Aquaculture Research

Aquaculture Research 2015, **46**, 994–1000 doi: 10.1111/are.12233

SHORT COMMUNICATION

A simple format feed to test the acceptability of ingredients for common octopus (*Octopus vulgaris* Cuvier, 1797)

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One of the main reasons for the slow development of cephalopod aquaculture is that there are no feeds available that are both palatable and contain a balanced nutritional composition for all stages of their growth (Vaz-Pires, Seixas & Barbosa 2004; Cerezo Valverde, Hernández, Aguado-Giménez & García García 2008). Current research is focused on the development and production of semi-moist or extruded diets made from dry ingredients (Morillo-Velarde, Cerezo Valverde, Hernández, Aguado-Giménez & García García 2012; Querol, Morillo-Velarde, Cerezo Valverde, Martinez Llorens, Moñino, Jover & Tomás 2012a,b) for their best conservation, stability and environmental impact on marine farms (Mazón, Piedecausa, Hernández & García García 2007). Although the results have been fairly satisfactory, they have still not managed to attain the high feeding and growth rates that are possible with crustacean diets. It is generally accepted that both the moisture content and the chemical agents used to make attractive diets will influence the degree to which they are found acceptable and be ingested (Domingues, López, Muñoz, Maldonado, Gaxiola & Rosas 2007). Indeed, the well-developed chemoreceptive capacity of octopus has been demonstrated in assays involving acids, sugars, salts, crab extracts, amino acids and nucleotides (Wells, O'Dor, Mangold & Well 1983; Lee 1992). Some of these substances may explain why the degree of acceptability and growth attained with both formulated and natural diets have been so dissimilar in cephalopods (Cerezo Valverde *et al.* 2008; Quintana, Domingues & García 2008; García-Garrido, Domingues, Navarro, Hachero, Garrido & Rosas 2010; Estefanell, Socorro, Tuya, Izquierdo & Roo 2011; Estefanell, Roo, Guirao, Alfonso, Fernández-Palacios, Izquierdo & Socorro 2012). In this respect, it should be noted that the ideal nutritional composition of a diet does not necessarily imply greater acceptability. For example, ongrowing experiments with diets containing *Mytilus galloprovincialis* have been associated with low ingestion and growth rates (Prato, Portacci & Biandolino 2010; Petza, Katsanevakis, Lykouri, Spiliotis & Verriopoulos 2011), despite its excellent nutritional composition for cephalopods as regards lipid classes and amino acids (Cerezo Valverde, Hernández, García-Garrido, Rodríguez, Estefanell, Gairín, Rodríguez, Tomás & García García 2012; Cerezo Valverde, Martínez-Llorens, Tomás Vidal, Jover, Rodríguez, Estefanell, Gairín, Domingues, Rodríguez & García García 2013).Therefore, there is a clear need for preliminary experiments in diet formulation using materials that will improve acceptability before any new material is incorporated. For this reason, this study looks at diet acceptability and consequent growth of *O. vulgaris* fed formulated diets differing exclusively in one test ingredient, using gelatine as binder due to its high digestibility and acceptability as demonstrated in previous assays (García-Garrido *et al.* 2010; Morillo-Velarde *et al.* 2012).

Specimens of *O. vulgaris* were captured in the Mediterranean Sea (Murcia, SE, Spain) and transferred to individual 216 L circular tanks in the laboratory. They were allowed to acclimatize for 2 weeks, during which time they were fed with bogue (*Boops*

Table 1 Test ingredients in the preparation of diets for *Octopus vulgaris*

Ingredients	Company
Plant meals	
Carob bean (CBM)	Pienso y Cereales Desco S.L., Valencia, Spain
Chickpea (CHM)	Panadería Rincón del Segura S.L., Elche de la Sierra, Albacete, Spain
Corn (COM)	White corn meal, Alimentos Polar Colombia S.A., Colombia
Gluten (GM)	Pienso y Cereales Desco S.L.
Oat (OM)	Agricultura de Conreu Ecológic, Maresme, Barcelona, Spain
Rice (RM)	Arrocería Pons S.A., Massanassa, Valencia, Spain
Soya bean (SBM)	Cocerva, Náquera, Valencia, Spain
Wheat (WM)	Marinera del Mar S.L., Almenara, Castellón, Spain
Animal meals	
Crab (CM)	*Polybius henslowii*, coming from fishing traditional, Galicia, Spain
Fish (FIM)	Skretting S.A., Burgos, Spain
Freeze-dried	
Chicken blood (FCB)	Chicken blood frozen and freeze-dried, Hijos de Pujante S.A., Beniel, Murcia, Spain
Discarded bogue (FDB)	*Boops boops*, coming from discarded of aquaculture, Murcia, Spain
Mussel (FM)	*Mytilus galloprovincialis*, coming from aquaculture, Galicia, Spain
Pea (FP)	Pea freeze and freeze-dried, Ultracongelados Virto S.A., Azagra, Navarra, Spain
Round sardinella (FS)	*Sardinella aurita*, coming from fishing traditional, Alicante, Spain
Wild bogue (FWB)	*Boops boops*, coming from fishing traditional, Alicante, Spain
Powder	
Whole egg (WEP)	Avícola San Isidro S.L. Los Belones, Cartagena, Murcia, Spain
Egg White (EWP)	Avícola San Isidro S.L. Los Belones
Egg yolk (EYP)	Avícola San Isidro S.L. Los Belones

Table 2 Proximate composition (% dry weight) of the diets tested

Diets	Moisture	Ash	Crude protein	Crude lipid	NFE
CBM	51.30 ± 2.04	4.60 ± 0.17	62.03 ± 1.60	0.49 ± 0.08	32.89 ± 1.62
CHM	53.46 ± 0.05	2.57 ± 0.13	49.75 ± 0.92	1.52 ± 0.14	46.17 ± 1.11
COM	51.28 ± 1.45	0.77 ± 0.06	42.28 ± 0.48	0.40 ± 0.11	56.56 ± 0.54
GM	52.22 ± 2.06	0.86 ± 0.04	79.70 ± 0.81	0.39 ± 0.06	19.05 ± 0.83
OM	55.67 ± 0.65	1.30 ± 0.06	42.28 ± 2.57	2.26 ± 0.64	54.15 ± 2.79
RM	51.32 ± 2.99	1.00 ± 0.03	43.42 ± 0.47	0.45 ± 0.11	55.13 ± 0.48
SBM	53.58 ± 1.28	4.01 ± 0.14	61.12 ± 2.38	2.76 ± 0.43	32.07 ± 2.13
WM	51.81 ± 1.62	0.89 ± 0.06	40.20 ± 0.63	0.25 ± 0.16	57.18 ± 2.74
CM	51.73 ± 0.85	12.61 ± 0.40	54.91 ± 0.08	4.20 ± 0.52	28.24 ± 0.45
FIM	56.40 ± 2.70	12.11 ± 1.52	84.02 ± 1.30	4.20 ± 0.37	0.00 ± 0.00
FCB	49.35 ± 1.06	4.09 ± 0.07	94.47 ± 0.46	0.38 ± 0.12	1.06 ± 0.61
FDB	51.37 ± 0.88	2.40 ± 0.20	59.80 ± 2.24	27.33 ± 1.09	10.48 ± 2.04
FM	51.47 ± 1.75	6.93 ± 0.26	70.08 ± 3.26	1.15 ± 0.18	21.84 ± 2.84
FP	53.94 ± 1.98	2.44 ± 0.02	51.57 ± 0.40	0.86 ± 0.09	46.97 ± 3.21
FS	48.93 ± 1.76	5.61 ± 0.61	73.32 ± 2.06	8.82 ± 0.24	14.96 ± 2.35
FWB	49.93 ± 0.78	4.54 ± 0.62	79.12 ± 1.11	4.22 ± 0.32	12.13 ± 0.90
WEP	51.82 ± 0.93	2.91 ± 0.07	64.49 ± 0.71	21.48 ± 0.71	11.12 ± 0.96
EWP	50.20 ± 1.47	2.74 ± 0.08	83.60 ± 1.09	0.63 ± 0.08	13.03 ± 1.14
EYP	51.79 ± 1.47	2.93 ± 0.06	63.14 ± 1.73	26.81 ± 1.04	7.32 ± 1.27

CBM, Carob bean meal; CHM, Chickpea meal; CM, *C. mediterranus* meal; COM, Corn meal; FIM, Fish meal; GM, Gluten meal; OM, Oat meal; RM, Rice meal; SBM, Soya bean meal; WM, Wheat meal; FCB, Freeze-dried chicken blood; FDB, Freeze-dried discarded *B. boops*; FM, Freeze-dried *M. galloprovincialis*; FP, Freeze-dried pea; FS, Freeze-dried *S. aurita*; FWB, Freeze-dried *wild B. boops*; WEP, Whole egg powder; EWP, Egg white powder; EYP, Egg yolk powder.

boops) and crab (*Carcinus mediterranus*) on alternate days. The tanks were equipped with a recirculation seawater system with mechanical and biological filtration; the water temperature was controlled at 18.2 ± 1.1°C and dissolved oxygen was at above 80% saturation. The diets were prepared so that they differed only in the ingredient being tested (30%), using gelatine as binder (20%) and distilled water (50%). Among the test ingredients were included eight plant meals (carob bean, chickpea, corn, oats, gluten, rice, soya bean, wheat), two animal meals (crab and fish), six freeze-dried substances (chicken blood, discarded bogue, mussels, pea, round sardinella and wild bogue) and three powered egg products (whole egg, egg white and egg yolk) (see Table 1 for abbreviations). To prepare the feeds, the gelatine was dissolved in water and then the test ingredient was added and mixed at 40°C with a cooking blender (Mycook® 1.8; Taurus, S.L. Lleida, Cataluña, Spain). The homogenized mixture was allowed to cool to 4°C in an aluminium mould for 24 h, and was then refrigerated at −4°C until use. Water stability was determined from the loss of dry matter in three samples of each feed after soaking in water for 4 and 24 h. Each diet was tested in experimental groups of between 6 and 14 male individuals (501–1329 g; one animal per tank) for a period of between 3 (when the feed was not accepted from the outset by any specimen) and 15 days. All experiments were not carried out simultaneously and the animals were not used for testing more than a diet. The octopuses were fed at

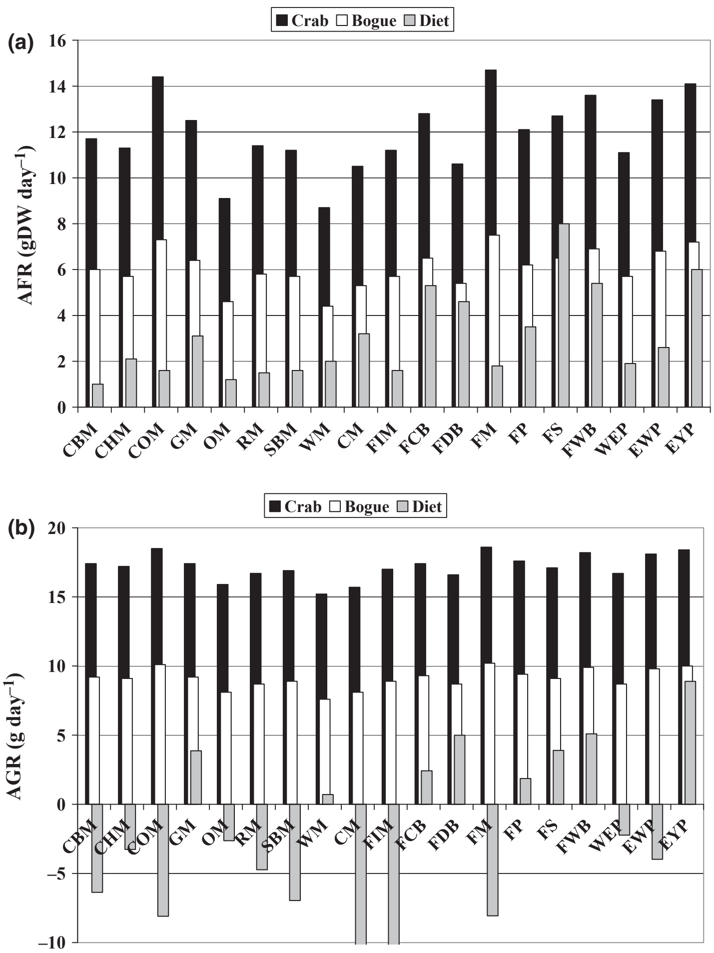

Figure 1 Mean values of the diets tested and estimation from the natural diets of *Boops boops* and *Carcinus mediterranus* in similar experimental conditions according to Aguado Giménez and García García (2002), (a) Absolute feeding rate in dry weight (AFR gDW day^{-1}); (b) Absolute growth rate (AGR g day^{-1}). CBM, Carob bean meal; CHM, Chickpea meal; CM, *C. mediterranus* meal; COM, Corn meal; FIM, Fish meal; GM, Gluten meal; OM, Oat meal; RM, Rice meal; SBM, Soya bean meal; WM, Wheat meal; FCB, Freeze-dried chicken blood; FDB, Freeze-dried discarded *B. boops*; FM, Freeze-dried *M. galloprovincialis*; FP, Freeze-dried pea; FS, Freeze-dried *S. aurita*; FWB, Freeze-dried wild *B. boops*; WEP, Whole egg powder; EWP, Egg white powder; EYP, Egg yolk powder.

09:00 hour 6 days a week. Feed remains were collected 4 h later, weighed daily and multiplied by a correction factor to discount the water absorbed or the disaggregation of the diet amending the changes of weight. The following indices were calculated:

- Absolute feeding rate in wet weight: AFR (g day^{-1}) = (Feed ingested in wet weight/no. days)
- Absolute growth rate: AGR (g day^{-1}) = Increase weight/no. days.
- Feed efficiency: FE (%) = (Increase weight/Total feed ingested)*100

To select the ingredients giving the best results, the feeding and growth values obtained with the formulated diets were compared with those obtained using natural diets based on fish (*B. boops*) or crab (*Carcinus mediterraneus*) in similar experimental conditions (of weight and temperature), as described by Aguado Giménez and García García (2002), obtaining the following indexes:

- Absolute feeding rate in dry weight obtained with the formulated diet: AFR (gDW day^{-1}) = (Feed ingested in dry weight/no. days).
- Absolute feeding rate in dry weight when octopuses were fed crab or bogue: AFRc (gDW day^{-1}) and AFRb (gDW day^{-1}) respectively.
- Absolute growth rate when octopuses were fed crab or bogue: AGRc (g day^{-1}) and AGRb (g day^{-1}) respectively.

The formulated diets were analysed in triplicate. A 1 g sample was used to obtain ash, moisture and crude protein and a 2 g sample to ascertain the crude lipid content. The moisture content was obtained by drying at 105 ± 1°C for 24 h to reach a constant weight (AOAC 1997; Method 930.15) and ash by incineration at 450 ± 1°C for 24 h in a muffle oven (HOBERSAL, HD-230, Forns Hobersal S.L., Barcelona, Spain). Crude protein was determined using the Kjeldhal method, with the corresponding conversion factor: 5.6 for meat, fish and eggs, 5.4 for cereals, 5.6 for corn, 5.5 for soybean, 5.4 for legumes, 5.55 for gelatine according to Mariotti, Tomé and Mirand (2008), and the material nitrogen-free extract (NFE) by difference. The results obtained were expressed as means ± standard deviation. Principal component analysis (PCA) was performed to obtain a more integrated interpretation of the biochemical composition and indices obtained from the different

Table 3 Growth and feed efficiency indices for *Octopus vulgaris* fed with the diets tested

Diets	*N*	Initial weight	AFR (g day^{-1})	SFR (%BW day^{-1})	AGR (g day^{-1})	SGR (%BW day^{-1})	FE %
CBM	8	849 ± 146	4.49 ± 1.59	0.55 ± 0.17	−3.27 ± 2.66	−0.41 ± 0.34	<0
CHM	8	841 ± 142	2.10 ± 0.55	0.26 ± 0.07	−6.37 ± 2.90	−0.77 ± 0.26	<0
COM	8	1261 ± 320	3.23 ± 2.95	0.24 ± 0.16	−8.10 ± 3.70	−0.71 ± 0.32	<0
GM	7	911 ± 142	6.89 ± 1.16	0.73 ± 0.11	3.87 ± 1.47	0.41 ± 0.13	56.85 ± 20.75
OM	14	501 ± 73	2.43 ± 1.19	0.49 ± 0.22	−2.63 ± 4.37	−0.52 ± 0.92	<0
RM	7	755 ± 178	3.41 ± 3.20	0.50 ± 0.49	−4.73 ± 4.09	−0.64 ± 0.57	<0
SBM	7	742 ± 192	3.35 ± 0.29	0.49 ± 0.14	−6.96 ± 3.81	−0.96 ± 0.41	<0
WM	8	623 ± 146	4.37 ± 1.62	0.70 ± 0.21	0.71 ± 3.17	0.06 ± 0.65	3.02 ± 110.60
CM	6	976 ± 99	7.05 ± 2.13	0.74 ± 0.17	−12.67 ± 9.71	−1.33 ± 0.97	<0
FIM	6	935 ± 107	3.43 ± 3.10	0.38 ± 0.32	−21.44 ± 6.73	−2.39 ± 0.70	<0
FCB	7	950 ± 164	10.49 ± 2.24	1.11 ± 0.26	2.42 ± 2.60	0.26 ± 0.29	24.55 ± 27.97
FDB	5	839 ± 92	9.92 ± 2.63	1.12 ± 0.20	4.99 ± 3.88	0.54 ± 0.41	44.44 ± 30.92
FM	8	1329 ± 186	3.77 ± 1.27	0.29 ± 0.11	−8.06 ± 6.89	−0.66 ± 0.58	<0
FP	7	792 ± 133	7.30 ± 1.53	0.91 ± 0.12	1.86 ± 1.55	0.22 ± 0.18	24.22 ± 19.00
FS	6	833 ± 134	16.55 ± 1.87	1.99 ± 0.37	3.89 ± 2.49	0.44 ± 0.27	23.30 ± 15.16
FWB	6	1141 ± 142	10.52 ± 1.96	0.90 ± 0.17	5.09 ± 2.56	0.43 ± 0.21	47.38 ± 16.62
WEP	8	728 ± 177	3.88 ± 2.62	0.56 ± 0.33	−2.23 ± 5.93	−0.29 ± 0.82	<0
EWP	6	1102 ± 84	6.13 ± 3.77	0.54 ± 0.27	−3.98 ± 7.71	−0.43 ± 0.67	<0
EYP	6	1211 ± 161	12.62 ± 3.54	0.98 ± 0.22	8.89 ± 4.73	0.67 ± 0.30	65.77 ± 20.48

CBM, Carob bean meal; CHM, Chickpea meal; CM, *C. mediterranus* meal; COM, Corn meal; FIM, Fish meal; GM, Gluten meal; OM, Oat meal; RM, Rice meal; SBM, Soya bean meal; WM, Wheat meal; FCB, Freeze-dried chicken blood; FDB, Freeze-dried discarded *B. boops*; FM, Freeze-dried *M. galloprovincialis*; FP, Freeze-dried pea; FS, Freeze-dried *S. aurita*; FWB, Freeze-dried *wild B. boops*; WEP, Whole egg powder; EWP, Egg white powder; EYP, Egg yolk powder.

formulated diets and groups of ingredients (plant meals, animal meals, freeze-dried ingredients and powdered egg products). SPSS version 16 (Pearson Deutschland GmbH, Munich, Germany) was used for statistical analyses of data.

All the formulated diets had a firm texture before and after being put into water, and there was no sign of disintegration when they were being manipulated by the octopus. This shows the stability of the diets that contain gelatine as binder. The survival rate was of 100% in all the experiments. The macronutrient composition of formulated diets used in this research is shown in Table 2. Figure 1 shows that the degree of acceptability of the diets containing freeze-dried ingredients (FS, FDB, FWB and FCB; see Table 1 for abbreviations) and egg yolk powder (EYP) was similar to that when *B. boops* is supplied (5.4–7.2 gDW day^{-1}), although a diet based on *C. mediterranus* doubled the values of all these diets (10.6–14.1 gDW day^{-1}; Aguado Giménez & García García 2002). In general, the growth rates obtained with the test diets were low compared with those estimated for natural diets (Table 3), with the exception of FDB (5.1 $\pm$ 2.6 g day^{-1}), FWB (5.0 $\pm$ 3.9 g day^{-1}) and EYP (8.9 $\pm$ 4.7 g day^{-1}). Similarly, FE was better in EYP, FP,

Table 4 Results obtained for principal component analysis taking into account the biochemical variables and rate analysed

	PC1	PC2	PC3
Eigenvalues	5.94	2.59	1.09
Per cent total variance	49.48	21.62	9.12
AFR (g day^{-1})	0.87	0.34	0.04
SFR (%BW day^{-1})	0.82	0.22	0.04
AGR (g day^{-1})	0.92	−0.34	0.01
SGR (%BW day^{-1})	0.92	−0.33	0.08
FE (%)	0.81	−0.37	0.16
Moisture	−0.60	−0.10	−0.58
Ash	−0.35	0.80	−0.04
Crude protein	0.28	0.79	0.31
Crude lipid	0.51	0.19	−0.72
NFE	−0.39	−0.86	0.08

NFE, nitrogen-free extract; FE, feed efficiency; ART, absolute growth rate; AFR, absolute feeding rate; PC, principal component.

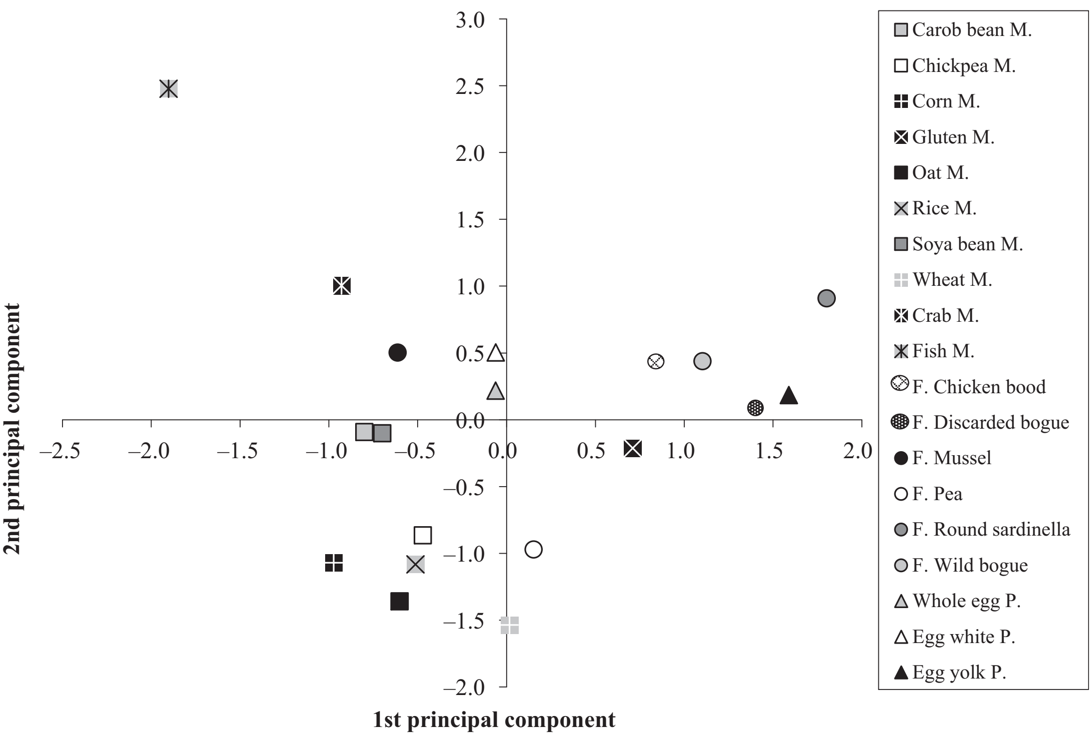

Figure 2 Separation of the different groups of diets (squares: meals; circles: freeze-dried ingredients; triangles: powdered egg products) on the basis of the first two principal components according to their biochemical composition and rates obtained.

FDB and FWB with respect to other diets tested, highlighting FE 65.8% in EYP (Table 3). Recently, some authors have elaborated artificial semi-moist (Morillo-Velarde *et al.* 2012) or extruded (Querol *et al.* 2012a,b) diets based exclusively on one or more of these dry or freeze-dried ingredients, obtaining good feeding and growth rates in *O. vulgaris*. In the PCA carried out, the three principal components (PC) selected explained 80.2% of the total variance. The first PC explained 49.5% of the variance and was related mainly to feeding and growth rates and FE, with high positive factor loadings (Table 4). The second PC explained 21.6% of the variance and was positively related to the percentage of ash and proteins and negatively related to the percentage of NFE. The third PC explained 9.1% of the variance and was negatively related to the percentage of lipids. Figure 2 shows a clear separation between the different groups of ingredients according to the first two principal components. All the meals, both plant and animal, with the exception of gluten meal, were separated from freeze-dried and powdered egg yolk based mainly on the first PC, with factor scores negatively related in meals and positively in freeze-dried ingredients. In this sense, the plant meals were characterized by low acceptability and poor growth rates according to the first CP and low percentages of protein and ash, and high NFE according to the second CP. This was also the case with the animal meals despite their greater protein and ash contents. The freeze-dried round sardinella and bogue (wild and discarded) and the powdered egg yolk were separated from the rest of the ingredients by high feeding and growth rates according to the first PC and by their greater percentage of protein and ash and lower NFE according to the second PC. These results suggest that future studies with artificial diets could contain a base of EYP and freeze-dried products, as they could help increase the acceptability of the diets and the growth of *O. vulgaris*. Furthermore, according to Querol *et al.* (2012a,b), these ingredients could serve as attractants or masking agents, encouraging the animals to feed on other, less acceptable ingredients, such as fish, crab or krill meals (Estefanell, Socorro, Roo, Naranjo, Martín, Fernández-Palacios & Izquierdo 2009; López, Rodríguez & Carrasco 2009), but which are considered to be nutritionally suitable. The possible advantages of freeze-dried ingredients or without thermal treatment than the meals or dehydrated ingredients have been argued previously by Domingues, Marquez, López and Rosas (2009) and Morillo-Velarde *et al.* (2012).

Acknowledgments

This work was financed by JACUMAR, the Spanish National Plans for Aquaculture, and was also partially sponsored by the IMIDA grant programme.

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Keywords: *O. vulgaris*, acceptability, semi-moist diet, formulated diet, freeze-dried ingredients, meal