feed costs leading to a better economic conversion ratio

Keywords: Blood meal, feather meal, gilthead seabream, growth.

Introduction

Aquaculture is one of the fastest growing food producing sectors in the world accounting for approximately 50% of fisheries products (FAO, 2010). As a result of the progressive intensification of lower value species and the increasingly widespread culture of higher value fish species there is an increase in the use of formulated feeds with higher demand for fishmeal. Nonetheless, the continued expansion of aquaculture will not be possible if fishmeal is relied upon as the main source of protein in aquafeeds. Due to its relatively high and variable cost, and growing environmental concerns about harvesting wild fish to produce fish meal, it is desirable to replace fishmeal with less expensive protein sources.

Substantial effort has been expended over the past decades in evaluating a wide range of potential alternatives to fishmeal and fish oils for use in aquaculture diets. The need for finding suitable and cost-effective alternatives must be with resultant efficiency and effectiveness in both environmental and industrial perspectives (Otubusin *et al*., 2009). Alternative ingredients can generally be classified into those being derived from either plant origin or terrestrial animal origin (Glencross *et al*., 2007). Currently, commercial diets manufactured for carnivorous fish such as gilthead sea bream, *Sparus aurata* commonly contain a wide range of protein sources to limit fishmeal inclusion. The majority of these secondary protein sources are a complex mixture of plant proteins sources that have proved efficient. Nevertheless, in animal nutrition a range of protein sources including meat and bone meals, blood meals (mainly from non-ruminant sources like porcine and avian derivatives) and avian by-products such as feather meals, that offer protein concentrates are currently considered to be effective substitutes for fish meal at appreciable levels in feeds (Webster *et al*., 1999; Kureshy *et al*., 2000; Wang *et al*., 2006; Glencross *et al*., 2007; Goda *et al*., 2007). In fact, animal protein sources not only perform better than plant protein in diets for carnivorous species (Hardy, 1998) but also complement certain plant protein ingredients (e.g., corn gluten meal and soybean meal).

Studies conducted recently have shown that rendered products are cost-effective sources of highly

available amino acids, fatty acids and several other key nutrients (Wang *et al*., 2006) and unlike plant proteins they are relatively free of anti-nutritional factors. Among the animal by-products, blood meal (BM) is an alternative cheaper protein source and large quantities of which are still being wasted in abattoirs throughout the countries. Spray and ring dried BMs are widely used in salmonid feeds due to their high digestibility and consistent quality. Good performances have been observed for fish fed diets containing approximately eight to twenty percent BM in conjunction with more than 20 percent fish meal levels (Luzier *et al*., 1995; Abery *et al*., 2002). Moreover, blood meal is low in phosphorous, which will please fish farmers from both an environmental and industrial perspective (Agbebi *et al*., 2009).

Feather meal is another by-product from poultry production that has been used in aquatic animals feeds (Langar and Metailler, 1989; Fowler, 1990; Steffens, 1994; Bureau *et al*., 2000) and often reported to combine synergistically with blood meal, particularly in diets for beef cattle. It is rich in indispensable amino acids such as cystine, theonine and arginine, and pepsin digestible protein (75-87%). Fowler (1990) reported that for Chinook salmon culture feather meal could replace 15% of fish meal in diet with similar growth and feed utilization efficiency. Somseueb and Boonyaratplain (2001) also reported a maximum 5% replacement of dietary fish meal for feather meal in walking catfish (hybrid *Clarias*).

Despite of its proven applicability, animal by-product usages within the European Union have been severely curtailed as a consequence of concerns related to the BSE crisis in Europe, in the late 1980's and early 1990's (Serwata, 2007). In 2000 EU passed a directive which declared that any animal protein (except fishmeal) used in feedstuffs and exports for use in diets were prohibited (EU Comission Decision 2000/766). The re-introduction of animal proteins of porcine and avian origin in fish feed by the ABPR (1774/2002) together with the TSE regulation (999/2001) and with the amendments produced in 2005 (Regulation 1292/2005) represents a significant and major step towards an efficient utilization of animal by products on fish feed production.

Notwithstanding this change in legislation, some effort is required at technical level to undertake further feasibility investigations to demonstrate the nutritional advantages for modern processed animal by-products and to ensure a higher degree of environmental sustainability and economic viability in the sector as pressures on the natural resource base and public awareness of environmental issues are reaching unprecedented levels.

This study aimed at evaluating the combined use of blood meal and feather meal in three practical diets for gilthead sea bream juveniles during a 12 week trial. Authors used least cost formulation, that is, formulas that are nutritionally complete with a minimum ingredient costs. Growth, tissue composition, fat deposition and of gilthead sea bream were evaluated at the end of the trial.

Materials and Methods

Fish and Husbandry

The feeding trial was conducted at the experimental facilities of Centro de Maricultura da Calheta (CMC), Madeira, Portugal. Gilthead seabream juveniles were acclimatized to the rearing conditions for a 2-week period prior to the feeding trial, during which all the fish were fed an extruded commercial diet without blood or avian meals (Perfom 205A from Aquasoja, Sorgal S.A.), twice a day to apparent satiation.

For the twelve week feeding trial, homogenous groups of 30 seabream with an average initial weight of 41.81±1.12 g (Mean±SD) were randomly distributed among nine indoor fibreglass tanks of 500L. Each tank was supplied with gravel-filtered seawater (37 salinity), at a water flow of 8 L min^{-1}. The water temperature was 22.2±1.25ºC, dissolved oxygen 5.3±0.6 mg L^{-1} and pH ranged from 7.6 to 8.5. The photoperiod was natural (10L: 14D) and all tanks had similar lighting conditions throughout the feeding trial.

Experimental Diets, Feeding and Design

Hydrolysed feather meal and blood meal were obtained rendering plants (Saria Industries, France). Chemical composition of the ingredients is presented in Table 1. Ingredients content, proximate compositions, lipid class and fatty acid profiles of the diets are represented in Table 2. Diets were designed according to least cost formulation, where the overall biological outcome of the product was kept in mind rather than the feed cost per unit weight. In this context, the most critical piece of information regarded digestibility / availability of nutrients within each feed ingredient (digestible protein, digestible energy, available phosphorus, digestible protein to digestible energy ratio, etc) rather than the proximate analyses of the finished diet. With this in mind, all three diets contained similar amounts of PD/ED (22.0 mg/kJ) but differed in the amount of digestible protein. FBCM diet (40% PD) and FBM diet (42% PD) contained similar percentages of blood and feather meal (10% and 5%, respectively) but differed in the proportion of soybean / rapeseed meal. These two protein sources were adjusted in order to lower digestible protein content and production costs, since rapeseed meal is approximately half the cost of fish meal per kg of protein. Nutritional density was adjusted between FB diets by substitution of raw materials with low protein content by equivalents with known higher digestible protein content (soya meal by rapeseed meal and corn gluten by corn flour). The third diet (P205A) was a conservative commercial

Table 1. Composition of the ingredients used in the trial

	Fish meal	Hidrolysed feather meal	Blood meal	Soybean meal	Rapeseed meal
Dry matter (%)	93	95	97	88	88
Crude protein (%)	66	80	90	48	34
Crude lipid (%)	9	5	1	1,5	2,3
Ash (%)	18	2	2	6	7
DP (%)	90	70	85	95	82
DE (%)	86	76	79	75	62

Table 2. Ingredients, proximate composition and fatty acid content (dry weight basis) of the three experimental diets fed to seabream juveniles for 90 experimental days

	FBCM	FBM	P205A
Ingredients (%)			
Fishmeal	30.90	30.70	36.40
Deffated soybean meal	16.60	21.00	24.00
Fish oil	8.40	10.00	10.00
Rapeseed meal	7.50	3.20	4.00
Hydrolysed feather meal	5.00	5.00	-
Blood meal	10.00	10.00	-
Corn flour	11.70	6.00	2.50
Corn gluten	2.40	3.40	14.70
Wheat gluten	0.20	0.20	0.20
Composition (% DM)			
Crude Protein	50.0	51.1	51.1
Crude Fat	18.8	21.1	21.1
Crude Ash	9.4	7.8	8.9
Digestible Protein	44.4	46.7	46.7
Digestible Energy (kJ/g DM)	20	21	21
PD/ED(mg/kJ	22	22	22
Fatty acid composition (mg g^{-1})			
C14:0	7.66±0.17^{a}	7.88±0.55^{a}	12.82±0.50^{b}
C16:0	27.06±1.23^{a}	26.09±2.09^{a}	41.32±1.68^{b}
C18:0	5.86±0.026^{a}	5.88±0.51^{a}	8.23±0.34^{b}
C16:1[1]	9.52±0.08^{a}	9.34±0.79^{a}	16.73±0.61^{b}
C18:1	22.48±0.12^{a}	24.18±1.95^{a}	33.88±1.20^{b}
C20:1[1]	6.26±0.06^{a}	7.86±0.67^{ab}	9.13±0.46^{b}
C18:2n-6	6.77±0.14	8.49±0.57	12.59±0.32
C18:3n-3	1.72±0.03^{a}	1.78±0.17^{a}	2.73±0.03^{b}
C18:4n-3	3.44±0.04^{a}	3.36±0.28^{a}	5.57±0.14^{b}
C20:4n-6	0.61±010^{a}	0.78±0.07^{a}	1.37±0.09^{b}
C20:4n-3	1.15±0.04^{a}	1.19±0.09^{a}	1.71±0.05^{b}
C20:5n-3	15.41±0.08^{a}	14.34±1.12^{a}	23.72±1.00^{b}
C22:5n-3	2.19±0.02^{a}	2.26±0.15^{a}	3.13±0.17^{b}
C22:6n-3	14.58±0.30^{a}	14.58±0.92^{a}	22.58±1.29^{b}
∑ SFA	43.45±1.43^{a}	42.66±3.39^{a}	66.83±2.68^{b}
Σ MUFA	46.00±0.31^{a}	51.95±4.28^{a}	70.22±3.30^{b}
Σ PUFA	48.25±0.28^{a}	48.63±3.40^{a}	76.73±3.18^{b}
Σ (n-6)	0.61±0.10^{a}	0.78±0.07^{a}	1.37±0.10^{b}
Σ (n-3)	40.58±0.34^{a}	39.06±2.88^{a}	62.35±2.77^{b}
Total[2]	137.70±2.02^{a}	143.24±11.07^{a}	213.78±0.19^{b}

Values (mean of three replications, n=10) in the same row, not sharing a common superscript are significantly different (P<0.05).
[1]Contains *n-9* and *n-7* isomers, [2]Contains some minor components not shown.

feed for seabream without rendered animal protein sources from Sorgal, S.A with similar PD and ED to diet FBM.

Diets were produced in Sorgal's industrial site according to standard protocol. Pellets were extruded using a monoscrew Andritz extruder and fat was added through a Vacuum coater to ensure proper coating. After cooling, experimental pellets were bagged and stored for shipment in a temperature-controlled warehouse.

Fish were hand fed *ad libitum*, twice a day, six times a week. The leftover feed was siphoned out, filtered, blotted and weighed before the next feeding.

Sampling

Prior to the start of the trial, all fish within each tank were individually weighted and measured under anaesthetic MS222 (tricaine methanesulfonate, Sigma Chemical, St Louis, MO, USA) to ensure uniformity of fish size. Sampling procedures were repeated every four weeks. At the end of the feeding trial (day 90), determination of growth parameters was performed on individual weight and measurement of all fish after starvation for 24h. Whole-body and liver samples were obtained from five fish of each tank. Samples were immediately frozen after collection and stored at -80ºC until further analyses were performed. Growth, biometric and economic indexes considered were as follows:

Weight Gain (%) (WG) = 100×(final weight-initial weight)/initial weight (1)

Specific Growth Rate (%day^{-1}) (SGR) = 100×ln(final weight/initial weight)/days (2)

Feed Intake (g) (FI) = Feed offered (g) –Feed wasted (g) (3)

Voluntary Feed Intake (g kg^{-1}day^{-1}) (VFI) = 100*(FI/days)–(1/(initial weight+final weight /2)) (4)

Feed Conversion Ratio (FCR) = feed offered (g)/weight gain (g) (5)

Protein Efficiency Ratio (PER) = weight gain (g)/protein offered (g) (6)

Condition factor (g cm^{-1}) (CF)=100× total weight (g)/total length^3 (cm) (7)

Hepatosomatic Index (%) (HSI) = 100×liver weight (g)/fish weight (g) (8)

Economic efficiency ratio (ECR)=feed offered (kg)×price index/weight gain (kg) (9)

Economic profit index (EPI) = final weight (kg fish^{-1})×fish sale price (€ kg^{-1})- ECR x weight gain (kg) (10)

Seabream sale price was considered at 4.5€ kg^{-1}.

Chemical Analysis

The chemical compositions of diets, whole-body and fish liver were analysed following AOAC (1995) procedures: dry matter (105ºC to constant weight), ash (550ºC to constant weight), crude protein (N × 6.25) by the Kjeldahl method after acid digestion and total lipids were extracted with a chloroform-methanol mixture (1:2 v/v), containing 0.01% BHT, according to Bligh and Dyer (1959).

Lipid classes were separated from total lipids using silica column at atmospheric pressure. Before make the column, the silica (60 Mesh, Sigma) was activated at 100ºC for one hour. The column was compacted by the dichloromethane. The elution sequence, of growing polarity, followed Guckert *et al*. (1985) and Smith *et al*. (1986) procedure: first 5 ml of dichloromethane, then 5 ml of acetone and finally 10 ml of methanol. The fractions were dried by low nitrogen flow. These elutions allow the separation of the different lipid fraction: neutral lipids and polar lipids.

Fatty acid content was determined as fatty acid methyl esters (FAME), according to the Lepage and Roy (1986), modified by Cohen *et al*. (1988). In brief, analysis were performed in a gas chromatograph (Agilent HP 6890) equipped with a flame ionisation detector and a mass selective detector (Agilent 5973). The separation was performed in a polyethylene glycol capillary column (Supercolwax) with 30 m of length, 0.25 mm i.d. and 0.25 µm film thickness from Supelco. FAME are expressed as mg *per* g of dry material.

Prior to all determinations, samples were freeze-dried (Labconco Freezone 4.5), homogenized and residual moisture was determined (Gibertini-Eurotherm dry weight balance).

Statistical Analysis

The obtained resulting data are presented as mean ± standard deviation (SD) of three separated determinations. Statistical analyses of data were carried out with SPSS 14.0 (2006) software package (SPSS; Chicago, IL). Normality was tested using Kolmogorov-Smirnoff test. Homogeneity was checked using the absolute residuals according to Levene's test. Effect of treatment was carried out using one-way ANOVA followed by a *post hoc* Tukey multiple comparison test. In all statistical tests used, $P<0.05$ was considered statistically different.

Results

The average cumulative mortality during the experiment was less than 5%. The effects of dietary treatments can be seen in Figure 1 which displays the live weight gain over the twelve week study. Fish grew from a mean initial weight of 41.81±1.12 g to a final weight of 128.96±1.96 g for fish fed the fishmeal commercial feed (P205A), 126.36±2.53 g for fish fed FBM diet and 135.76±7.05 g for fish fed FBCM diet. Table 3 presents growth performance parameters for the respective experimental treatments. The actual feed consumption was similar in all groups; none of the feeds was specially preferred or ignored, with consequent similar protein efficiency ratio between experimental treatments. The similar weight gain (Eq. 1) for the three experimental feeds agrees with SGR (Eq. 2) ($P>0.05$), varying between 1.45±0.03 for FBM feed and 1.50±0.03 for 20PA feed. Similarities in feed consumption were also reflected in FCR values (Eq.

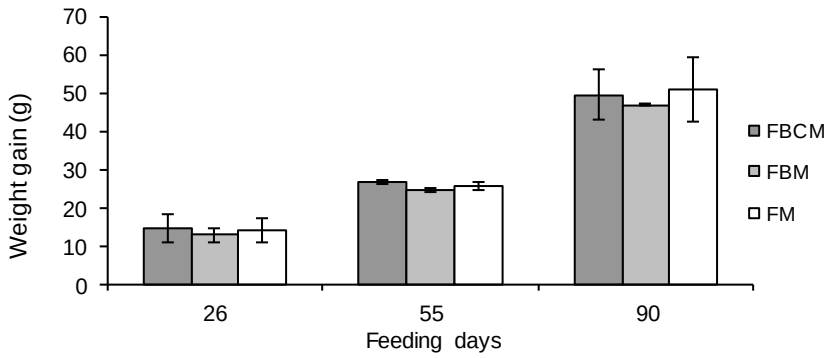

Figure 1. Weight gain (g) after 26, 55 and 90 feeding days of seabream juveniles fed three diets: FBCM- Supplemented Feather, Blood and Corm Meal diet; FBM- Supplemented Feather and Blood Meal diet; P205A- Fishmeal based commercial diet.

Table 3. Growth performance of seabream juveniles fed the experimental diets containing different protein sources for 90 experimental days

	Diets		
	FBCM	FBM	P205A
W_i (g)	42.28±1.33	41.85±1.86	41.29±0.25
W_f (g)	135.76±7.50	126.36±2.54	128.96±1.96
$CF_{initial}$	1.50±0.07	1.48±0.06	1.49±0.09
CF_{final}	1.74±0.03	1.744±0.01	1.73±0.03
SGR (%day^{-1})	1.53±0.11	1.45±0.03	1.50±0.03
FI (gfish^{-1})	147.82±3.36	138.20±3.93	144.01±0.55
VFI (gKgBW^{-1}d^{-1})	20.06±0.24	19.84±0.04	20.44±0.28
PER	1.53±0.11	1.42±0.03	1.48±0.04
FCR	1.46±0.10	1.50±0.03	1.51±0.04
HSI (%)	1.41±0.33	1.63±0.39	1.32±0.26

FBCM- Supplemented Feather, Blood and Corm Meal diet; FBM- Supplemented Feather and Blood Meal diet; P205A- Fishmeal based commercial diet. Values are expressed as mean ±SD (n=30).

3), averaging 0.62 in FBCM dietary treatment and 0.63 for the fish fed FBM diet.

Although growth performance and feed utilization were very similar in all treatments, the chemical composition of whole-body analyses showed significant differences (Table 4). Protein content of fish fed P205A diet was similar to fish fed FBM diet, but differences were found regarding fish fed FBCM diet, which presented significantly higher protein contents (24.28±0.15%, wet weight). Blood and feather meal supplementation increased significantly whole-body total lipid contents compared with fishmeal diet (P205A). Other body composition traits (water and ash content) were not affected by the different experimental diets.

The proximate composition of the three experimental diets was very similar with protein ranging from 45% to 47% and total lipid values between 17% and 19% (Table 2). As expected, FBCM and FBM diets had similar composition concerning the majority of the fatty acids ($P>0.05$), including total fatty acid concentration (mg g^{-1}), but lower than P205A diet.

Effects of blood and feather meal supplementation on seabream whole-body and liver fatty acid content are shown in Table 5. Whole-body total fatty acids concentration did not differ between treatments, ranging between 242.57±14.17 mg g^{-1} in FBM diet and 274.62±23.95 mg g^{-1} for FBCM diet. Whole-body sum of saturated fatty acids (SFA) and long chain polyunsaturated fatty acids (PUFA) concentrations were not affected by blood and feather meal supplementation, whereas differences were found in total monounsaturated fatty acids (MUFA). Fish fed FBCM diet presented higher total MUFA content than fish fed both FBM and P205A diets. Similar results were found for alpha-linolenic acid (C18:3n-3). Fish fed FBCM diet presented similar results with fish fed (Perform 205A), but differing from fish fed FBM diet for the following fatty acids: eicosatetraenoic acid (C20:4n3); arachidonic acid (ARA-C20:4n-6); erucic acid (C22:1n-9) and docosapentaenoic acid (C22:5n-3).

Though HSI (Eq. 7) was not significantly affected by blood and feather meal supplements (Table 3), liver total lipid content *post hoc* tests revealed that fish fed FBCM diet presented significantly lower values (15.36±0.51%) than fish fed FBM and P205A diet (25.58±2.63% and 20.90±1.87%, respectively) (Table 4).

Table 4. Proximate composition (%, wet weight basis) of juvenile seabream fed the diets containing different protein sources for 90 experimental days

	Diets		
	FBCM	FBM	P205A
Protein	24.28±0.15[a]	20.45±1.92[b]	22.21±4.21[b]
Lipid	11.68±0.83[a]	10.59±0.10[a]	8.30±2.21[b]
Water	56.03±3.60[a]	58.46±3.13[a]	55.74±4.29[a]
Ash	8.01±3.09[a]	10.50±0.68[a]	13.76±3.05 [a]

FBCM- Supplemented Feather, Blood and Corm Meal diet; FBM- Supplemented Feather and Blood Meal diet; P205A- Fishmeal based commercial diet. Values (mean±SD, n=10) with different letter are significantly different (P<0.05).

Table 5. Whole-body and liver fatty acid content (mg g^{-1}, dry weight basis) of total lipids of seabream juveniles fed different protein sources for 90 experimental days

	Whole-Body			Liver		
	FBCM	FBM	P205A	FBCM	FBM	P205A
Total lipids	27.08±2.82[a]	25.55±1.75[a]	18.61±3.56[b]	15.36±0.50[a]	25.58±2.63[b]	20.9±1.87[b]
Fatty acids						
C14:0	14.91±1.25[a]	13.00±1.15[a]	12.74±0.21[a]	9.08±1.14[a]	9.99±1.06[a]	8.37±0.75[a]
C16:0	56.81±4.13[a]	50.86±3.75[a]	48.39±0.50[a]	46.47±4.83[a]	54.72±4.73[a]	42.51±1.48[a]
C18:0	11.62±0.76[a]	16.86±1.06[a]	6.30±0.31[a]	13.64±1.19[a]	*tr*[b]	12.95±0.42[a]
C16:1[1]	24.47±1.91[a]	20.30±1.61[a]	21.49±0.26[a]	16.08±1.71[a]	17.98±1.83[a]	15.55±0.85[a]
C18:1	65.63±4.37[a]	52.68±1.18[a]	57.08±0.82[a]	50.28±4.06[a]	86.92±4.91[b]	49.57±1.65[a]
C20:1[1]	11.81±0.79[a]	11.99±0.81[a]	11.41±0.27[a]	6.61±0.56[ab]	9.58±0.49[a]	3.42±0.10[b]
C18:2n-6	15.72±1.85[a]	17.11±1.36[a]	17.74±0.20[a]	9.29±0.79[a]	11.63±0.68[a]	9.89±0.55[a]
C18:3n-3	2.78±0.46[a]	*tr*[b]	0.06±0.00[b]	1.72±0.14[a]	1.77±0.10[a]	1.56±0.18[a]
C18:4n-3	*tr*[a]	*tr*[a]	0.96±0.03[b]	2.37±0.17[a]	2.37±0.08[a]	0.89±0.02[b]
C20:4n-6	2.33±0.20[a]	*tr*[b]	2.15±0.08[a]	0.99±0.07[a]	1.73±0.27[b]	1.00±0.08[a]
C20:4n-3	2.49±0.35[a]	*tr*[b]	2.68±0.06[a]	1.92±0.03[a]	2.30±0.33[a]	1.73±0.21[a]
C20:5n-3	16.92±2.31[a]	18.05±1.85[a]	18.14±0.40[a]	11.92±0.73[a]	11.48±1.85[a]	10.12±1.44[a]
C22:5n-3	7.08±0.82[a]	*tr*[b]	7.17±0.16[a]	5.56±0.28[a]	7.46±1.87[a]	5.37±0.42[a]
C22:6n-3	23.10±3.48[a]	27.09±3.03[a]	26.83±0.63[a]	20.81±0.85[a]	22.11±3.42[a]	19.68±2.38[a]
∑ SFA	88.14±6.50[a]	85.90±5.49[a]	72.23±0.85[a]	73.51±7.48[a]	69.36±5.79[a]	67.44±2.31[a]
Σ MUFA	114.77±7.80[a]	94.42±4.06 [b]	101.77±1.59[a b]	80.09±6.72[a]	126.02±5.17[b]	75.68±2.20[a]
Σ PUFA	71.72±9.65[a]	62.25±5.86[a]	77.01±1.53[a]	57.44±3.13[a]	64.12±8.50[a]	52.84±5.02[a]
Σ (n-6)	18.75±2.15[a]	17.11±1.36[a]	20.59±0.30[a]	11.11±0.82[a]	14.23±1.01[b]	11.59±0.52[ab]
Σ (n-3)	52.36±7.43[a]	45.14±4.88[a]	55.85±1.23[a]	45.72±2.22[a]	48.96±7.44[a]	40.62±4.59[a]
Total[2]	274.62±23.95[a]	242.57±14.17[a]	251.01±2.59[a]	211.04±17.33[a]	259.49±11.72[b]	195.97±8.34[a]

Values (mean of three replications, n=10) in the same row, not sharing a common superscript are significantly different (P<0.05).
[1]Contains *n-9* and *n-7* isomers, *tr*, Trace (<0.005 mg g^{-1})
[2]Contains some minor components not shown.

Fatty acid content in liver differed between treatments. Fish on the FBM diet presented higher stearic (C18:0); oleic (C18:1); arachidonic acid (C20:4n-6); MUFA and total fatty acid content. Proportion of the n-6 series in fish fed P205A diet was similar to fish fed supplemented feather and blood meal diets, but between these last differed significantly (P<0.05). Fatty acid determination of lipid classes was also determined for experimental diets and whole-body (Table 6 and Table 7). Polar and neutral fatty acids fraction of the diets was similar for most fatty acids. In the neutral fraction differences were only found for the FBCM diet. Arachidonic acid and sum of n-6, were lower than the remaining diets and docohexanoic acid (DHA-C22:6n-3) and sum of n-3 fatty acids amount were significantly higher compared to the other two diets. In the polar lipids fraction, FBCM diet presented lower contents for ARA and DHA. FBM diet presented lower content of myristic acid (C14:0) and palmitoleic acid (C16:1).

As for whole-body lipid class determination, no differences were found for percentage of neutral lipids detected, which ranged between 79.64±7.83% of the total lipids for fish fed FBM diet and 81.17±13.44% for seabream fed FBCM diet. The amount of polar lipids revealed that fish fed FBCM diet had significantly lower content than fish fed 20PA diet (4.99±1.07% and 9.48±3.26%, respectively), whereas polar lipid content in fish fed FBM diet did not reveal statistical difference with the remaining diets (7.80±3.48%).

Fatty acid (FA) composition in the whole-body of seabream fed the diets containing different protein sources revealed that palmitic acid (C16:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6); EPA (C20:5n-3) and DHA (C22:6n-3) were the most abundant of saturates, monoenes and highly unsaturated fatty acids (HUFA), respectively, in both polar and neutral lipid

Table 6. Fatty acid composition (%) of polar and neutral lipid fraction in the diets containing different protein sources

	Neutral Lipids			Polar Lipids		
	FBCM	FBM	20PA	FBCM	FBM	20PA
Lipid fraction (% of the total lipids)	84.82±1.78[a]	74.73±8.29[a]	81.47±2.16[a]	6.66±1.05[a]	5.26±1.96[a]	6.11±0.79[a]
Fatty acids (% in the fraction)						
C14:0	5.21±0.38[a]	5.77±0.68[a]	5.66±0.18[a]	2.53±0.06[a]	1.92±0.07[b]	2.44±0.16[a]
C16:0	19.20±0.12[a]	18.31±0.50[a]	19.46±0.36[a]	23.82±2.62[a]	24.39±1.1[a]	27.99±1.10[a]
C18:0	4.57±0.08[a]	3.99±0.32[a]	4.26±0.04[a]	6.50±0.44[a]	6.33±0.60[a]	7.09±0.09[a]
C16:1[1]	7.07±0.03[a]	6.98±0.65[a]	7.58±0.11[a]	4.46±0.00[a]	2.82±0.11[b]	4.06±0.20[a]
C18:1	16.60±1.53[a]	17.64±0.28[a]	17.53±0.12[a]	21.16±1.42[a]	19.06±1.43[a]	19.44±0.32[a]
C20:1[1]	5.78±0.46[a]	6.34±0.84[a]	5.41±0.01[a]	1.38±0.08[a]	1.17±0.22[a]	0.06±0.03[b]
C18:2n-6	4.05±0.28[a]	4.96±0.27[a]	4.95±0.09[a]	20.54±1.27[a]	21.40±3.03[a]	18.50±0.06[a]
C18:3n-3	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a] ..	*tr*.[a]	*tr*.[a]
C18:4n-3	1.29±0.05[a]	1.26±0.11[a]	1.33±0.01[a]	2.18±0.22[a]	2.46±0.38[a]	1.72±0.08[a]
C20:4n-6	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a] ..	*tr*.[a]	*tr*.[a]
C20:4n-3	0.24±0.01[a]	0.78±0.08[b]	0.78±0.03[b]	*tr*[a]	0.83±0.08[b]	1.04±0.11[b]
C20:5n-3	12.62±0.49[a]	11.16±0.91[a]	12.56±0.33[a]	5.98±0.05[ab]	6.16±0.41[a]	5.05±0.11[b]
C22:5n-3	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a]
C22:6n-3	14.48±0.23[a]	11.59±0.19[b]	11.93±0.63[b]	9.77±0.24[a]	11.70±0.13[b]	12.68±0.62[b]
∑ SFA	29.88±0.27[a]	28.99±0.92[a]	30.34±0.60[a]	32.84±3.00[a]	33.04±1.84[a]	37.53±0.85[a]
Σ MUFA	37.45±0.78[a]	41.26±2.09[a]	38.12±0.24[a]	28.69±1.66[a]	24.42±1.78[a]	23.50±0.12[a]
Σ PUFA	32.67±0.50[a]	29.75±1.17[a]	31.54±0.84[a]	38.47±1.34[a]	42.54±3.62[a]	38.98±0.98[a]
Σ (n-6)	4.29±0.27[a]	5.74±0.34[b]	5.73±0.12[b]	20.54±1.27[a]	22.23±2.95[a]	19.53±0.17[a]
Σ (n-3)	28.39±0.77[a]	24.01±0.83[b]	25.82±0.96[ab]	17.93±0.07[a]	20.32±0.67[a]	19.45±0.81[a]

Values (mean of three replications. n=10) in the same row not sharing a common superscript are significantly different (P<0.05).
[1]Contains *n-9* and *n-7* isomers. *tr*. Trace (<0.005 mg g^{-1}) [2]Contains some minor components not shown.

Table 7. Fatty acid composition of polar and neutral lipid fraction (%) in the whole-body of juvenile seabream fed the diets containing different protein sources for 90 experimental days

	Neutral Lipids			Polar Lipids		
	FBCM	FBM	P205A	FBCM	FBM	P205A
Lipid fraction (% of the total lipids)	81.17±13.44[a]	79.64±7.83[a]	80.40±10.80[a]	4.99±1.07[a]	7.80±3.48[ab]	9.48±3.36[b]
Fatty acids (% in the fraction)						
C14:0	7.27±0.28[a]	6.81±0.65[a]	6.72±0.27[a]	6.40±0.27[a]	3.21±0.11[b]	5.42±0.20[a]
C16:0	27.89±0.36[a]	26.20±0.98[a]	27.27±0.76[a]	34.87±2.19[a]	28.08±0.55[b]	34.23±1.09[a]
C18:0	5.66±0.25[a]	5.84±0.12[a]	5.77±0.30[a]	7.47±1.17[a]	7.45±0.26[a]	6.13±0.63[a]
C16:1[1]	10.57±0.57[a]	10.40±0.24[a]	10.30±0.51[a]	9.24±0.61[a]	7.11±0.48[b]	11.73±0.73[c]
C18:1	29.39±0.19[a]	31.65±0.57[b]	29.68±0.53[a]	25.78±1.46[a]	25.12±0.69[a]	25.12±0.66[a]
C20:1[2]	4.85±0.05[a]	5.30±0.29[b]	5.14±0.11[ab]	3.04±0.32[a]	2.37±0.10[a]	2.24±0.09[a]
C18:2n-6	3.39±0.27[a]	3.62±0.5[a]	4.07±0.27[a]	2.13±0.28[a]	5.40±0.18[b]	3.73±0.18[ab]
C18:3n-3	0.46±0.05[a]	*tr*.[a]	*tr*.[a]	0.09±0.01[a]	*tr*.[a]	*tr*.[a]
C18:4n-3	*tr*.[a]	*tr* [a]	0.47±0.05[a]	*tr* .[a]	*tr* [a]	0.90±0.04[a]
C20:4n-6	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr*.[a]	*tr* [a]	*tr*.[a]
C20:4n-3	0.35±0.03[a]	*tr* [a]	0.23±0.03[a]	0.57±0.09[a]	*tr*.[a]	0.54±0.04[a a]
C20:5n-3	1.03±0.12[a]	0.33±0.06[a]	1.01±0.06[a]	2.11±0.33[a]	4.98±0.70[b]	2.70±0.14[ab]
C22:5n-3	*tr*. [a]	*tr*. [a]	*t*. [a]	*tr*.[a]	*tr*.[a]	*tr*.[a]
C22:6n-3	1.74±0.20[a]	1.25±0.04[a]	1.37±0.17[a]	3.03±0.17[a]	10.91±0.74[b]	3.43±0.45[a]
∑ SFA	42.14±0.53[a]	40.09±1.60[a]	41.06±0.78[a]	50.11±2.48[a]	39.84±0.83[b]	47.13±0.72[ab]
Σ MUFA	50.88±0.39[a]	54.34±0.90[b]	51.79±0.44[a]	41.96±2.37[a]	37.38±0.79[a]	41.58±0.39[a]
Σ PUFA	6.97±0.48[a]	5.57±0.70[a]	7.15±0.34[a]	7.94±0.23[a]	22.78±0.55[b]	11.29±0.34[a]
Σ (n-6)	3.74±0.26[a]	3.73±0.59[a]	4.31±0.24[a]	2.70±0.22[a]	6.26±0.22[b]	4.26±0.22[ab]
Σ (n-3)	3.23±0.34[a]	1.83±0.10[a]	2.84±0.14[a]	5.23±0.41[a]	16.52±0.32[b]	7.03±0.56[a]

Values (mean of three replications. n=10) in the same row. not sharing a common superscript are significantly different (P<0.05). [1]Contains *n-9* and *n-7* isomers. *tr*. Trace (<0.005 mg g^{-1}) [2]Contains some minor components not shown.

fractions of the fish. The percentages of SFA, PUFA, n-6 and n-3 fatty acids series in neutral lipids of fish were not significantly different (P>0.05) among fish fed the three diets, but differed in the amount of MUFA which was higher in fish fed the FBM diet. Likewise, increased proportions of DHA, PUFA and the sum of n-3 fatty acids were observed in the polar fraction fish fed FBM diet.

Results of economical evaluation including feed costs, costs *per* kg gain in weight and its ratio (ECR, Eq. 8) to that of fish fed commercial diet (Perform 205A) are presented in Table 8. These results

Table 8. Global results of economic parameters at the end of the experiment

	Diets		
	FBCM	FBM	P205A
FCR	1.46	1.50	1.51
Price index[2]	1.00	1.04	1.13
ECR [3]	1.46	1.56	1.70
EPI [4]	0.47	0.44	0.43
Relative ECR to 20P5A (%)	14.5	8.4	-

[1] Calculated from following price of the ingredients (January 2010): Fish meal (Peruvian Super-Prime) = 1.65 USD kg^{-1}; Blood meal = 0.75 € kg^{-1}. Feather meal = 0.50 € kg^{-1}. Corn meal = 0.65 € kg^{-1}

indicated that the incorporation of low levels of blood and feather meal as a substitute of fish meal decreased feed costs leading to a better economic conversion ratio. Costs of one kg gain in weight were reduced by 14.5% and 8.4% compared to the control diet (P205A). Economical profit index (EPI, Eq. 9) revealed that FBCM diet presented best economic viability, considering both fish sale price and cost of diets, although no significant differences were found.

Discussion

Reduction of the fishmeal dependency is becoming more important for the sustainability and profitability of commercial fish farming. Several recent studies have shown that rendered animal protein ingredients, such as BM, FM, PBM and MBM are highly digestible and have good nutritive value for fish (Luzier *et al*., 1995; Bureau *et al*., 1999; Nengas *et al*., 1999; Bureau *et al*., 2000; Kureshy *et al*., 2000; Wang *et al*., 2006). Most studies have focused on the use of these ingredients individually, reporting incorporation levels of 5-25% (El-Haroun *et al*., 2009). However, individual rendered animal protein meals, such as blood meal or hydrolysed feather meal often have deficiencies or excesses in essential amino acids that may affect the overall productivity of cultured fish (Fasakin *et al*., 2005). Moreover, these diets are not always representative of what is commonly used in the industry and the use of rendered animal protein ingredients needs to be evaluated in more practical diets. In the present study two practical diets were designed, accordingly to least cost formulation, to include low levels of blood and hydrolyzed feather meal (10% and 5%, respectively) and formulated to be DP/DE equivalent (22 mg/Kj), which has been demonstrated adequate for growth of seabream (Santinha *et al*., 1999). The three designed diets differed in the amount of digestible protein and energy.

In our experimental conditions, all three dietary treatments presented at least a three-fold increase in biomass after 90 experimental days, needed to make meaningful evaluation of diets and to show any major differences in growth performance (Bureau *et al*., 2000). No significant differences were found for weight gain and condition factor. Moreover, voluntary feed intake, feed conversion rate and protein efficiency rate were similar between dietary treatments, meaning that feeds were equally accepted by seabream juveniles and eaten in approximately equal amounts. These results suggest that palatability of the diets was not affected by the inclusion of blood and feather meal and that feed utilization and growth of juvenile gilthead seabream was not influenced by the dietary treatment.

Our findings agree with several studies which demonstrated that both feather meal and blood meal are nutritionally adequate protein sources for many fish species (Davies *et al*., 1989; Wang *et al*., 2006). Compared to the fish meal fed group (diet P205A), whole body composition of seabream fed diets containing blood and feather meal differed only in lipid content, which was lower in the P205A diet group. Effects of the experimental diets in whole-body protein content revealed that fish fed FBCM diet presented significantly higher protein content than both FBM and P205A diets.

Whole-body fatty acid composition demonstrated that apart from a very few exceptions, no marked differences were observed between all dietary groups. The few differences in composition do not reflect the fatty acid composition of the diets. These were observed in alpha linolenic acid; stearidonic acid; eicosatetraenoic acid; arachidonic acid and docosapentaenoic acid content, mostly because these fatty acids were not detected in fish fed FBM diet. Generally fish require n-3 fatty acids, rather than n-6 fatty acids with requirements varying among species. Gilthead seabream juveniles have been reported to have n-3 PUFA requirements of 19-23% total fatty acids in diets containing 80-100g kg^{-1} of lipids (Ibeas *et al*., 1994). In the present study, n-3 PUFA contents of the diets ranged between 27 and 29.5% total fatty acids.

Palmitic and oleic acid were the most abundant of saturates and monoenes, respectively, in polar and neutral lipid fractions of whole-body analysis, reflecting diets composition. This might indicate that these fatty acids are the main source of energy and the primary fatty acids selectively incorporated into membrane phospholipids with n-3 HUFA, as previously suggested by Ibeas *et al.* (1996). Differences regarding the polar fraction of fish fed FBM diet (lower myristic acid and palmitic acid content and higher DHA, n-3 fatty acids and PUFA) do not translate necessarily diet fatty acid composition, as no significant differences were observed between diets regarding DHA and PUFA content.

Several authors have mentioned that poor growth and feed utilization of fish fed feeds containing spray-dried blood meal or feather meals may be due to low protein digestibility and essential amino acid deficiency (mainly to deficient processing

of rendered meals). Though, in on our study these parameters were not evaluated, growth performance, feed utilization and carcass composition of fish fed diets containing blood and hydrolysed feather meal seem to indicate that inclusion of low levels of these two animal production by-products in seabream juveniles diets did not adversely affect growth or proximate composition within whole fish. Moreover, the feather and blood meal contributed only 28% of the digestive protein content in the diets.

Bureau *et al*. (2000) suggested the use of two or three protein sources in fish feed formulation to reduce the effects of nutrient imbalance, excessive levels of anti-nutritional factors or lower palatability. Several studies have proved the good potential of different combinations - PBM, FM and BM (Fowler, 1991); PBM and FM (Steffens, 1994; Nengas *et al*., 1999), MBM and BM (Millamena, 2002) PM, MBM, FM and BM (Guo *et al*., 2007) - in various fish species. The results in our study are in agreement with these findings indicating that a good combination of different rendered animal proteins appears to be complementary. Laporte *et al*. (2008) tested the utilization of several blends of feather meal with spray dried haemoglobin in seabream juveniles. Authors found that combining feather meal and blood meal did not prove to be advantageous due to low digestibility coefficients of the several kinds of blend tested. Nevertheless, those authors believe that, on the evidence of results obtained with trout, fish diets would benefit, in terms of both economics and nutrition, from a moderate inclusion of blended animal proteins.

In fact, the formulation of diets needs consideration of the relative cost and availability of different ingredients as well as their nutritional value. As described in our economical analyses, feed costs were the highest for the fishmeal traditional diet, but even a low percentage of fish meal substitution, by introduction of rendered protein sources and adjustments of the plant protein sources, lead to better economical conversion rates as 15% higher, with consequent better economic profit index. Replacement of fish meal with alternative plant and animal by-products offers the scope to produce flexible solutions whilst minimizing the final cost of the diet. Though prices of raw materials and feed ingredients vary, depending on each country importation tariffs, energy costs, seasonal factors and the economic status of the country, prices are based on fluctuating global markets and are considered to be major commodities for trading. The main constraint on using rendered animal products in fish feeds is consumer acceptance. Although these ingredients have proven to be effective substitutes and secondary protein sources to fish meal in temperate, tropical and marine fish species, their role must be addressed in the light of new information and public confidence in commercial terrestrial animal based feeds.

Given the urgent need to provide sustainable aquafeeds it is imperative to undertake further research to determine the quality and potential nutritional value of modern animal by-products. Poultry meat meal and feather meal are widely used on a global basis and in North America and Australia are included in commercial diets for trout and salmon as well as other species and the pet food market (Serwata, 2007). Moreover, it would be pertinent to include sensory evaluation of fish subjected to dietary formulations containing terrestrial animal derived proteins compared to standard marine protein based feeds of the final product.

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